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**Danubia Szabadalmi és Jogi Iroda Kft.,
Budapest**(54) **Pektát-liázok, az azokat kódoló nukleinsavak és eljárások előállításukra és alkalmazásukra**

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(54) **Pectate lyases, Nucleic Acids encoding them and methods for making and using them**

Pektatlyasen, Nukleinsäuren, die für diese kodieren und Verfahren zu deren Herstellung und Verwendung

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Description

TECHNICAL FIELD

[0001] This invention relates to molecular and cellular biology, biochemistry and biotechnology. In particular, the invention is directed to polypeptides having a pectate lyase activity, e.g., a pectinase, polynucleotides encoding the polypeptides, and methods for making and using these polynucleotides and polypeptides. The polypeptides of the invention can be used as pectate lyases to catalyze the beta-elimination or hydrolysis of pectin and/or polygalacturonic acid, such as 1,4-linked alpha-D-galacturonic acid. They can be used in variety of industrial applications, e.g., to treat plant cell walls, such as those in cotton or other natural fibers. In another exemplary industrial application, the polypeptides of the invention can be used in textile scouring.

BACKGROUND

[0002] Cotton fiber consists of a primary and a secondary cell wall. The secondary cell wall is practically pure cellulose, whereas the primary cell wall is a complex lattice of pectin, protein, waxes, pigments, hemicellulose and cellulose. In textile scouring of cellulosic material (e.g. knitted or woven cotton fabric) alkaline conditions (up to 10% NaOH) and high temperatures (up to 100°C) are needed for effective removal of primary cell wall components. This harsh chemical treatment results in raw material losses and in substantial environmental burden. There are several different enzymes that have the ability to degrade pectin; these are the pectinases, pectin methylesterases, pectin lyases and pectate lyases.

[0003] "Size" is the name given to the substance or mixture of substances that is applied to the warp thread before weaving. The size forms a coating around the surface of the thread before weaving. This coating provides the lubrication and prevents the breakage of warp thread during the weaving operation. Some common chemicals used to prepare sizes are Polyacrylic Acid (PA), Polyvinyl Alcohol (PVA), Starch, and Modified Starch. Cellulosic fibers including cotton, rayon and blend of these with synthetic fibers such as polyester, is usually sized with starch-based sizes. Desizing process removes the size before dyeing, printing and/or finishing. Starch sizes can be removed by hot acid wash, which will hydrolyze starch. However, acid hydrolysis results in loss of raw material since cellulose is also prone to acid hydrolysis. Starch sizes can also be removed by using hydrogen peroxide to degrade starch by oxidation. Desizing can also be an enzymatic process. Amylases have been used for many years in textile industry for removal of starch sizes. Conditions (e.g., pH and temperature) for enzymatic desizing are dictated by the operating conditions of the enzyme. Most amylases used in the application are relatively thermostable, however, they are neutral or acidic optimum enzymes.

[0004] "Scouring" is a process in which desized cotton fabric is processed to solubilize and extract undesired non-cellulosic material naturally found in cotton and also to remove applied impurities such as machinery lubricants. Scouring uses highly alkaline chemicals to remove the non-cellulosic material, which has a serious environmental impact. Additionally, the chemicals partially degrade the cellulose in the cotton fiber which causes a loss of fiber strength and raw materials and as such is a non-optimal process. The final step in the cotton fabric pretreatment process is bleaching in which the natural pigments and matter present in the fiber are bleached. A thermostable alkaline pectinolytic enzyme that could target specifically the non-cellulosic material could reduce or eliminate the use of harsh chemicals lessening the burden on the environment while maintaining the integrity and strength of the cotton fiber.

SUMMARY

[0005] The invention provides isolated, synthetic or recombinant nucleic acids comprising a nucleic acid sequence a) as set forth in SEQ NO:99 or b) a nucleic acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ NO:99, encoding at least one polypeptide having a pectate lyase activity. The sequence identities may be determined by analysis with a sequence comparison algorithm or by a visual inspection.

[0006] Exemplary nucleic acids of the invention also include isolated or recombinant nucleic acids encoding a polypeptide having a sequence as set forth in SEQ ID NO: 100. In one aspect, the polypeptide has a pectate lyase activity.

[0007] Claim 1 recites other nucleic acids of the invention.

[0008] The disclosure provides pectate lyase-encoding nucleic acids isolated from mixed cultures comprising a nucleic acid sequence, e.g., having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55,

SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:131, SEQ ID NO:133, over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, or more.

[0009] Pectate lyase-encoding nucleic acids have a common novelty in that they are derived from environmental sources, e.g., mixed environmental sources. Pectate lyase-encoding nucleic acids are isolated from environmental sources, e.g., mixed environmental sources, comprising a sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the disclosure over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200 or more, residues, wherein the nucleic acid encodes at least one polypeptide having a pectate lyase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

[0010] In one aspect, the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

[0011] An isolated or recombinant nucleic acid may include at least 10 consecutive bases of a nucleic acid sequence of the invention, sequences substantially identical thereto, and the sequences complementary thereto.

[0012] In one aspect, pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of pectin or polygalacturonic acid (pectate). The pectate lyase activity can comprise the breakup or dissolution of plant cell walls. The pectate lyase activity can comprise beta-elimination (trans-elimination) or hydrolysis of 1,4-linked alpha-D-galacturonic acid. The pectate lyase activity can comprise catalysis of beta-elimination (trans-elimination) or hydrolysis of methyl-esterified galacturonic acid. The pectate lyase activity can be exo-acting or endo-acting. In one aspect, the pectate lyase activity is endo-acting and acts at random sites within a polymer chain to give a mixture of oligomers. In one aspect, the pectate lyase activity is exo-acting and acts from one end of a polymer chain and produces monomers or dimers. The pectate lyase activity can catalyze the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid) by trans-elimination or hydrolysis. The pectate lyase activity can comprise activity the same or similar to pectate lyase (EC 4.2.2.2), poly(1,4-alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonate lyase (EC 4.2.2.9) or exo-poly-alpha-galacturonosidase (EC 3.2.1.82). The pectate lyase activity can comprise beta-elimination (trans-elimination) or hydrolysis of galactan to galactose or galactooligomers. The pectate lyase activity can comprise beta-elimination (trans-elimination) or hydrolysis of a plant fiber. The plant fiber can comprise cotton fiber, hemp fiber or flax fiber.

[0013] In one aspect, the isolated or recombinant nucleic acid encodes a polypeptide having a pectate lyase activity that is thermostable. The polypeptide can retain a pectate lyase activity under conditions comprising a temperature range of between about 37°C to about 95°C; between about 55°C to about 85°C, between about 70°C to about 95°C, or, between about 90°C to about 95°C.

[0014] In another aspect, the isolated or recombinant nucleic acid encodes a polypeptide having a pectate lyase activity that is thermotolerant. The polypeptide can retain a pectate lyase activity after exposure to a temperature in the range from greater than 37°C to about 95°C or anywhere in the range from greater than 55°C to about 85°C. In one aspect, the polypeptide retains a pectate lyase activity after exposure to a temperature in the range from greater than 90°C to about 95°C at pH 4.5.

[0015] The invention provides isolated, synthetic or recombinant nucleic acids comprising a sequence that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:99, wherein the nucleic acid encodes a polypeptide having a pectate lyase activity. The stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

[0016] A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a pectate lyase activity comprises at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more, consecutive bases of a sequence comprising a sequence of the invention, or fragments or subsequences thereof, wherein the probe identifies the nucleic acid by binding or hybridization. The probe can comprise an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a sequence comprising a sequence of the invention, or fragments or subsequences thereof.

[0017] A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a pectate lyase activity com-

prises a nucleic acid comprising a sequence at least about 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more residues having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to a nucleic acid of the invention, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

[0018] The probe can comprise an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a nucleic acid sequence of the invention, or a subsequence thereof.

[0019] An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity is capable of amplifying a nucleic acid comprising a sequence of the invention, or fragments or subsequences thereof. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence.

[0020] Methods of amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity comprise amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence of the invention, or fragments or subsequences thereof.

[0021] The invention provides expression cassettes comprising a nucleic acid of the invention or a subsequence thereof. In one aspect, the expression cassette can comprise the nucleic acid that is operably linked to a promoter. The promoter can be a viral, bacterial, mammalian or plant promoter. In one aspect, the plant promoter can be a potato, rice, corn, wheat, tobacco or barley promoter. The promoter can be a constitutive promoter. The constitutive promoter can comprise CaMV35S. In another aspect, the promoter can be an inducible promoter. In one aspect, the promoter can be a tissue-specific promoter or an environmentally regulated or a developmentally regulated promoter. Thus, the promoter can be, e.g., a seed-specific, a leaf-specific, a root-specific, a stem-specific or an abscission-induced promoter. In one aspect, the expression cassette can further comprise a plant or plant virus expression vector.

[0022] The invention provides cloning vehicles comprising an expression cassette (e.g., a vector) of the invention or a nucleic acid of the invention. The cloning vehicle can be a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector can comprise an adenovirus vector, a retroviral vector or an adeno-associated viral vector. The cloning vehicle can comprise a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

[0023] The invention provides transformed cell comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention, or a cloning vehicle of the invention. In one aspect, the transformed cell can be a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell. In one aspect, the plant cell can be a potato, wheat, rice, corn, tobacco or barley cell.

[0024] The invention provides transgenic non-human animals comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. In one aspect, the animal is a mouse.

[0025] Transgenic plants comprise a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. The transgenic plant can be a corn plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant or a tobacco plant.

[0026] Transgenic seeds may comprise a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. The transgenic seed can be a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a peanut or a tobacco plant seed.

[0027] An antisense oligonucleotide may comprise a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. Methods of inhibiting the translation of a pectate lyase message in a cell comprise administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention.

[0028] The invention provides an isolated, synthetic or recombinant polypeptide comprising (a) an amino acid sequence as set for in SEQ ID NO:100 or (b) an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:100, wherein the polypeptide has lyase activity. Further polypeptides of the invention are set out in claim 6. Exemplary polypeptides also include fragments of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 or more residues in length, or over the full length of an enzyme. A peptide can be, e.g., an immunogenic fragment, a motif (e.g., a binding site), a signal sequence, a prepro sequence or an active site. Exemplary polypeptide or peptide sequences of the invention include sequence encoded by a nucleic acid of the invention. Exemplary polypeptide or peptide sequences include polypeptides or peptides specifically bound by an antibody of the disclosure. In one aspect, the isolated or recombinant polypeptide of the invention (with or without a signal sequence) has pectate lyase activity.

[0029] An isolated or recombinant polypeptide or peptide may include at least 10 consecutive bases of a polypeptide

or peptide sequence of the invention, sequences substantially identical thereto, and the sequences complementary thereto.

[0030] In one aspect, pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of pectin or polygalacturonic acid (pectate). The pectate lyase activity can comprise the breakup or dissolution of plant cell walls. The pectate lyase activity can comprise beta-elimination (trans-elimination) or hydrolysis of 1,4-linked alpha-D-galacturonic acid. The pectate lyase activity can comprise catalysis of beta-elimination (trans-elimination) or hydrolysis of methyl-esterified galacturonic acid. The pectate lyase activity can be exo-acting or endo-acting. In one aspect, the pectate lyase activity is endo-acting and acts at random sites within a polymer chain to give a mixture of oligomers. In one aspect, the pectate lyase activity is exo-acting and acts from one end of a polymer chain and produces monomers or dimers. The pectate lyase activity can catalyze the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid) by trans-elimination or hydrolysis. The pectate lyase activity can comprise activity the same or similar to pectate lyase (EC 4.2.2.2), poly(1,4-alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonate lyase (EC 4.2.2.9) or exo-poly-alpha-galacturonosidase (EC 3.2.1.82). The pectate lyase activity can comprise beta-elimination (trans-elimination) or hydrolysis of galactan to galactose or galactooligomers. The pectate lyase activity can comprise beta-elimination (trans-elimination) or hydrolysis of a plant fiber. The plant fiber can comprise cotton fiber, hemp fiber or flax fiber.

[0031] In one aspect, the pectate lyase activity is thermostable. The polypeptide can retain a pectate lyase activity under conditions comprising a temperature range of between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 95°C, or between about 90°C to about 95°C. In another aspect, the pectate lyase activity can be thermotolerant. The polypeptide can retain a pectate lyase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, or in the range from greater than 55°C to about 85°C. In one aspect, the polypeptide can retain a pectate lyase activity after exposure to a temperature in the range from greater than 90°C to about 95°C at pH 4.5.

[0032] In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention that lacks a signal sequence. In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention comprising a heterologous signal sequence, such as a heterologous pectate lyase or non-pectate lyase signal sequence.

[0033] Chimeric proteins may comprise a first domain comprising a signal sequence of the invention (e.g., as set forth in Table 2, below) and at least a second domain. The protein can be a fusion protein. The second domain can comprise an enzyme. The enzyme can be a pectate lyase.

[0034] In one aspect, the pectate lyase activity comprises a specific activity at about 37°C in the range from about 100 to about 1000 units per milligram of protein. In another aspect, the pectate lyase activity comprises a specific activity from about 500 to about 750 units per milligram of protein. Alternatively, the pectate lyase activity comprises a specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein. In one aspect, the pectate lyase activity comprises a specific activity at 37°C in the range from about 750 to about 1000 units per milligram of protein. In another aspect, the thermotolerance comprises retention of at least half of the specific activity of the pectate lyase at 37°C after being heated to the elevated temperature. Alternatively, the thermotolerance can comprise retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to the elevated temperature.

[0035] The invention provides the isolated or recombinant polypeptide of the invention, wherein the polypeptide comprises at least one glycosylation site. In one aspect, glycosylation can be an N-linked glycosylation. In one aspect, the polypeptide can be glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.

[0036] In one aspect, the polypeptide can retain a pectate lyase activity under conditions comprising about pH 6.5, pH 6, pH 5.5, pH 5, pH 4.5 or pH 4. In another aspect, the polypeptide can retain a pectate lyase activity under conditions comprising about pH 7, pH 7.5 pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10, pH 10.5 or pH 11.

[0037] In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention that lacks a signal sequence and/or a prepro domain. In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention comprising a heterologous signal sequence and/or prepro domain, such as a heterologous pectate lyase signal sequence.

[0038] A signal sequence may comprise a peptide comprising/ consisting of a sequence as set forth in residues 1 to 12, 1 to 13, 1 to 14, 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 39, 1 to 40, 1 to 41, 1 to 42, 1 to 43, 1 to 44 of a polypeptide of the invention. Chimeric proteins comprise a first domain comprising such a signal sequence and at least a second domain. The protein can be a fusion protein. The second domain can comprise an enzyme. The enzyme can be a pectate lyase, e.g., an enzyme of the disclosure.

[0039] Chimeric polypeptides may comprise at least a first domain comprising signal peptide (SP), a prepro domain, a catalytic domain (CD), or an active site of a pectate lyase of the invention and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with

the signal peptide (SP), prepro domain or catalytic domain (CD). In one aspect, the heterologous polypeptide or peptide is not a pectate lyase. The heterologous polypeptide or peptide can be amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP), prepro domain or catalytic domain (CD).

[0040] The invention provides protein preparations comprising a polypeptide of the invention, wherein the protein preparation comprises a liquid, a solid or a gel.

[0041] Heterodimers may comprise a polypeptide of the invention and a second domain. In one aspect, the second domain can be a polypeptide and the heterodimer can be a fusion protein. In one aspect, the second domain can be an epitope or a tag. Homodimers may comprise a polypeptide of the invention.

[0042] The invention provides immobilized polypeptides having a pectate lyase activity, wherein the polypeptide comprises a polypeptide of the invention, a polypeptide encoded by a nucleic acid of the invention, or a polypeptide comprising a polypeptide of the invention and a second domain. In one aspect, the polypeptide can be immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

[0043] Arrays may comprise an immobilized nucleic acid of the invention. Arrays may comprise an antibody of the invention.

[0044] The invention provides isolated or recombinant antibodies that specifically bind to a polypeptide of the invention or to a polypeptide encoded by a nucleic acid of the invention. The antibody can be a monoclonal or a polyclonal antibody. Hybridomas may comprise an antibody of the invention, e.g., an antibody that specifically binds to a polypeptide of the invention or to a polypeptide encoded by a nucleic acid of the invention.

[0045] The invention provides food supplements for an animal comprising a polypeptide of the invention, e.g., a polypeptide encoded by the nucleic acid of the invention. In one aspect, the polypeptide in the food supplement can be glycosylated. The invention provides edible enzyme delivery matrices comprising a polypeptide of the invention, e.g., a polypeptide encoded by the nucleic acid of the invention. In one aspect, the delivery matrix comprises a pellet. In one aspect, the polypeptide can be glycosylated. In one aspect, the pectate lyase activity is thermotolerant. In another aspect, the pectate lyase activity is thermostable.

[0046] A method of isolating or identifying a polypeptide having a pectate lyase activity comprises the steps of: (a) providing an antibody of the invention; (b) providing a sample comprising polypeptides; and (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a pectate lyase activity.

[0047] Methods of making an anti-pectate lyase antibody comprise administering to a non-human animal a nucleic acid of the invention or a polypeptide of the invention or subsequences thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-pectate lyase antibody. Methods of making an anti-pectate lyase immune comprise administering to a non-human animal a nucleic acid of the invention or a polypeptide of the invention or subsequences thereof in an amount sufficient to generate an immune response.

[0048] The invention provides methods of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid of the invention operably linked to a promoter; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide. In one aspect, the method can further comprise transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

[0049] Methods for identifying a polypeptide having a pectate lyase activity comprise the following steps: (a) providing a polypeptide of the invention; or a polypeptide encoded by a nucleic acid of the invention; (b) providing a pectate lyase substrate; and (c) contacting the polypeptide or a fragment or variant thereof of step (a) with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a pectate lyase activity.

[0050] Methods for identifying a pectate lyase substrate comprise the following steps: (a) providing a polypeptide of the invention; or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test substrate; and (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a pectate lyase substrate.

[0051] Methods of determining whether a test compound specifically binds to a polypeptide comprise the following steps: (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid comprises a nucleic acid of the invention, or, providing a polypeptide of the invention; (b) providing a test compound; (c) contacting the polypeptide with the test compound; and (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

[0052] Methods for identifying a modulator of a pectate lyase activity comprise the following steps: (a) providing a polypeptide of the invention or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test compound; (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the pectate

lyase, wherein a change in the pectate lyase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the pectate lyase activity. In one aspect, the pectate lyase activity can be measured by providing a pectate lyase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product. A decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of pectate lyase activity. An increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of pectate lyase activity.

[0053] Computer systems may comprise a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence of the invention (e.g., a polypeptide encoded by a nucleic acid of the invention). In one aspect, the computer system can further comprise a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon. In another aspect, the sequence comparison algorithm comprises a computer program that indicates polymorphisms. In one aspect, the computer system can further comprise an identifier that identifies one or more features in said sequence. Computer readable media may have stored thereon a polypeptide sequence or a nucleic acid sequence of the invention. Methods for identifying a feature in a sequence comprise the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence of the invention; and (b) identifying one or more features in the sequence with the computer program. Methods for comparing a first sequence to a second sequence comprise the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence of the invention; and (b) determining differences between the first sequence and the second sequence with the computer program. The step of determining differences between the first sequence and the second sequence can further comprise the step of identifying polymorphisms. In one aspect, the method can further comprise an identifier that identifies one or more features in a sequence. In another aspect, the method can comprise reading the first sequence using a computer program and identifying one or more features in the sequence.

[0054] Methods for isolating or recovering a nucleic acid encoding a polypeptide having a pectate lyase activity from an environmental sample comprise the steps of: (a) providing an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity, wherein the primer pair is capable of amplifying a nucleic acid of the invention; (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and, (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide having a pectate lyase activity from an environmental sample. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence of the invention.

[0055] Methods for isolating or recovering a nucleic acid encoding a polypeptide having a pectate lyase activity from an environmental sample comprise the steps of: (a) providing a polynucleotide probe comprising a nucleic acid of the invention or a subsequence thereof; (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a); (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide having a pectate lyase activity from an environmental sample. The environmental sample can comprise a water sample, a liquid sample, a soil sample, an air sample or a biological sample. In one aspect, the biological sample can be derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

[0056] Methods of generating a variant of a nucleic acid encoding a polypeptide having a pectate lyase activity comprise the steps of: (a) providing a template nucleic acid comprising a nucleic acid of the invention; and (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid. The method can further comprise expressing the variant nucleic acid to generate a variant pectate lyase polypeptide. The modifications, additions or deletions can be introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis (GSSM™), synthetic ligation reassembly (SLR) or a combination thereof. The modifications, additions or deletions may be introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, ra-

diogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

[0057] The method can be iteratively repeated until a pectate lyase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced. The variant pectate lyase polypeptide may be thermotolerant, and retain some activity after being exposed to an elevated temperature. Or, the variant pectate lyase polypeptide may have increased glycosylation as compared to the pectate lyase encoded by a template nucleic acid. Alternatively, the variant pectate lyase polypeptide has a pectate lyase activity under a high temperature, wherein the pectate lyase encoded by the template nucleic acid is not active under the high temperature. The method can be iteratively repeated until a pectate lyase coding sequence having an altered codon usage from that of the template nucleic acid is produced. Alternatively, the method can be iteratively repeated until a pectate lyase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

[0058] Methods for modifying codons in a nucleic acid encoding a polypeptide having a pectate lyase activity to increase its expression in a host cell, comprise the following steps: (a) providing a nucleic acid of the invention encoding a polypeptide having a pectate lyase activity; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

[0059] Methods for modifying codons in a nucleic acid encoding a polypeptide having a pectate lyase activity comprise the following steps: (a) providing a nucleic acid of the invention; and, (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a pectate lyase.

[0060] Methods for modifying codons in a nucleic acid encoding a polypeptide having a pectate lyase activity to increase its expression in a host cell comprise the following steps: (a) providing a nucleic acid of the invention encoding a pectate lyase polypeptide; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

[0061] Methods for modifying a codon in a nucleic acid encoding a polypeptide having a pectate lyase activity to decrease its expression in a host cell comprise the following steps: (a) providing a nucleic acid of the invention; and (b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell. In one aspect, the host cell can be a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

[0062] Methods for producing a library of nucleic acids encoding a plurality of modified pectate lyase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site, comprise the following steps: (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a nucleic acid of the invention, and the nucleic acid encodes a pectate lyase active site or a pectate lyase substrate binding site; (b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, (c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified pectate lyase active sites or substrate binding sites. In one aspect, the method comprises mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSM™), synthetic ligation re-assembly (SLR), error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis (GSSM™), synthetic ligation reassembly (SLR) and a combination thereof. In another aspect, the method comprises mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, Chimeric nucleic acid multimer creation and a combination thereof.

[0063] Methods for making a small molecule comprise the following steps: (a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a pectate lyase enzyme encoded by a nucleic acid of the invention; (b) providing a substrate for at least one of the enzymes of step (a); and (c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions. Methods for modifying a small molecule comprise the following steps: (a) providing a pectate lyase enzyme, wherein the enzyme comprises a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention, or a subsequence thereof; (b) providing a small molecule; and (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the pectate lyase enzyme, thereby modifying a small molecule by a pectate lyase enzymatic reaction. In one aspect, the method can comprise a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the pectate lyase enzyme. In one aspect, the method can comprise a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions. In another aspect, the method can further comprise the step of testing the library to determine if a particular modified small molecule that exhibits a desired activity is present within the library. The step of testing the library can further comprise the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

[0064] Methods for determining a functional fragment of a pectate lyase enzyme comprise the steps of: (a) providing a pectate lyase enzyme, wherein the enzyme comprises a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention, or a subsequence thereof; and (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a pectate lyase activity, thereby determining a functional fragment of a pectate lyase enzyme. In one aspect, the pectate lyase activity is measured by providing a pectate lyase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

[0065] Methods for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis comprise the following steps: (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid of the invention; (b) culturing the modified cell to generate a plurality of modified cells; (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and, (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis. In one aspect, the genetic composition of the cell can be modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene. In one aspect, the method can further comprise selecting a cell comprising a newly engineered phenotype. In another aspect, the method can comprise culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

[0066] Methods of increasing thermotolerance or thermostability of a pectate lyase polypeptide comprise glycosylating a pectate lyase polypeptide, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide of the invention; or a polypeptide encoded by a nucleic acid sequence of the invention, thereby increasing the thermotolerance or thermostability of the pectate lyase polypeptide. In one aspect, the pectate lyase specific activity can be thermostable or thermotolerant at a temperature in the range from greater than about 37°C to about 95°C.

[0067] Methods for overexpressing a recombinant pectate lyase polypeptide in a cell comprise expressing a vector comprising a nucleic acid comprising a nucleic acid of the invention or a nucleic acid sequence of the invention, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

[0068] Methods of making a transgenic plant comprise the following steps: (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a nucleic acid sequence of the invention, thereby producing a transformed plant cell; and (b) producing a transgenic plant from the transformed cell. In one aspect, the step (a) can further comprise introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts. In another aspect, the step (a) can further comprise introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment. Alternatively, the step (a) can further comprise introducing the heterologous nucleic acid sequence into the plant cell DNA using an *Agrobacterium tumefaciens* host. In one aspect, the plant cell can be a potato, corn, rice, wheat, tobacco, or barley cell.

[0069] Methods of expressing a heterologous nucleic acid sequence in a plant cell comprise the following steps: (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a nucleic acid of the invention; (b) growing the plant under conditions wherein the

heterologous nucleic acids sequence is expressed in the plant cell.

[0070] An isolated or recombinant signal sequence comprises or consists of signal peptides (SP), as set forth in Table 2. Isolated or recombinant signal sequence may consist of a sequence as set forth in residues 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 39, 1 to 40, 1 to 41, 1 to 42, 1 to 43, and/or 1 to 44, of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134.

[0071] Isolated or recombinant peptides may consist of a pectin methyl esterase domain (PED) or a catalytic domain (CD) as set forth in Table 2.

[0072] Chimeric polypeptides may comprise at least a first domain comprising signal peptide (SP), a pectin methyl esterase domain (PED) or a catalytic domain (CD) as set forth in Table 2 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP), pectin methyl esterase domain (PED) or catalytic domain (CD). In one aspect, the heterologous polypeptide or peptide is not a pectate lyase. The heterologous polypeptide or peptide can be amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP), pectin methyl esterase domain (PED) or catalytic domain (CD).

[0073] Isolated or recombinant nucleic acids may encode a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP), a pectin methyl esterase domain (PED) or a catalytic domain (CD) as set forth in Table 2 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP), pectin methyl esterase domain (PED) or catalytic domain (CD).

[0074] A method of increasing thermotolerance or thermostability of a pectate lyase comprises glycosylating a pectate lyase, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide of the invention, thereby increasing the thermotolerance or thermostability of the pectate lyase.

[0075] Methods for overexpressing a recombinant pectate lyase in a cell comprise expressing a vector comprising a nucleic acid of the invention, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

[0076] Methods of making a transgenic plant comprise the following steps: (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a nucleic acid of the invention, thereby producing a transformed plant cell; (b) producing a transgenic plant from the transformed cell. In one aspect, step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts. Step (a) can comprise introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an *Agrobacterium tumefaciens* host.

[0077] Methods of expressing a heterologous nucleic acid sequence in a plant cell comprise the following steps: (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence of the invention; (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

[0078] The invention provides methods for hydrolyzing, breaking up or disrupting a pectin- or pectate (polygalacturonic acid)-comprising composition comprising the following steps: (a) providing a polypeptide of the invention having a pectate lyase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition comprising a pectin or a pectate; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide hydrolyzes, breaks up or disrupts the pectin- or pectate-comprising composition. In one aspect, the composition comprises a plant cell wall or a bacterial cell wall. The plant can be a cotton plant, a hemp plant or a flax plant.

[0079] The invention provides methods for liquefying or removing a pectin or pectate (polygalacturonic acid) from a composition comprising the following steps: (a) providing a polypeptide of the invention having a pectate lyase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition comprising a pectin or pectate (polygalacturonic acid); and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide removes or liquefies the pectin or pectate (polygalacturonic acid).

[0080] The invention provides detergent compositions comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention, wherein the polypeptide has a pectate lyase activity. In one aspect, the

pectate lyase is a nonsurface-active pectate lyase or a surface-active pectate lyase. The pectate lyase can be formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form.

[0081] The invention provides methods for washing an object comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a pectate lyase activity; (b) providing an object; and (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

[0082] The invention provides textiles or fabrics comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention. The invention provides methods for fiber, thread, textile or fabric scouring comprising the following steps: (a) providing a polypeptide of the invention having a pectate lyase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a fiber, a thread, a textile or a fabric; and (c) contacting the polypeptide of step (a) and the textile or fabric of step (b) under conditions wherein the pectate lyase can scour the fiber, thread, textile or fabric. In one aspect, the pectate lyase is an alkaline active and thermostable pectate lyase. The desizing and scouring treatments can be combined in a single bath. The method can further comprise addition of an alkaline and thermostable amylase in the contacting of step (c). The desizing or scouring treatments can comprise conditions of between about pH 8.5 to pH 10.0 and temperatures of at about 40°C. The method can further comprise addition of a bleaching step. The desizing, scouring and bleaching treatments can be done simultaneously or sequentially in a single-bath container. The bleaching treatment can comprise hydrogen peroxide or at least one peroxy compound that can generate hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator. The fiber, thread, textile or fabric can comprise a cellulosic material. The cellulosic material can comprise a crude fiber, a yarn, a woven or knit textile, a cotton, a linen, a flax, a ramie, a rayon, a hemp, a jute or a blend of natural or synthetic fibers.

[0083] The invention provides feeds or foods comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention. The invention provides methods improving the extraction of oil from an oil-rich plant material comprising the following steps: (a) providing a polypeptide of the invention having a pectate lyase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing an oil-rich plant material; and (c) contacting the polypeptide of step (a) and the oil-rich plant material. In one aspect, the oil-rich plant material comprises an oil-rich seed. The oil can be a soybean oil, an olive oil, a rapeseed (canola) oil or a sunflower oil.

[0084] The invention provides methods for preparing a fruit or vegetable juice, syrup, puree or extract comprising the following steps: (a) providing a polypeptide of the invention having a pectate lyase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition or a liquid comprising a fruit or vegetable material; and (c) contacting the polypeptide of step (a) and the composition, thereby preparing the fruit or vegetable juice, syrup, puree or extract.

[0085] The invention provides papers or paper products or paper pulps comprising a pectate lyase of the invention, or a polypeptide encoded by a nucleic acid of the invention. The invention provides methods for treating a paper or a paper or wood pulp comprising the following steps: (a) providing a polypeptide of the invention having a pectate lyase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition comprising a paper or a paper or wood pulp; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the pectate lyase can treat the paper or paper or wood pulp.

[0086] The invention provides pharmaceutical compositions comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention. The pharmaceutical composition can act as a digestive aid.

[0087] The invention provides oral care products comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention. The oral care product can comprise a toothpaste, a dental cream, a gel or a tooth powder, an odontic, a mouth wash, a pre- or post brushing rinse formulation, a chewing gum, a lozenge or a candy.

[0088] The disclosure provides isolated or recombinant nucleic acids having a sequence comprising a sequence modification of SEQ ID NO:131, wherein the modification of SEQ ID NO:131 comprises one or more of the following changes: the nucleotides at residues 352 to 354 are CAT or CAC, the nucleotides at residues 544 to 546 are GTG, GTT, GTC, or GTA, the nucleotides at residues 568 to 570 are TTG, TTA, CTT, CTC, CTA, or CTG, the nucleotides at residues 589 to 591 are GGT, GGC, GGA, or GGG, the nucleotides at residues 622 to 624 are AAG or AAA, the nucleotides at residues 655 to 657 are ATG, the nucleotides at residues 667 to 669 are GAG or GAA, the nucleotides at residues 763 to 765 are CGG, CGT, CGC, CGA, AGA, AGG, the nucleotides at residues 787 to 789 are AAG or AAA, the nucleotides at residues 823 to 825 are TAT or TAC, the nucleotides at residues 925 to 927 are TGG, or the nucleotides at residues 934 to 936 are GTT, GTG, GTC, or GTA. In one aspect, the nucleic acid encodes a polypeptide having a pectate lyase activity, which can be thermotolerant or thermostable.

[0089] The disclosure provides isolated or recombinant nucleic acids having a sequence comprising a sequence modification of a nucleic acid of the disclosure (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67,

SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129), wherein the sequence modification comprises one or more of the following changes: the nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO:131 are changed to CAT or CAC, the nucleotides at the equivalent of residues 544 to 546 of SEQ ID NO:131 are changed to GTG, GTT, GTC, or GTA, the nucleotides at the equivalent of residues 568 to 570 of SEQ ID NO:131 are changed to TTG, TTA, CTT, CTC, CTA, or CTG, the nucleotides at the equivalent of residues 589 to 591 of SEQ ID NO:131 are changed to GGT, GGC, GGA, or GGG, the nucleotides at the equivalent of residues 622 to 624 of SEQ ID NO:131 are changed to AAG or AAA, the nucleotides at the equivalent of residues 655 to 657 of SEQ ID NO:131 are changed to ATG, the nucleotides at the equivalent of residues 667 to 669 of SEQ ID NO:131 are GAG or GAA, the nucleotides at the equivalent of residues 763 to 765 of SEQ ID NO:131 are changed to CGG, CGT, CGC, CGA, AGA, AGG, the nucleotides at the equivalent of residues 787 to 789 of SEQ ID NO:131 are changed to AAG or AAA, the nucleotides at the equivalent of residues 823 to 825 of SEQ ID NO:131 are changed to TAT or TAC, the nucleotides at the equivalent of residues 925 to 927 of SEQ ID NO:131 are changed to TGG, or the nucleotides at the equivalent of residues 934 to 936 of SEQ ID NO:131 are changed to GTT, GTG, GTC, or GTA. In one aspect, the nucleic acid encodes a polypeptide having a pectate lyase activity, which can be thermotolerant or thermostable.

[0090] The disclosure provides isolated or recombinant polypeptides having a sequence comprising a sequence modification of SEQ ID NO:132, wherein the modification of SEQ ID NO:132 comprises one or more of the following mutations: the alanine at amino acid position 118 is histidine, the alanine at amino acid position 182 is valine, the threonine at amino acid position 190 is leucine, the alanine at amino acid position 197 is glycine, the serine at amino acid position 208 is lysine, the threonine at amino acid position 219 is methionine, the threonine at amino acid position 223 is glutamic acid, the serine at amino acid position 255 is arginine, the serine at amino acid position 263 is lysine, the asparagine at amino acid position 275 is tyrosine, the tyrosine at amino acid position 309 is tryptophan, or, the serine at amino acid position 312 is valine. In one aspect, the polypeptide has a pectate lyase activity, which can be thermotolerant or thermostable.

[0091] The disclosure provides isolated or recombinant polypeptides having a sequence comprising a sequence modification of a polypeptide of the disclosure (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130), wherein the sequence modification comprises one or more of the following changes: the amino acid at the equivalent of the alanine at residue 118 of SEQ ID NO:132 is changed to a histidine, the amino acid at the equivalent of the alanine at residue 182 of SEQ ID NO:132 is changed to a valine, the amino acid at the equivalent of the threonine at residue 190 of SEQ ID NO:132 is changed to a leucine, the amino acid at the equivalent of the alanine at residue 197 of SEQ ID NO:132 is changed to a glycine, the amino acid at the equivalent of the serine at residue 208 of SEQ ID NO:132 is changed to a lysine, the amino acid at the equivalent of the threonine at residue 219 of SEQ ID NO:132 is changed to a methionine, the amino acid at the equivalent of the threonine at residue 223 of SEQ ID NO:132 is changed to a glutamic acid, the amino acid at the equivalent of the serine at residue 255 of SEQ ID NO:132 is changed to a arginine, the amino acid at the equivalent of the serine at residue 263 of SEQ ID NO:132 is changed to a lysine, the amino acid at the equivalent of the asparagine at residue 275 of SEQ ID NO:132 is changed to a tyrosine, the amino acid at the equivalent of the tyrosine at residue 309 of SEQ ID NO:132 is changed to a tryptophan, or, the amino acid at the equivalent of the serine at residue 312 of SEQ ID NO:132 is changed to a valine. In one aspect, the polypeptide has a pectate lyase activity, which can be thermotolerant or thermostable.

[0092] Methods for generating a modified pectate-lyase encoding nucleic acid comprise making one or more sequence modifications to a pectate-lyase encoding nucleic acid, wherein the changes in the pectate-lyase encoding nucleic acid are equivalent to one or more of the following: changing nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO:131 to CAT or CAC, changing nucleotides at the equivalent of residues 544 to 546 of SEQ ID NO:131 to GTG, GTT, GTC, or GTA, changing nucleotides at the equivalent of residues 568 to 570 of SEQ ID NO:131 to TTG, TTA, CTT, CTC, CTA, or CTG, changing nucleotides at the equivalent of residues 589 to 591 of SEQ ID NO:131 to GGT, GGC, GGA, or GGG, changing nucleotides at the equivalent of residues 622 to 624 of SEQ ID NO:131 to AAG or AAA, changing nucleotides at the equivalent of residues 655 to 657 of SEQ ID NO:131 to ATG, changing nucleotides at the equivalent of residues 667 to 669 of SEQ ID NO:131 to GAG or GAA, the nucleotides at the equivalent of residues 763 to 765 of

SEQ ID NO:131 to CGG, CGT, CGC, CGA, AGA, AGG, changing nucleotides at the equivalent of residues 787 to 789 of SEQ ID NO:131 to AAG or AAA, changing nucleotides at the equivalent of residues 823 to 825 of SEQ ID NO:131 to TAT or TAC, changing nucleotides at the equivalent of residues 925 to 927 of SEQ ID NO:131 to TGG, or changing nucleotides at the equivalent of residues 934 to 936 of SEQ ID NO:131 to GTT, GTG, GTC, or GTA. In one aspect, the modified pectate lyase activity has a thermotolerant or thermostable activity. In one aspect, the pectate-lyase encoding nucleic acid comprises a nucleic acid having a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133.

[0093] Methods for generating a modified pectate lyase comprise making one or more sequence modifications to a pectate lyase, wherein the changes in the pectate lyase are equivalent to one or more of the following changes: the amino acid at the equivalent of the alanine at residue 118 of SEQ ID NO:132 is changed to a histidine, the amino acid at the equivalent of the alanine at residue 182 of SEQ ID NO:132 is changed to a valine, the amino acid at the equivalent of the threonine at residue 190 of SEQ ID NO:132 is changed to a leucine, the amino acid at the equivalent of the alanine at residue 197 of SEQ ID NO:132 is changed to a glycine, the amino acid at the equivalent of the serine at residue 208 of SEQ ID NO:132 is changed to a lysine, the amino acid at the equivalent of the threonine at residue 219 of SEQ ID NO:132 is changed to a methionine, the amino acid at the equivalent of the threonine at residue 223 of SEQ ID NO:132 is changed to a glutamic acid, the amino acid at the equivalent of the serine at residue 255 of SEQ ID NO:132 is changed to a arginine, the amino acid at the equivalent of the serine at residue 263 of SEQ ID NO:132 is changed to a lysine, the amino acid at the equivalent of the asparagine at residue 275 of SEQ ID NO:132 is changed to a tyrosine, the amino acid at the equivalent of the tyrosine at residue 309 of SEQ ID NO:132 is changed to a tryptophan, or, the amino acid at the equivalent of the serine at residue 312 of SEQ ID NO:132 is changed to a valine. In one aspect, the pectate lyase comprises a sequence of the disclosure (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130). In one aspect, the modified pectate lyase activity has a thermotolerant or thermostable activity.

[0094] The invention provides formulations comprising at least one enzyme of the invention comprising dosages in the range of between about 1 gram per ton and 100 or more grams per ton (per ton treated material, e.g., per ton fabric, cloth or the like), between about 10 grams per ton and 90 grams per ton, between about 20 grams per ton and 80 gram per ton, between about 30 grams per ton and 70 grams per ton, between about 40 grams per ton and 50 grams per ton. For example, exemplary formulations comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, etc. to 100, 200, 300, 400, 500, etc. or more grams per ton.

[0095] Alternatively, the invention provides formulations comprising at least one enzyme of the invention comprising dosages in the range of between about 1 μ g per gram and 100 or more μ g per gram (per gram treated material, e.g., per gram fabric, cloth or the like), between about 10 μ g per gram and 90 μ g per gram, between about 20 μ g per gram and 80 μ g per gram, between about 30 μ g per gram and 70 μ g per gram, between about 40 μ g per gram and 50 μ g per gram. For example, exemplary formulations comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, etc. to 100, 200, 300, 400, 500, etc. or more μ g per gram (e.g., per gram fabric, cloth or the like).

[0096] Alternatively, the invention provides formulations comprising at least one enzyme of the invention comprising dosages in the range of between about 0.5 mg per pound and 50 or more mg per pound (per pound treated material, e.g., per pound fabric, cloth or the like), between about 1 mg per pound and 45 mg per pound, between about 5 mg per pound and 40 mg per pound, between about 10 mg per pound and 35 mg per pound, between about 15 mg per pound and 30 mg per pound. For example, exemplary formulations comprise about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, etc. to 50 or more mg per pound (e.g., per pound fabric, cloth or the like).

[0097] The invention provides formulations comprising at least one enzyme of the invention comprising dosages comprising an enzyme strength of between about 100 to 40,000 units/ml, 200 to 30,000 units/ml, 500 to 30,000 units/ml, 1000 to 20,000 units/ml, 1000 to 25,000 units/ml, 1000 to 15,000 units/ml, 1000 to 10000 units/ml, 1000 to 5000 units/ml, between about 2000 to 20000 units/ml, between about 2000 to 15000 units/ml, between about 2000 to 10000 units/ml, or between about 2000 to 4000 units/ml, or, between about 200 to 25,000 units/ml, 200 to 20,000 units/ml, 200 to 15000 units/ml, 200 to 10,000 units/ml, between about 400 to 8000 units/ml, between about 600 to 6000 units/ml, between about 800 to 4000 units/ml, or between about 1000 to 2000 units/ml., or, wherein the dosage comprises an enzyme strength of about 1000 u/ml. or, wherein the dosage comprises an enzyme strength of about 3000 units/ml.

[0098] In one aspect, the formulation comprises a lyophilized enzyme (e.g., an enzyme of the invention), or, the formulation is a water-based formulation comprising an enzyme of the invention. In one aspect, the formulation comprises a lyophilized enzyme resuspended in water. In one aspect, a formulation of the invention further comprises a glycerol, sucrose, sodium chloride, dextrin, propylene glycol, sorbitol, sodium sulphate or TRIS, or an equivalent. In one aspect, a formulation of the invention further comprises a buffer, e.g., a buffer comprising pH 7,35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7,35% glycerol, 300 ppm proxel; pH 7, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7,10% sodium chloride, 25% glycerol, 300 ppm proxel; pH 5.5, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 5.5, 35% glycerol, 300 ppm proxel; pH 5.5, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; or, 20mM acetate buffer, pH 5.5, 35% glycerol; 20 mM MOPS, pH 7 or 25 mM MOPS, 50 mM NaCl, pH 7.5; pH 5.0, 40mM TRIS; pH 7.0, 40mM TRIS; pH 8.0, 40mM TRIS; pH 7.5, 50% glycerol; pH 7.5, 20% NaCl; pH 7.5, 30% propylene glycol; pH 7.5, 100mM sodium sulfate; pH 5.5, 35% glycerol; or, any combination thereof, or, equivalents thereof.

[0099] The invention provides bioscouring processes comprising the following steps: (a) providing a pectate lyase of the invention; (b) providing a pectin- or polygalacturonic acid- comprising material; (c) contacting the pectate lyase of (a) with the material of (b) under alkaline conditions, e.g., a pH great than 7.5, or, conditions comprising between about pH 8 and pH 9 or greater, e.g., pH 8.5, in bicarbonate buffer or equivalent. In one aspect, the method also comprises a non-ionic wetting agent, e.g., at about 1 g/L. In one aspect, the pectate lyase ratio is in an enzyme bath between about 10:1 to 50:1 L pectate lyase:kg of material. In one aspect, the pectate lyase dose is between about 0.1 and 0.2 ml of a concentrated extract per kg of material, or equivalent. Alternatively, the pectate lyase dose is between about 0.1 ml to 1 ml of a concentrated extract per kg of material, or equivalent. In one aspect, the temperature range is between about 50°C to 70°C. In one aspect, the treatment time is about 20 min. In one aspect of the bioscouring processes of the invention, the material comprises a fabric or a cloth. In one aspect, the pectate lyase dose is about 0.137 ml of a concentrated extract per kg of material, or equivalent. In one aspect, the contacting step further comprises use of a chelant, wherein the chelant is excluded from the enzyme bath and is added after about 20 minutes of enzyme treatment and retained for about 10 minutes before discharging bath.

[0100] Other aspects of the invention are set out in the accompanying claims.

[0101] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0102] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 is a block diagram of a computer system.

Figure 2 is a flow diagram illustrating one aspect of a process for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database.

Figure 3 is a flow diagram illustrating one aspect of a process in a computer for determining whether two sequences are homologous.

Figure 4 is a flow diagram illustrating one aspect of an identifier process 300 for detecting the presence of a feature in a sequence.

Figure 5 is a chart summary of the relative substrate specificity, relative substrate specificity value, characterization activity temperature, characterization activity pH, enzyme activity, characterization description and characterization substrate of exemplary pectate lyases of the invention.

Figure 6 is a summary of pectate lyase polypeptides, characterized as "upmutants," as discussed in detail, below.

Figure 7 is a table summarizing exemplary melting temperatures and specific activities (SA) of exemplary enzymes

of the invention at various temperatures.

Figure 8 summarizes data from activity assays of exemplary thermotolerant enzymes of the invention, as described in Example 4, below.

[0103] Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

[0104] The invention provides polypeptides having a pectate lyase activity, polynucleotides encoding the polypeptides, and methods for making and using these polynucleotides and polypeptides. In one aspect, the pectate lyases of the invention are used to catalyze the beta-elimination (trans-elimination) and/or hydrolysis of pectin and/or polygalacturonic acid (pectate) or other plant wall constituents, e.g., homogalacturonan or rhamnogalacturonan, including 1,4-linked alpha-D-galacturonic acid. The pectate lyases of the invention can also be used for the hydrolysis of plant cell walls, e.g., in treating natural fibers comprising pectin, for example, cotton fibers.

[0105] Use of the pectate lyases of the invention to hydrolyze primary cell wall pectin can eliminate the need for caustics and high temperatures in cotton fiber scouring. Use of the pectate lyases of the invention also can significantly reduce the amount of water used to rinse treated fibers, e.g., knitted or woven cotton fabric, after chemical scouring. Use of the pectate lyases of the invention also can reduce raw material losses in chemical scouring. In one aspect, a pectate lyase of the invention, e.g., an alkaline and/or thermostable pectate lyase, is used for bioscouring. Thus, the invention provides processes in which desized cotton fabrics are processed to solubilize and extract undesired non-cellulosic material in fabrics and other cellulosic materials using an enzyme of the invention. The processes of the invention can be used to solubilize and/or extract materials naturally found in cotton and/or to remove applied impurities, such as machinery lubricants.

[0106] Figures 5 and 7 are chart summaries of, inter alia, the relative substrate specificity, relative substrate specificity value, characterization activity temperature, characterization activity pH, enzyme activity, characterization description and characterization substrate of exemplary pectate lyases.

[0107] The pectate lyase preparations of the invention (including those for treating or processing feeds or foods, treating fibers and textiles, waste treatments, plant treatments, and the like) can further comprise one or more enzymes, for example, proteases, cellulases (endo-beta-1,4-glucanases), beta-glucanases (endo-beta-1,3(4)-glucanases), lipases, cutinases, peroxidases, laccases, amylases, pectate lyases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, arabinanases, hemicellulases, mannanases, xyloglucanases, xylanases, pectin acetyl esterases, rhamnogalacturonan acetyl esterases, polygalacturonases, rhamnogalacturonases, galactanases, pectin lyases, pectin methylesterases, cellobiohydrolases, transglutaminases; or mixtures thereof.

Definitions

[0108] The term "pectate lyase" includes all polypeptides having a pectate lyase, or pectinase, activity, including the beta-elimination (trans-elimination) and/or hydrolysis of pectin and/or polygalacturonic acid (pectate) or other plant wall constituents, e.g., homogalacturonan or rhamnogalacturonan, including 1,4-linked alpha-D-galacturonic acid. In one aspect, pectate lyase activity includes catalysis of the cleavage of glycosidic linkages of pectic substances, e.g., catalyzing the beta-elimination (trans-elimination) and/or hydrolysis of plant cell walls (e.g., the breakup or dissolution of cell walls comprising pectin, e.g., plant cell walls). In one aspect, pectate lyase activity includes catalyzing the beta-elimination (trans-elimination) and/or hydrolysis of methyl-esterified galacturonic acid, including partially or completely methyl-esterified polygalacturonic acid. In one aspect, the pectate lyase activity is mainly endo-acting, e.g., cutting the polymer (e.g., polygalacturonic acid) at random sites within a chain to give a mixture of oligomers, or the pectate lyase activity may be exo-acting, attacking from one end of the polymer and producing monomers or dimers, or, a combination thereof. In one aspect, the pectate lyase activity comprises catalyzing the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid) by trans-elimination. In one aspect, pectate lyase activity includes polypeptides having activity the same or similar to pectate lyase (EC 4.2.2.2), poly(1,4-alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonate lyase (EC 4.2.2.9) and/or exo-poly-alpha-galacturonosidase (EC 3.2.1.82).

[0109] A polypeptide can be routinely assayed for pectate lyase activity (e.g., tested to see if the protein is within the scope of the invention) by any method, e.g., a PGA assay for pectate lyases. In this test pectate lyase activity is measured at desired temperature and pH using 0.2% polygalacturonic acid (Sigma, P3850) in 25mM TrisHCl - 25mM Glycine NaOH buffer. One unit of enzyme activity is defined as the amount of protein that produced 1 μ mol of unsaturated oligogalacturonides per minute equivalent to 1 μ mol of unsaturated digalacturonide, using molecular extinction coefficient value of 4600 $M^{-1}cm^{-1}$ at 235 nm for dimer. Protein can be determined for homogenous purified protein by measuring absorbance at 280 nm, using extinction coefficient value specific for each protein based on sequence.

[0110] The term "antibody" includes a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope, see, e.g. Fundamental Immunology, Third Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994) J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

[0111] The terms "array" or "microarray" or "biochip" or "chip" as used herein is a plurality of target elements, each target element comprising a defined amount of one or more polypeptides (including antibodies) or nucleic acids immobilized onto a defined area of a substrate surface, as discussed in further detail, below.

[0112] As used herein, the terms "computer," "computer program" and "processor" are used in their broadest general contexts and incorporate all such devices, as described in detail, below. A "coding sequence of" or a "sequence encodes" a particular polypeptide or protein, is a nucleic acid sequence which is transcribed and translated into a polypeptide or protein when placed under the control of appropriate regulatory sequences.

[0113] The term "expression cassette" as used herein refers to a nucleotide sequence which is capable of affecting expression of a structural gene (i.e., a protein coding sequence, such as a pectate lyase of the invention) in a host compatible with such sequences. Expression cassettes include at least a promoter operably linked with the polypeptide coding sequence; and, optionally, with other sequences, e.g., transcription termination signals. Additional factors necessary or helpful in effecting expression may also be used, e.g., enhancers. Thus, expression cassettes also include plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like.

[0114] "Operably linked" as used herein refers to a functional relationship between two or more nucleic acid (e.g., DNA) segments. Typically, it refers to the functional relationship of transcriptional regulatory sequence to a transcribed sequence. For example, a promoter is operably linked to a coding sequence, such as a nucleic acid of the invention, if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are *cis*-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[0115] A "vector" comprises a nucleic acid that can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (e.g., a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not limited to replicons (e.g., RNA replicons, bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA (e.g., plasmids, viruses, and the like, see, e.g., U.S. Patent No. 5,217,879), and include both the expression and non-expression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extra-chromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

[0116] As used herein, the term "promoter" includes all sequences capable of driving transcription of a coding sequence in a cell, e.g., a plant cell. Thus, promoters used in the constructs of the invention include *cis*-acting transcriptional control elements and regulatory sequences that are involved in regulating or modulating the timing and/or rate of transcription of a gene. For example, a promoter can be a *cis*-acting transcriptional control element, including an enhancer, a promoter, a transcription terminator, an origin of replication, a chromosomal integration sequence, 5' and 3' untranslated regions, or an intronic sequence, which are involved in transcriptional regulation. These *cis*-acting sequences typically interact with proteins or other biomolecules to carry out (turn on/off, regulate, modulate, etc.) transcription. "Constitutive" promoters are those that drive expression continuously under most environmental conditions and states of development or cell differentiation. "Inducible" or "regulatable" promoters direct expression of the nucleic acid of the invention under the influence of environmental conditions or developmental conditions. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, drought, or the presence of light.

[0117] "Tissue-specific" promoters are transcriptional control elements that are only active in particular cells or tissues or organs, e.g., in plants or animals. Tissue-specific regulation may be achieved by certain intrinsic factors that ensure that genes encoding proteins specific to a given tissue are expressed. Such factors are known to exist in mammals and

plants so as to allow for specific tissues to develop.

[0118] The term "plant" includes whole plants, plant parts (e.g., leaves, stems, flowers, roots, etc.), plant protoplasts, seeds and plant cells and progeny of same. The class of plants which can be used in the method of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including angiosperms (mono-

cotyledonous and dicotyledonous plants), as well as gymnosperms. It includes plants of a variety of ploidy levels, including polyploid, diploid, haploid and hemizygous states. As used herein, the term "transgenic plant" includes plants or plant cells into which a heterologous nucleic acid sequence has been inserted, e.g., the nucleic acids and various recombinant constructs (e.g., expression cassettes) of the invention.

[0119] "Plasmids" can be commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. Equivalent plasmids to those described herein are known in the art and will be apparent to the ordinarily skilled artisan.

[0120] The term "gene" includes a nucleic acid sequence comprising a segment of DNA involved in producing a transcription product (e.g., a message), which in turn is translated to produce a polypeptide chain, or regulates gene transcription, reproduction or stability. Genes can include regions preceding and following the coding region, such as leader and trailer, promoters and enhancers, as well as, where applicable, intervening sequences (introns) between individual coding segments (exons).

[0121] The phrases "nucleic acid" or "nucleic acid sequence" includes oligonucleotide, nucleotide, polynucleotide, or to a fragment of any of these, to DNA or RNA (e.g., mRNA, rRNA, tRNA) of genomic or synthetic origin which may be single-stranded or double-stranded and may represent a sense or antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material, natural or synthetic in origin, including, e.g., iRNA, ribonucleoproteins (e.g., iRNPs). The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones, see e.g., Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197; Strauss-Soukup (1997) Biochemistry 36:8692-8698; Samstag (1996) Antisense Nucleic Acid Drug Dev 6:153-156.

[0122] "Amino acid" or "amino acid sequence" include an oligopeptide, peptide, polypeptide, or protein sequence, or to a fragment, portion, or subunit of any of these, and to naturally occurring or synthetic molecules. The terms "polypeptide" and "protein" include amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain modified amino acids other than the 20 gene-encoded amino acids. The term "polypeptide" also includes peptides and polypeptide fragments, motifs and the like. The term also includes glycosylated polypeptides. The peptides and polypeptides of the invention also include all "mimetic" and "peptidomimetic" forms, as described in further detail, below.

[0123] The term "isolated" includes a material removed from its original environment, e.g., the natural environment if it is naturally occurring. For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. As used herein, an isolated material or composition can also be a "purified" composition, i.e., it does not require absolute purity; rather, it is intended as a relative definition. Individual nucleic acids obtained from a library can be conventionally purified to electrophoretic homogeneity. In alternative aspects, the invention provides nucleic acids which have been purified from genomic DNA or from other sequences in a library or other environment by at least one, two, three, four, five or more orders of magnitude.

[0124] As used herein, the term "recombinant" can include nucleic acids adjacent to a "backbone" nucleic acid to which it is not adjacent in its natural environment. In one aspect, nucleic acids represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid "backbone molecules." "Backbone molecules" according to the invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids, and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest. In one aspect, the enriched nucleic acids represent 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. "Recombinant" polypeptides or proteins refer to polypeptides or proteins produced by recombinant DNA techniques; e.g., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide or protein. "Synthetic" polypeptides or protein are those prepared by chemical synthesis, as described in further detail, below.

[0125] A promoter sequence can be "operably linked to" a coding sequence when RNA polymerase which initiates transcription at the promoter will transcribe the coding sequence into mRNA, as discussed further, below.

[0126] "Oligonucleotide" includes either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide can ligate to a fragment that has not been dephosphorylated.

[0127] The phrase "substantially identical" in the context of two nucleic acids or polypeptides, can refer to two or more

sequences that have, e.g., at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more nucleotide or amino acid residue (sequence) identity, when compared and aligned for maximum correspondence, as measured using one any known sequence comparison algorithm, as discussed in detail below, or by visual inspection. In alternative aspects, the disclosure provides nucleic acid and polypeptide sequences having substantial identity to an exemplary sequence of the disclosure, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133 (nucleic acids) SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134 (polypeptides), over a region of at least about 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more residues, or a region ranging from between about 50 residues to the full length of the nucleic acid or polypeptide. Nucleic acid sequences can be substantially identical over the entire length of a polypeptide coding region.

[0128] A "substantially identical" amino acid sequence also can include a sequence that differs from a reference sequence by one or more conservative or non-conservative amino acid substitutions, deletions, or insertions, particularly when such a substitution occurs at a site that is not the active site of the molecule, and provided that the polypeptide essentially retains its functional properties. A conservative amino acid substitution, for example, substitutes one amino acid for another of the same class (e.g., substitution of one hydrophobic amino acid, such as isoleucine, valine, leucine, or methionine, for another, or substitution of one polar amino acid for another, such as substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine). One or more amino acids can be deleted, for example, from a pectate lyase, resulting in modification of the structure of the polypeptide, without significantly altering its biological activity. For example, amino- or carboxyl-terminal amino acids that are not required for pectate lyase activity can be removed.

[0129] "hybridization" includes the process by which a nucleic acid strand joins with a complementary strand through base pairing. Hybridization reactions can be sensitive and selective so that a particular sequence of interest can be identified even in samples in which it is present at low concentrations. Stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. For example, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature, altering the time of hybridization, as described in detail, below. In alternative aspects, nucleic acids of the invention are defined by their ability to hybridize under various stringency conditions (e.g., high, medium, and low), as set forth herein.

[0130] "variant" includes polynucleotides or polypeptides of the invention modified at one or more base pairs, codons, introns, exons, or amino acid residues (respectively) yet still retain the biological activity of a pectate lyase of the invention. Variants can be produced by any number of means included methods such as, for example, error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, GSSM™ and any combination thereof. Techniques for producing variant pectate lyase having activity at a pH or temperature, for example, that is different from a wild-type pectate lyase, are included herein.

[0131] The term "saturation mutagenesis" or "GSSM™" includes a method that uses degenerate oligonucleotide primers to introduce point mutations into a polynucleotide, as described in detail, below.

[0132] The term "optimized directed evolution system" or "optimized directed evolution" includes a method for reassembling fragments of related nucleic acid sequences, e.g., related genes, and explained in detail, below.

[0133] The term "synthetic ligation reassembly" or "SLR" includes a method of ligating oligonucleotide fragments in a non-stochastic fashion, and explained in detail, below.

Generating and Manipulating Nucleic Acids

[0134] The disclosure provides isolated and recombinant nucleic acids, e.g., polynucleotides having a sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133; nucleic acids encoding polypeptides SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134.

[0135] The nucleic acids can also comprise expression cassettes, such as expression vectors, where in one aspect they encode a polypeptide of the disclosure. The disclosure also includes methods for discovering new pectate lyase sequences using the nucleic acids. The disclosure also includes methods for inhibiting the expression of pectate lyase genes, transcripts and polypeptides using the nucleic acids. Also provided are methods for modifying the nucleic acids by, e.g., synthetic ligation reassembly, optimized directed evolution system and/or gene site saturation mutagenesis (GSSM™).

[0136] The nucleic acids can be made, isolated and/or manipulated by, e.g., cloning and expression of cDNA libraries, amplification of message or genomic DNA by PCR, and the like. In practicing the methods, homologous genes can be modified by manipulating a template nucleic acid, as described herein. This can be practiced in conjunction with any method or protocol or device known in the art, which are well described in the scientific and patent literature.

General Techniques

[0137] The nucleic acids, whether RNA, iRNA (i.e., RNAi), antisense nucleic acid, cDNA, genomic DNA, vectors, viruses or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed/generated recombinantly. Recombinant polypeptides generated from these nucleic acids can be individually isolated or cloned and tested for a desired activity. Any recombinant expression system can be used, including bacterial, mammalian, yeast, insect or plant cell expression systems.

[0138] Alternatively, these nucleic acids can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066.

[0139] Techniques for the manipulation of nucleic acids, such as, e.g., subcloning, labeling probes (e.g., random-primer labeling using Klenow polymerase, nick translation, amplification), sequencing, hybridization and the like are well described in the scientific and patent literature, see, e.g., Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

[0140] Another useful means of obtaining and manipulating nucleic acids used to practice the methods is to clone from genomic samples, and, if desired, screen and re-clone inserts isolated or amplified from, e.g., genomic clones or cDNA clones. Sources of nucleic acid used in the methods include genomic or cDNA libraries contained in, e.g., mammalian artificial chromosomes (MACs), see, e.g., U.S. Patent Nos. 5,721,118; 6,025,155; human artificial chromosomes,

see, e.g., Rosenfeld (1997) Nat. Genet. 15:333-335; yeast artificial chromosomes (YAC); bacterial artificial chromosomes (BAC); P1 artificial chromosomes, see, e.g., Woon (1998) Genomics 50:306-316; P1-derived vectors (PACs), see, e.g., Kern (1997) Biotechniques 23:120-124; cosmids, recombinant viruses, phages or plasmids.

[0141] In one aspect, a nucleic acid encoding a polypeptide is assembled in appropriate phase with a leader sequence capable of directing secretion of the translated polypeptide or fragment thereof.

[0142] The disclosure provides fusion proteins and nucleic acids encoding them. A polypeptide of the disclosure can be fused to a heterologous peptide or polypeptide, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification. Peptides and polypeptides of the disclosure can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, e.g., producing a more immunogenic peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, e.g., metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Coip, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between a purification domain and the motif-comprising peptide or polypeptide to facilitate purification. For example, an expression vector can include an epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see e.g., Williams (1995) Biochemistry 34:1787-1797; Dobeli (1998) Protein Expr. Purif. 12:404-414). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well described in the scientific and patent literature, see e.g., Kroll (1993) DNA Cell. Biol., 12:441-53.

Transcriptional and translational control sequences

[0143] The disclosure provides nucleic acid (e.g., DNA) sequences operatively linked to expression (e.g., transcriptional or translational) control sequence(s), e.g., promoters or enhancers, to direct or modulate RNA synthesis/ expression. The expression control sequence can be in an expression vector. Exemplary bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, PL and tip. Exemplary eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein I.

[0144] Promoters suitable for expressing a polypeptide in bacteria include the *E. coli* lac or tip promoters, the lacI promoter, the lacZ promoter, the T3 promoter, the T7 promoter, the gpt promoter, the lambda PR promoter, the lambda PL promoter, promoters from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), and the acid phosphatase promoter. Eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, heat shock promoters, the early and late SV40 promoter, LTRs from retroviruses, and the mouse metallothionein-I promoter. Other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses may also be used.

Tissue-Specific Plant Promoters

[0145] The disclosure provides expression cassettes that can be expressed in a tissue-specific manner, e.g., that can express a pectate lyase of the disclosure in a tissue-specific manner. The disclosure also provides plants or seeds that express a pectate lyase of the disclosure in a tissue-specific manner. The tissue-specificity can be seed specific, stem specific, leaf specific, root specific, fruit specific and the like.

[0146] In one aspect, a constitutive promoter such as the CaMV 35S promoter can be used for expression in specific parts of the plant or seed or throughout the plant. For example, for overexpression, a plant promoter fragment can be employed which will direct expression of a nucleic acid in some or all tissues of a plant, e.g., a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumefaciens*, and other transcription initiation regions from various plant genes known to those of skill. Such genes include, e.g., *ACT11* from *Arabidopsis* (Huang (1996) Plant Mol. Biol. 33:125-139); *Cat3* from *Arabidopsis* (GenBank No. U43147, Zhong (1996) Mol. Gen. Genet. 251:196-203); the gene encoding stearyl-acyl carrier protein desaturase from *Brassica napus* (Genbank No. X74782, Solocombe (1994) Plant Physiol. 104:1167-1176); *Gpc1* from maize (GenBank No. X15596; Martinez (1989) J. Mol. Biol. 208:551-565); the *Gpc2* from maize (GenBank No. U45855, Manjunath (1997) Plant Mol. Biol. 33:97-112); plant promoters described in U.S. Patent Nos. 4,962,028; 5,633,440.

[0147] The disclosure uses tissue-specific or constitutive promoters derived from viruses which can include, e.g., the tobamovirus subgenomic promoter (Kumagai (1995) Proc. Natl. Acad. Sci. USA 92:1679-1683; the rice tungro bacilliform virus (RTBV), which replicates only in phloem cells in infected rice plants, with its promoter which drives strong phloem-

specific reporter gene expression; the cassava vein mosaic virus (CVMV) promoter, with highest activity in vascular elements, in leaf mesophyll cells, and in root tips (Verdaguer (1996) Plant Mol. Biol. 31:1129-1139).

[0148] Alternatively, the plant promoter may direct expression of pectate lyase-expressing nucleic acid in a specific tissue, organ or cell type (*i.e.* tissue-specific promoters) or may be otherwise under more precise environmental or developmental control or under the control of an inducible promoter. Examples of environmental conditions that may affect transcription include anaerobic conditions, elevated temperature, the presence of light, or sprayed with chemicals/hormones. For example, the disclosure incorporates the drought-inducible promoter of maize (Busk (1997) *supra*); the cold, drought, and high salt inducible promoter from potato (Kirch (1997) Plant Mol. Biol. 33:897-909).

[0149] Tissue-specific promoters can promote transcription only within a certain time frame of developmental stage within that tissue. See, *e.g.*, Blazquez (1998) Plant Cell 10:791-800, characterizing the *Arabidopsis* LEAFY gene promoter. See also Cardon (1997) Plant J 12:367-77, describing the transcription factor SPL3, which recognizes a conserved sequence motif in the promoter region of the *A. thaliana* floral meristem identity gene AP1; and Mandel (1995) Plant Molecular Biology, Vol. 29, pp 995-1004, describing the meristem promoter *elf4*. Tissue specific promoters which are active throughout the life cycle of a particular tissue can be used. In one aspect, the nucleic acids are operably linked to a promoter active primarily only in cotton fiber cells. In one aspect, the nucleic acids are operably linked to a promoter active primarily during the stages of cotton fiber cell elongation, *e.g.*, as described by Rinehart (1996) *supra*. The nucleic acids can be operably linked to the *Fbl2A* gene promoter to be preferentially expressed in cotton fiber cells (*ibid*). See also, John (1997) Proc. Natl. Acad. Sci. USA 89:5769-5773; John, et al., U.S. Patent Nos. 5,608,148 and 5,602,321, describing cotton fiber-specific promoters and methods for the construction of transgenic cotton plants. Root-specific promoters may also be used to express the nucleic acids. Examples of root-specific promoters include the promoter from the alcohol dehydrogenase gene (DeLisle (1990) Int. Rev. Cytol. 123:39-60). Other promoters that can be used to express the nucleic acids include, *e.g.*, ovule-specific, embryo-specific, endosperm-specific, integument-specific, seed coat-specific promoters, or some combination thereof; a leaf-specific promoter (see, *e.g.*, Busk (1997) Plant J. 11:1285-1295, describing a leaf-specific promoter in maize); the ORF13 promoter from *Agrobacterium rhizogenes* (which exhibits high activity in roots, see, *e.g.*, Hansen (1997) *supra*); a maize pollen specific promoter (see, *e.g.*, Guerrero (1990) Mol. Gen. Genet. 224:161-168); a tomato promoter active during fruit ripening, senescence and abscission of leaves and, to a lesser extent, of flowers can be used (see, *e.g.*, Blume (1997) Plant J. 12:731-746); a pistil-specific promoter from the potato SK2 gene (see, *e.g.*, Ficker (1997) Plant Mol. Biol. 35:425-431); the *Blec4* gene from pea, which is active in epidermal tissue of vegetative and floral shoot apices of transgenic alfalfa making it a useful tool to target the expression of foreign genes to the epidermal layer of actively growing shoots or fibers; the ovule-specific *BEL1* gene (see, *e.g.*, Reiser (1995) Cell 83:735-742, GenBank No. U39944); and/or, the promoter in Klee, U.S. Patent No. 5,589,583, describing a plant promoter region is capable of conferring high levels of transcription in meristematic tissue and/or rapidly dividing cells.

[0150] Alternatively, plant promoters which are inducible upon exposure to plant hormones, such as auxins, are used to express the nucleic acids. Examples are the auxin-response elements E1 promoter fragment (AuxREs) in the soybean (*Glycine max* L.) (Liu (1997) Plant Physiol. 115:397-407); the auxin-responsive *Arabidopsis* GST6 promoter (also responsive to salicylic acid and hydrogen peroxide) (Chen (1996) Plant J. 10: 955-966); the auxin-inducible *parC* promoter from tobacco (Sakai (1996) 37:906-913); a plant biotin response element (Streit (1997) Mol. Plant Microbe Interact. 10:933-937); and, the promoter responsive to the stress hormone abscisic acid (Sheen (1996) Science 274:1900-1902).

[0151] The nucleic acids can also be operably linked to plant promoters which are inducible upon exposure to chemicals reagents which can be applied to the plant, such as herbicides or antibiotics. For example, the maize *In2-2* promoter, activated by benzenesulfonamide herbicide safeners, can be used (De Veylder (1997) Plant Cell Physiol. 38:568-577); application of different herbicide safeners induces distinct gene expression patterns, including expression in the root, hydathodes, and the shoot apical meristem. Coding sequence can be under the control of, *e.g.*, a tetracycline-inducible promoter, *e.g.*, as described with transgenic tobacco plants containing the *Avena sativa* L. (oat) arginine decarboxylase gene (Masgrau (1997) Plant J. 11:465-473); or, a salicylic acid-responsive element (Stange (1997) Plant J. 11:1315-1324). Using chemically- (*e.g.*, hormone- or pesticide-) induced promoters, *i.e.*, promoter responsive to a chemical which can be applied to the transgenic plant in the field, expression of a polypeptide of the disclosure can be induced at a particular stage of development of the plant. Thus, this also provides for transgenic plants containing an inducible gene encoding for polypeptides of the disclosure whose host range is limited to target plant species, such as corn, rice, barley, wheat, potato or other crops, inducible at any stage of development of the crop.

[0152] One of skill will recognize that a tissue-specific plant promoter may drive expression of operably linked sequences in tissues other than the target tissue. Thus, a tissue-specific promoter is one that drives expression preferentially in the target tissue or cell type, but may also lead to some expression in other tissues as well.

[0153] The nucleic acids can also be operably linked to plant promoters which are inducible upon exposure to chemicals reagents. These reagents include, *e.g.*, herbicides, synthetic auxins, or antibiotics which can be applied, *e.g.*, sprayed, onto transgenic plants. Inducible expression of the pectate lyase-producing nucleic acids will allow the grower to select plants with the optimal pectate lyase expression and/or activity. The development of plant parts can thus be controlled. This

provides the means to facilitate the harvesting of plants and plant parts. For example, in various embodiments, the maize In2-2 promoter, activated by benzenesulfonamide herbicide safeners, is used (De Veylder (1997) Plant Cell Physiol. 38:568-577); application of different herbicide safeners induces distinct gene expression patterns, including expression in the root, hydathodes, and the shoot apical meristem. Coding sequences are also under the control of a tetracycline-inducible promoter, e.g., as described with transgenic tobacco plants containing the *Avena sativa* L. (oat) arginine decarboxylase gene (Masgrau (1997) Plant J. 11:465-473); or, a salicylic acid-responsive element (Stange (1997) Plant J. 11:1315-1324).

[0154] If proper polypeptide expression is desired, a polyadenylation region at the 3'-end of the coding region should be included. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from genes in the *Agrobacterium* T-DNA.

Expression vectors and cloning vehicles

[0155] Expression vectors and cloning vehicles comprise nucleic acids of the disclosure, e.g., sequences encoding the pectate lyases of the disclosure. Expression vectors and cloning vehicles can comprise viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral DNA (e.g., vaccinia, adenovirus, fowl pox virus, pseudorabies and derivatives of SV40), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as bacillus, *Aspergillus* and yeast). Vectors can include chromosomal, non-chromosomal and synthetic DNA sequences. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available. Exemplary vectors are include: bacterial: pQE vectors (Qiagen), pBluescript plasmids, pNH vectors, (lambda-ZAP vectors (Stratagene); ptrc99a, pKK223-3, pDR540, pRIT2T (Pharmacia); Eukaryotic: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, pSVLSV40 (Pharmacia). However, any other plasmid or other vector may be used so long as they are replicable and viable in the host. Low copy number or high copy number vectors may be employed.

[0156] The expression vector can comprise a promoter, a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. Mammalian expression vectors can comprise an origin of replication, any necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. In some aspects, DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

[0157] In one aspect, the expression vectors contain one or more selectable marker genes to permit selection of host cells containing the vector. Such selectable markers include genes encoding dihydrofolate reductase or genes conferring neomycin resistance for eukaryotic cell culture, genes conferring tetracycline or ampicillin resistance in *E. coli*, and the *S. cerevisiae* TRP1 gene. Promoter regions can be selected from any desired gene using chloramphenicol transferase (CAT) vectors or other vectors with selectable markers.

[0158] Vectors for expressing the polypeptide or fragment thereof in eukaryotic cells can also contain enhancers to increase expression levels. Enhancers are cis-acting elements of DNA, usually from about 10 to about 300 bp in length that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and the adenovirus enhancers.

[0159] A nucleic acid sequence can be inserted into a vector by a variety of procedures. In general, the sequence is ligated to the desired position in the vector following digestion of the insert and the vector with appropriate restriction endonucleases. Alternatively, blunt ends in both the insert and the vector may be ligated. A variety of cloning techniques are known in the art, e.g., as described in Ausubel and Sambrook. Such procedures and others are deemed to be within the scope of those skilled in the art.

[0160] The vector can be in the form of a plasmid, a viral particle, or a phage. Other vectors include chromosomal, non-chromosomal and synthetic DNA sequences, derivatives of SV40; bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. A variety of cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by, e.g., Sambrook.

[0161] Particular bacterial vectors which can be used include the commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017), pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), GEM1 (Promega Biotec, Madison, WI, USA) pQE70, pQE60, pQE-9 (Qiagen), pD10, psiX174 pBluescript II KS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene), ptrc99a, pKK223-3, pKK233-3, DR540, pRIT5 (Pharmacia), pKK232-8 and pCM7. Particular eukaryotic vectors include pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). However, any other vector may be used as long as it is replicable and viable in the host cell.

[0162] The nucleic acids can be expressed in expression cassettes, vectors or viruses and transiently or stably expressed in plant cells and seeds. One exemplary transient expression system uses episomal expression systems, e.g.,

cauliflower mosaic virus (CaMV) viral RNA generated in the nucleus by transcription of an episomal mini-chromosome containing supercoiled DNA, see, e.g., Covey (1990) Proc. Natl. Acad. Sci. USA 87:1633-1637. Alternatively, coding sequences, i.e., all or sub-fragments of sequences can be inserted into a plant host cell genome becoming an integral part of the host chromosomal DNA. Sense or antisense transcripts can be expressed in this manner. A vector comprising the sequences (e.g., promoters or coding regions) from nucleic acids of the disclosure can comprise a marker gene that confers a selectable phenotype on a plant cell or a seed. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosulfuron or Basta.

[0163] Expression vectors capable of expressing nucleic acids and proteins in plants are well known in the art, and can include, e.g., vectors from *Agrobacterium* spp., potato virus X (see, e.g., Angell (1997) EMBO J. 16:3675-3684), tobacco mosaic virus (see, e.g., Casper (1996) Gene 173:69-73), tomato bushy stunt virus (see, e.g., Hillman (1989) Virology 169:42-50), tobacco etch virus (see, e.g., Dolja (1997) Virology 234:243-252), bean golden mosaic virus (see, e.g., Morinaga (1993) Microbiol Immunol. 37:471-476), cauliflower mosaic virus (see, e.g., Cecchini (1997) Mol. Plant Microbe Interact. 10:1094-1101), maize Ac/Ds transposable element (see, e.g., Rubin (1997) Mol. Cell. Biol. 17:6294-6302; Kunze (1996) Curr. Top. Microbiol. Immunol. 204:161-194), and the maize suppressor-mutator (Spm) transposable element (see, e.g., Schlappi (1996) Plant Mol. Biol. 32:717-725); and derivatives thereof.

[0164] In one aspect, the expression vector can have two replication systems to allow it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector can contain at least one sequence homologous to the host cell genome. It can contain two homologous sequences which flank the expression construct. The integrating vector can be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

[0165] Expression vectors may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed, e.g., genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers can also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways.

Host cells and transformer cells

[0166] A transformed cell comprises a nucleic acid sequence of the disclosure, e.g., a sequence encoding a pectate lyase of the disclosure, or a vector of the disclosure. The host cell may be any of the host cells familiar to those skilled in the art, including prokaryotic cells, eukaryotic cells, such as bacterial cells, fungal cells, yeast cells, mammalian cells, insect cells, or plant cells. Exemplary bacterial cells include *E. coli*, *Streptomyces*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhimurium* and various species within the genera *Bacillus*, *Streptomyces*, and *Staphylococcus*. Exemplary insect cells include *Drosophila* S2 and *Spodoptera* Sf9. Exemplary yeast cells include *Pichia pastoris*, *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. Exemplary animal cells include CHO, COS or Bowes melanoma or any mouse or human cell line. The selection of an appropriate host is within the abilities of those skilled in the art. Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, e.g., Weising (1988) Ann. Rev. Genet. 22:421-477, U.S. Patent No. 5,750,870.

[0167] The vector can be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation (Davis, L., Dibner, M., Battey, L., Basic Methods in Molecular Biology, (1986)).

[0168] In one aspect, the nucleic acids or vectors are introduced into the cells for screening, thus, the nucleic acids enter the cells in a manner suitable for subsequent expression of the nucleic acid. The method of introduction is largely dictated by the targeted cell type. Exemplary methods include CaPO₄ precipitation, liposome fusion, lipofection (e.g., LIPOFECTIN™), electroporation, viral infection, etc. The candidate nucleic acids may stably integrate into the genome of the host cell (for example, with retroviral introduction) or may exist either transiently or stably in the cytoplasm (i.e. through the use of traditional plasmids, utilizing standard regulatory sequences, selection markers, etc.). As many pharmaceutically important screens require human or model mammalian cell targets, retroviral vectors capable of transfecting such targets are preferred.

[0169] Where appropriate, the engineered host cells can be cultured in conventional nutriment media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the disclosure. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be induced by appropriate means (e.g., temperature shift or chemical induction) and the cells may be cultured for an additional period to allow them to produce the desired polypeptide or fragment thereof.

[0170] Cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract is retained for further purification. Microbial cells employed for expression of proteins can be disrupted by any

convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art. The expressed polypeptide or fragment thereof can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. If desired, high performance liquid chromatography (HPLC) can be employed for final purification steps.

[0171] Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts and other cell lines capable of expressing proteins from a compatible vector, such as the C127, 3T3, CHO, HeLa and BHK cell lines.

[0172] The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Depending upon the host employed in a recombinant production procedure, the polypeptides produced by host cells containing the vector may be glycosylated or may be non-glycosylated. Polypeptides of the disclosure may or may not also include an initial methionine amino acid residue.

[0173] Cell-free translation systems can also be employed to produce a polypeptide of the disclosure. Cell-free translation systems can use mRNAs transcribed from a DNA construct comprising a promoter operably linked to a nucleic acid encoding the polypeptide or fragment thereof. In some aspects, the DNA construct may be linearized prior to conducting an in vitro transcription reaction. The transcribed mRNA is then incubated with an appropriate cell-free translation extract, such as a rabbit reticulocyte extract, to produce the desired polypeptide or fragment thereof.

[0174] The expression vectors can contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

Amplification of Nucleic Acids

[0175] Nucleic acids of the disclosure and nucleic acids encoding the pectate lyases of the disclosure, or modified nucleic acids of the disclosure, can be reproduced by amplification. Amplification can also be used to clone or modify the nucleic acids. Thus, the disclosure provides amplification primer sequence pairs for amplifying nucleic acids of the disclosure. One of skill in the art can design amplification primer sequence pairs for any part of or the full length of these sequences. The disclosure provides a nucleic acid amplified by such a primer pair, e.g., a primer pair as set forth by about the first (the 5') 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of a nucleic acid of the disclosure, and about the first (the 5') 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of the complementary strand (e.g., of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133).

[0176] Amplification reactions can also be used to quantify the amount of nucleic acid in a sample (such as the amount of message in a cell sample), label the nucleic acid (e.g., to apply it to an array or a blot), detect the nucleic acid, or quantify the amount of a specific nucleic acid in a sample. Message isolated from a cell or a cDNA library may be amplified.

[0177] The skilled artisan can select and design suitable oligonucleotide amplification primers. Amplification methods are also well known in the art, and include, e.g., polymerase chain reaction, PCR (see, e.g., PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS, ed. Innis, Academic Press, N.Y. (1990) and PCR STRATEGIES (1995), ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (see, e.g., Wu (1989) Genomics 4:560; Landegren (1988) Science 241:1077; Barringer (1990) Gene 89:117); transcription amplification (see, e.g., Kwok (1989) Proc. Natl. Acad. Sci. USA 86:1173); and, self-sustained sequence replication (see, e.g., Guatelli (1990) Proc. Natl. Acad. Sci. USA 87:1874); Q Beta replicase amplification (see, e.g., Smith (1997) J. Clin. Microbiol. 35:1477-1491), automated Q-beta replicase amplification assay (see, e.g., Burg (1996) Mol. Cell. Probes 10:257-271) and other RNA polymerase mediated techniques (e.g., NASBA, Cangene, Mississauga, Ontario); see also Berger (1987) Methods Enzymol. 152:307-316; Sambrook; Ausubel; U.S. Patent Nos. 4,683,195 and 4,683,202; Sooknanan (1995) Biotechnology 13:563-564.

Determining the degree of sequence identity

[0178] The disclosure provides nucleic acids comprising sequences having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, and nucleic acids encoding SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134 over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more, residues. The disclosure provides polypeptides comprising sequences having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary polypeptide. The extent of sequence identity (homology) may be determined using any computer program and associated parameters, including those described herein, such as BLAST 2.2.2. or FASTA version 3.0t78, with the default parameters.

[0179] Homologous sequences also include RNA sequences in which uridines replace the thymines in the nucleic acid sequences. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error. It will be appreciated that the nucleic acid sequences as set forth herein can be represented in the traditional single character format (see, e.g., Stryer, Lubert. Biochemistry, 3rd Ed., W. H. Freeman & Co., New York) or in any other format which records the identity of the nucleotides in a sequence.

[0180] Various sequence comparison programs identified herein are used. Protein and/or nucleic acid sequence identities (homologies) may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are not limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85(8):2444-2448, 1988; Altschul et al., J. Mol. Biol. 215(3):403-410, 1990; Thompson et al., Nucleic Acids Res. 22(2):4673-4680, 1994; Higgins et al., Methods Enzymol. 266:383-402, 1996; Altschul et al., J. Mol. Biol. 215(3):403-410, 1990; Altschul et al., Nature Genetics 3:266-272, 1993).

[0181] Homology or identity can be measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various deletions, substitutions and other modifications. The terms "homology" and "identity" in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same when compared and aligned for maximum correspondence over a comparison window or designated region as measured using any number of sequence comparison algorithms or by manual alignment and visual inspection. For sequence comparison, one sequence can act as a reference sequence, e.g., a sequence of the disclosure, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0182] A "comparison window", as used herein, includes reference to a segment of any one of the numbers of contiguous

residues. For example, contiguous residues ranging anywhere from 20 to the full length of an exemplary polypeptide or nucleic acid sequence are compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. If the reference sequence has the requisite sequence identity to an exemplary polypeptide or nucleic acid sequence, e.g., 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a sequence, that sequence is within the desired scope. In alternative embodiments, subsequences ranging from about 20 to 600, about 50 to 200, and about 100 to 150 are compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequence for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443, 1970, by the search for similarity method of person & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection. Other algorithms for determining homology or identity include, for example, in addition to a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (Blocks IMPROVED Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic Algorithm) and WHAT-IF. Such alignment programs can also be used to screen genome databases to identify polynucleotide sequences having substantially identical sequences. A number of genome databases are available, for example, a substantial portion of the human genome is available as part of the Human Genome Sequencing Project (Gibbs, 1995). Several genomes have been sequenced, e.g., *M. genitalium* (Fraser et al., 1995), *M. jannaschii* (Bult et al., 1996), *H. influenzae* (Fleischmann et al., 1995), *E. coli* (Blattner et al., 1997), and yeast (*S. cerevisiae*) (Mewes et al., 1997), and *D. melanogaster* (Adams et al., 2000). Significant progress has also been made in sequencing the genomes of model organism, such as mouse, *C. elegans*, and *Arabidopsis* sp. Databases containing genomic information annotated with some functional information are maintained by different organization, and are accessible via the internet.

[0183] BLAST, BLAST 2.0 and BLAST 2.2.2 algorithms are also used. They are described, e.g., in Altschul (1977) Nuc. Acids Res. 25:3389-3402; Altschul (1990) J. Mol. Biol. 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul (1990) supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectations (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873). One measure of similarity provided by BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a references sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001. In one aspect, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"). For example, five specific BLAST programs can be used to perform the following task: (1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database; (2) BLASTN compares a nu-

cleotide query sequence against a nucleotide sequence database; (3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database; (4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and, (5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (i.e., aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet et al., Science 256:1443-1445, 1992; Henikoff and Henikoff, Proteins 17:49-61, 1993). Less preferably, the PAM or PAM250 matrices may also be used (see, e.g., Schwartz and Dayhoff, eds., 1978, Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure, Washington: National Biomedical Research Foundation).

[0184] To determine if a nucleic acid has the requisite sequence identity to be within the desired scope, the NCBI BLAST 2.2.2 programs is used, default options to blastp. There are about 38 setting options in the BLAST 2.2.2 program. In this example, all default values are used except for the default filtering setting (i.e., all parameters set to default except filtering which is set to OFF); in its place a "-F F" setting is used, which disables filtering. Use of default filtering often results in Karlin-Altschul violations due to short length of sequence.

[0185] The default values used include:

"Filter for low complexity: ON
Word Size: 3
Matrix: Blosum62
Gap Costs: Existence: 11
Extension:1"

[0186] Other default settings can be: filter for low complexity OFF, word size of 3 for protein, BLOSUM62 matrix, gap existence penalty of -11 and a gap extension penalty of -1. An exemplary NCBI BLAST 2.2.2 program setting has the "-W" option default to 0. This means that, if not set, the word size defaults to 3 for proteins and 11 for nucleotides.

Computer systems and computer program products

[0187] To determine and identify sequence identities, structural homologies, motifs and the like in silico, the sequence of the disclosure can be stored, recorded, and manipulated on any medium which can be read and accessed by a computer. Accordingly, the disclosure provides computers, computer systems, computer readable mediums, computer programs products and the like recorded or stored thereon the nucleic acid and polypeptide sequences of the disclosure. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any known methods for recording information on a computer readable medium to generate manufactures comprising one or more of the nucleic acid and/or polypeptide sequences of the disclosure.

[0188] Another aspect of the disclosure is a computer readable medium having recorded thereon at least one nucleic acid and/or polypeptide sequence of the disclosure. Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disk, a floppy disk, a magnetic tape, CD-ROM, Digital Versatile Disk (DVD), Random Access Memory (RAM), or Read Only Memory (ROM) as well as other types of other media known to those skilled in the art.

[0189] Aspects include systems (e.g., internet based systems), particularly computer systems, which store and manipulate the sequences and sequence information described herein. One example of a computer system 100 is illustrated in block diagram form in Figure 1. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze a nucleotide or polypeptide sequence. The computer system 100 can include a processor for processing, accessing and manipulating the sequence data. The processor 105 can be any well-known type of central processing unit, such as, for example, the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq, AMD or International Business Machines. The computer system 100 is a general purpose system that comprises the processor 105 and one or more internal data storage components 110 for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

[0190] In one aspect, the computer system 100 includes a processor 105 connected to a bus which is connected to a main memory 115 (preferably implemented as RAM) and one or more internal data storage devices 110, such as a hard drive and/or other computer readable media having data recorded thereon. The computer system 100 can further include one or more data retrieving device 118 for reading the data stored on the internal data storage devices 110. The data retrieving device 118 may represent, for example, a floppy disk drive, a compact disk drives, a magnetic tape drives,

or a modem capable of connection to a remote data storage system (e.g., via the internet) etc. In some embodiments, the internal data storage device 110 is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system 100 may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device. The computer system 100 includes a display 120 which is used to display output to a computer user. It should also be noted that the computer system 100 can be linked to other computer systems 125a-c in a network or wide area network to provide centralized access to the computer system 100. Software for accessing and processing the nucleotide or amino acid sequences can reside in main memory 115 during execution. In some aspects, the computer system 100 may further comprise a sequence comparison algorithm for comparing a nucleic acid sequence. The algorithm and sequence(s) can be stored on a computer readable medium. A "sequence comparison algorithm" refers to one or more programs which are implemented (locally or remotely) on the computer system 100 to compare a nucleotide sequence with other nucleotide sequences and/or compounds stored within a data storage means. For example, the sequence comparison algorithm may compare the nucleotide sequences of the disclosure stored on a computer readable medium to reference sequences stored on a computer readable medium to identify homologies or structural motifs.

[0191] The parameters used with the above algorithms may be adapted depending on the sequence length and degree of homology studied. In some aspects, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user. Figure 2 is a flow diagram illustrating one aspect of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system 100, or a public database such as GENBANK that is available through the Internet. The process 200 begins at a start state 201 and then moves to a state 202 wherein the new sequence to be compared is stored to a memory in a computer system 100. As discussed above, the memory could be any type of memory, including RAM or an internal storage device. The process 200 then moves to a state 204 wherein a database of sequences is opened for analysis and comparison. The process 200 then moves to a state 206 wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state 210 to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system. Once a comparison of the two sequences has been performed at the state 210, a determination is made at a decision state 210 whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process 200. If a determination is made that the two sequences are the same, the process 200 moves to a state 214 wherein the name of the sequence from the database is displayed to the user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process 200 moves to a decision state 218 wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process 200 terminates at an end state 220. However, if more sequences do exist in the database, then the process 200 moves to a state 224 wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database. It should be noted that if a determination had been made at the decision state 212 that the sequences were not homologous, then the process 200 would move immediately to the decision state 218 in order to determine if any other sequences were available in the database for comparison. Accordingly, a computer system comprises a processor, a data storage device having stored thereon a nucleic acid sequence of the disclosure and a sequence comparer for conducting the comparison. The sequence comparer may indicate a homology level between the sequences compared or identify structural motifs, or it may identify structural motifs in sequences which are compared to these nucleic acid codes and polypeptide codes. Figure 3 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous. The process 250 begins at a start state 252 and then moves to a state 254 wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state 256. The process 250 then moves to a state 260 wherein the first character in the first sequence is read and then to a state 262 wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it can be a single letter amino acid code so that the first and sequence sequences can be easily compared. A determination is then made at a decision state 264 whether the two characters are the same. If they are the same, then the process 250 moves to a state 268 wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If

they are, then the process 250 continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process 250 moves to a decision state 274 to determine whether there are any more characters either sequence to read. If there are not any more characters to read, then the process 250 moves to a state 276 wherein the level of homology between the first and second sequences is displayed to the user.

The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with an every character in a second sequence, the homology level would be 100%.

[0192] Alternatively, the computer program can compare a reference sequence to a sequence of the disclosure to determine whether the sequences differ at one or more positions. The program can record the length and identity of inserted, deleted or substituted nucleotides or amino acid residues with respect to the sequence of either the reference or the disclosure. The computer program may be a program which determines whether a reference sequence contains a single nucleotide polymorphism (SNP) with respect to a sequence of the disclosure, or, whether a sequence of the disclosure comprises a SNP of a known sequence. Thus, in some aspects, the computer program is a program which identifies SNPs. The method may be implemented by the computer systems described above and the method illustrated in Figure 3. The method can be performed by reading a sequence of the disclosure and the reference sequences through the use of the computer program and identifying differences with the computer program.

[0193] In other aspects the computer based system comprises an identifier for identifying features within a nucleic acid or polypeptide of the disclosure. An "identifier" refers to one or more programs which identifies certain features within a nucleic acid sequence. For example, an identifier may comprise a program which identifies an open reading frame (ORF) in a nucleic acid sequence. Figure 4 is a flow diagram illustrating one aspect of an identifier process 300 for detecting the presence of a feature in a sequence. The process 300 begins at a start state 302 and then moves to a state 304 wherein a first sequence that is to be checked for features is stored to a memory 115 in the computer system 100. The process 300 then moves to a state 306 wherein a database of sequence features is opened. Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG". Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group. Alternatively, the features may be structural polypeptide motifs such as alpha helices, beta sheets, or functional polypeptide motifs such as enzymatic active sites, helix-turn-helix motifs or other motifs known to those skilled in the art. Once the database of features is opened at the state 306, the process 300 moves to a state 308 wherein the first feature is read from the database. A comparison of the attribute of the first feature with the first sequence is then made at a state 310. A determination is then made at a decision state 316 whether the attribute of the feature was found in the first sequence. If the attribute was found, then the process 300 moves to a state 318 wherein the name of the found feature is displayed to the user. The process 300 then moves to a decision state 320 wherein a determination is made whether more features exist in the database. If no more features do exist, then the process 300 terminates at an end state 324. However, if more features do exist in the database, then the process 300 reads the next sequence feature at a state 326 and loops back to the state 310 wherein the attribute of the next feature is compared against the first sequence. If the feature attribute is not found in the first sequence at the decision state 316, the process 300 moves directly to the decision state 320 in order to determine if any more features exist in the database. Thus, in one aspect, the computer program identifies open reading frames (ORFs).

[0194] A polypeptide or nucleic acid sequence can be stored and manipulated in a variety of data processor programs in a variety of formats. For example, a sequence can be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparison algorithms, identifiers, or sources of reference nucleotide sequences or polypeptide sequences to be compared to a nucleic acid sequence of the disclosure. The programs and databases used include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applications Group), Mac Look (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et al, J. Mol. Biol. 215: 403, 1990), FASTA (Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 85: 2444, 1988), FASTDB (Brutlag et al. Comp. App. Biosci. 6:237-245, 1990), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius2.DBAccess (Molecular Simulations Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMm (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer (Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwent's World Drug Index database, the BioByteMasterFile database, the Genbank database, and the Genseqn database. Many other programs and data bases would be apparent to one of skill in the art given the present

disclosure.

[0195] Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

Hybridization of nucleic acids

[0196] Isolated or recombinant nucleic acids hybridize under stringent conditions to an exemplary sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, or a nucleic acid that encodes a polypeptide SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134. The stringent conditions can be highly stringent conditions, medium stringent conditions and/or low stringent conditions, including the high and reduced stringency conditions described herein. In one aspect, it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the desired scope, as discussed below.

[0197] In alternative embodiments, nucleic acids as defined by their ability to hybridize under stringent conditions can be between about five residues and the full length of nucleic acid; e.g., they can be at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, or more, residues in length. Nucleic acids shorter than full length are also included. These nucleic acids can be useful as, e.g., hybridization probes, labeling probes, PCR oligonucleotide probes, iRNA, antisense or sequences encoding antibody binding peptides (epitopes), motifs, active sites and the like.

[0198] In one aspect, nucleic acids are defined by their ability to hybridize under high stringency comprising conditions of about 50% formamide at about 37°C to 42°C. In one aspect, nucleic acids are defined by their ability to hybridize under reduced stringency comprising conditions in about 35% to 25% formamide at about 30°C to 35°C.

[0199] Alternatively, nucleic acids are defined by their ability to hybridize under high stringency comprising conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and a repetitive sequence blocking nucleic acid, such as cot-1 or salmon sperm DNA (e.g., 200 n/ml sheared and denatured salmon sperm DNA). In one aspect, nucleic acids are defined by their ability to hybridize under reduced stringency conditions comprising 35% formamide at a reduced temperature of 35°C.

[0200] Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

[0201] The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Nucleic acids are also defined by their ability to hybridize under high, medium, and low stringency conditions as set forth in Ausubel and Sambrook. Variations on the above ranges and conditions are well known in the art. Hybridization conditions are discussed further, below.

[0202] The above procedure may be modified to identify nucleic acids having decreasing levels of homology to the probe sequence. For example, to obtain nucleic acids of decreasing homology to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C

to 42°C in a hybridization buffer having a Na⁺ concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 55°C. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 45°C.

[0203] Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate" hybridization, conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

[0204] However, the selection of a hybridization format is not critical - it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the desired scope. Wash conditions used to identify nucleic acids include, e.g.: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50°C or about 55°C to about 60°C; or, a salt concentration of about 0.15 M NaCl at 72°C for about 15 minutes; or, a salt concentration of about 0.2X SSC at a temperature of at least about 50°C or about 55°C to about 60°C for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2X SSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1X SSC containing 0.1% SDS at 68°C for 15 minutes; or, equivalent conditions. See Sambrook, Tijssen and Ausubel for a description of SSC buffer and equivalent conditions.

[0205] These methods may be used to isolate nucleic acids of the disclosure.

Oligonucleotides probes and methods for using them

[0206] The disclosure also provides nucleic acid probes that can be used, e.g., for identifying nucleic acids encoding a polypeptide with a pectate lyase activity or fragments thereof or for identifying pectate lyase genes. In one aspect, the probe comprises at least 10 consecutive bases of a nucleic acid of the disclosure. Alternatively, a probe can be at least about 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150 or about 10 to 50, about 20 to 60 about 30 to 70, consecutive bases of a sequence as set forth in a nucleic acid of the disclosure. The probes identify a nucleic acid by binding and/or hybridization. The probes can be used in arrays, see discussion below, including, e.g., capillary arrays. The probes can also be used to isolate other nucleic acids or polypeptides.

[0207] The probes can be used to determine whether a biological sample, such as a soil sample, contains an organism having a nucleic acid sequence of the disclosure or an organism from which the nucleic acid was obtained. In such procedures, a biological sample potentially harboring the organism from which the nucleic acid was isolated is obtained and nucleic acids are obtained from the sample. The nucleic acids are contacted with the probe under conditions which permit the probe to specifically hybridize to any complementary sequences present in the sample. Where necessary, conditions which permit the probe to specifically hybridize to complementary sequences may be determined by placing the probe in contact with complementary sequences from samples known to contain the complementary sequence, as well as control sequences which do not contain the complementary sequence. Hybridization conditions, such as the salt concentration of the hybridization buffer, the formamide concentration of the hybridization buffer, or the hybridization temperature, may be varied to identify conditions which allow the probe to hybridize specifically to complementary nucleic acids (see discussion on specific hybridization conditions).

[0208] If the sample contains the organism from which the nucleic acid was isolated, specific hybridization of the probe is then detected. Hybridization may be detected by labeling the probe with a detectable agent such as a radioactive isotope, a fluorescent dye or an enzyme capable of catalyzing the formation of a detectable product. Many methods for using the labeled probes to detect the presence of complementary nucleic acids in a sample are familiar to those skilled in the art. These include Southern Blots, Northern Blots, colony hybridization procedures, and dot blots. Protocols for each of these procedures are provided in Ausubel and Sambrook.

[0209] Alternatively, more than one probe (at least one of which is capable of specifically hybridizing to any complementary sequences which are present in the nucleic acid sample), may be used in an amplification reaction to determine whether the sample contains an organism containing a nucleic acid sequence of the disclosure (e.g., an organism from which the nucleic acid was isolated). In one aspect, the probes comprise oligonucleotides. In one aspect, the amplification reaction may comprise a PCR reaction. PCR protocols are described in Ausubel and Sambrook (see discussion on amplification reactions). In such procedures, the nucleic acids in the sample are contacted with the probes, the amplification reaction is performed, and any resulting amplification product is detected. The amplification product may be detected by performing gel electrophoresis on the reaction products and staining the gel with an intercalator such as ethidium bromide. Alternatively, one or more of the probes may be labeled with a radioactive isotope and the presence

of a radioactive amplification product may be detected by autoradiography after gel electrophoresis.

[0210] Probes derived from sequences near the 3' or 5' ends of a nucleic acid sequence of the disclosure can also be used in chromosome walking procedures to identify clones containing additional, e.g., genomic sequences. Such methods allow the isolation of genes which encode additional proteins of interest from the host organism.

[0211] In one aspect, nucleic acid sequences of the disclosure are used as probes to identify and isolate related nucleic acids. In some aspects, the so-identified related nucleic acids may be cDNAs or genomic DNAs from organisms other than the one from which the nucleic acid of the disclosure was first isolated. In such procedures, a nucleic acid sample is contacted with the probe under conditions which permit the probe to specifically hybridize to related sequences. Hybridization of the probe to nucleic acids from the related organism is then detected using any of the methods described above.

[0212] In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency can vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content), and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter. Hybridization can be carried out under conditions of low stringency, moderate stringency or high stringency. As an example of nucleic acid hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 0.5% SDS, 10X Denhardt's, and 0.5 mg/ml polyribadenylic acid. Approximately 2 X 10⁷ cpm (specific activity 4-9 X 10⁸ cpm/ug) of ³²P end-labeled oligonucleotide probe can then be added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature (RT) in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m-10°C for the oligonucleotide probe. The membrane is then exposed to autoradiographic film for detection of hybridization signals.

[0213] By varying the stringency of the hybridization conditions used to identify nucleic acids, such as cDNAs or genomic DNAs, which hybridize to the detectable probe, nucleic acids having different levels of homology to the probe can be identified and isolated. Stringency may be varied by conducting the hybridization at varying temperatures below the melting temperatures of the probes. The melting temperature, T_m, is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly complementary probe. Very stringent conditions are selected to be equal to or about 5°C lower than the T_m for a particular probe. The melting temperature of the probe may be calculated using the following exemplary formulas. For probes between 14 and 70 nucleotides in length the melting temperature (T_m) is calculated using the formula: T_m=81.5+16.6(log [Na⁺])+0.41(fraction G+C)-(600/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation: T_m=81.5+16.6(log [Na⁺])+0.41(fraction G+C)-(0.63% formamide)-(600/N) where N is the length of the probe. Prehybridization may be carried out in 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100μg denatured fragmented salmon sperm DNA or 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100μg denatured fragmented salmon sperm DNA, 50% formamide. Formulas for SSC and Denhardt's and other solutions are listed, e.g., in Sambrook.

[0214] Hybridization is conducted by adding the detectable probe to the prehybridization solutions listed above. Where the probe comprises double stranded DNA, it is denatured before addition to the hybridization solution. The filter is contacted with the hybridization solution for a sufficient period of time to allow the probe to hybridize to cDNAs or genomic DNAs containing sequences complementary thereto or homologous thereto. For probes over 200 nucleotides in length, the hybridization may be carried out at 15-25°C below the T_m. For shorter probes, such as oligonucleotide probes, the hybridization may be conducted at 5-10°C below the T_m. In one aspect, hybridizations in 6X SSC are conducted at approximately 68°C. In one aspect, hybridizations in 50% formamide containing solutions are conducted at approximately 42°C. All of the foregoing hybridizations would be considered to be under conditions of high stringency.

[0215] Following hybridization, the filter is washed to remove any non-specifically bound detectable probe. The stringency used to wash the filters can also be varied depending on the nature of the nucleic acids being hybridized, the length of the nucleic acids being hybridized, the degree of complementarity, the nucleotide sequence composition (e.g., GC v. AT content), and the nucleic acid type (e.g., RNA v. DNA). Examples of progressively higher stringency condition washes are as follows: 2X SSC, 0.1% SDS at room temperature for 15 minutes (low stringency); 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour (moderate stringency); 0.1X SSC, 0.5% SDS for 15 to 30 minutes at between the hybridization temperature and 68°C (high stringency); and 0.15M NaCl for 15 minutes at 72°C (very high stringency). A final low stringency wash can be conducted in 0.1X SSC at room temperature. The examples above are merely illustrative of one set of conditions that can be used to wash filters. One of skill in the art would know that there are numerous recipes for different stringency washes.

[0216] Nucleic acids which have hybridized to the probe can be identified by autoradiography or other conventional techniques. The above procedure may be modified to identify nucleic acids having decreasing levels of homology to the probe sequence. For example, to obtain nucleic acids of decreasing homology to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C

to 42°C in a hybridization buffer having a Na⁺ concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C. An example of "moderate" hybridization conditions is when the above hybridization is conducted at 55°C. An example of "low stringency" hybridization conditions is when the above hybridization is conducted at 45°C.

[0217] Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

[0218] These probes and methods can be used to isolate nucleic acids having a sequence with at least about 99%, 98%, 97%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% homology to a nucleic acid sequence of the disclosure comprising at least about 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, or more consecutive bases thereof, and the sequences complementary thereto. Homology may be measured using an alignment algorithm, as discussed herein. For example, the homologous polynucleotides may have a coding sequence which is a naturally occurring allelic variant of one of the coding sequences described herein. Such allelic variants may have a substitution, deletion or addition of one or more nucleotides when compared to a nucleic acid of the disclosure.

[0219] Additionally, the probes and methods can be used to isolate nucleic acids which encode polypeptides having at least about 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% sequence identity (homology) to a polypeptide of the disclosure comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids, as determined using a sequence alignment algorithm (e.g., such as the FASTA version 3.0t78 algorithm with the default parameters, or a BLAST 2.2.2 program with exemplary settings as set forth herein).

Inhibiting Expression of Pectate lyase

[0220] Nucleic acids may be complementary to (e.g., antisense sequences to) the nucleic acid sequences of the disclosure. Antisense sequences are capable of inhibiting the transport, splicing or transcription of pectate lyase-encoding genes. The inhibition can be effected through the targeting of genomic DNA or messenger RNA. The transcription or function of targeted nucleic acid can be inhibited, for example, by hybridization and/or cleavage. One particularly useful set of inhibitors includes oligonucleotides which are able to either bind pectate lyase gene or message, in either case preventing or inhibiting the production or function of pectate lyase. The association can be through sequence specific hybridization. Another useful class of inhibitors includes oligonucleotides which cause inactivation or cleavage of pectate lyase message. The oligonucleotide can have enzyme activity which causes such cleavage, such as ribozymes. The oligonucleotide can be chemically modified or conjugated to an enzyme or composition capable of cleaving the complementary nucleic acid. A pool of many different such oligonucleotides can be screened for those with the desired activity. This provides various compositions for the inhibition of pectate lyase expression on a nucleic acid and/or protein level, e.g., antisense, iRNA and ribozymes comprising pectate lyase sequences of the disclosure and the anti-pectate lyase antibodies of the disclosure.

[0221] Inhibition of pectate lyase expression can have a variety of industrial applications. For example, inhibition of pectate lyase expression can slow or prevent "soft-rot" spoilage. "Soft-rot" spoilage occurs when pectin, a major structural polysaccharide in the plant cell wall, is enzymatically degraded. This can lead to the deterioration, or rot, of fruits and vegetables. In one aspect, use of compositions that inhibit the expression and/or activity of pectate lyases, e.g., antibodies, antisense oligonucleotides, ribozymes and RNAi, are used to slow or prevent "soft-rot" spoilage. This provides methods and compositions comprising application onto a plant or plant product (e.g., a fruit, seed, root, leaf, etc.) antibodies, antisense oligonucleotides, ribozymes and RNAi to slow or prevent "soft-rot" spoilage. These compositions also can be expressed by the plant (e.g., a transgenic plant) or another organism (e.g., a bacterium or other microorganism transformed with a pectate lyase gene of the disclosure).

[0222] Inhibition of pectate lyase expression also can prevent or slow the normal growth of the powdery mildew pathogen *Erysiphe cichoracearum*. This powdery mildew resistance represents a form of disease resistance based on the loss of a gene required during a compatible interaction rather than the activation of known host defense pathways. See, e.g., Vogel (2002) Plant Cell 14:2095-2106. This provides methods and compositions comprising application onto a plant or plant product (e.g., a fruit, seed, root, leaf, etc.) antibodies, antisense oligonucleotides, ribozymes and RNAi to slow or prevent growth of the powdery mildew pathogen.

Antisense Oligonucleotides

[0223] Antisense oligonucleotides may be capable of binding pectate lyase message which can inhibit proteolytic activity by targeting mRNA. Strategies for designing antisense oligonucleotides are well described in the scientific and patent literature, and the skilled artisan can design such pectate lyase oligonucleotides using the novel reagents of the disclosure. For example, gene walking/ RNA mapping protocols to screen for effective antisense oligonucleotides are well known in the art, see, e.g., Ho (2000) *Methods Enzymol.* 314:168-183, describing an RNA mapping assay, which is based on standard molecular techniques to provide an easy and reliable method for potent antisense sequence selection. See also Smith (2000) *Eur. J. Pharm. Sci.* 11:191-198.

[0224] Naturally occurring nucleic acids are used as antisense oligonucleotides. The antisense oligonucleotides can be of any length; for example, in alternative aspects, the antisense oligonucleotides are between about 5 to 100, about 10 to 80, about 15 to 60, about 18 to 40. The optimal length can be determined by routine screening. The antisense oligonucleotides can be present at any concentration. The optimal concentration can be determined by routine screening. A wide variety of synthetic, non-naturally occurring nucleotide and nucleic acid analogues are known which can address this potential problem. For example, peptide nucleic acids (PNAs) containing non-ionic backbones, such as N-(2-aminoethyl) glycine units can be used. Antisense oligonucleotides having phosphorothioate linkages can also be used, as described in WO 97/03211; WO 96/39154; Mata (1997) *Toxicol Appl Pharmacol* 144:189-197; *Antisense Therapeutics*, ed. Agrawal (Humana Press, Totowa, N.J., 1996). Antisense oligonucleotides having synthetic DNA backbone analogues provided can also include phosphoro-dithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, and morpholino carbamate nucleic acids, as described above.

[0225] Combinatorial chemistry methodology can be used to create vast numbers of oligonucleotides that can be rapidly screened for specific oligonucleotides that have appropriate binding affinities and specificities toward any target, such as the sense and antisense pectate lyase sequences of the disclosure (see, e.g., Gold (1995) *J. of Biol. Chem.* 270:13581-13584).

Inhibitory Ribozymes

[0226] Ribozymes are capable of binding pectate lyase message. These ribozymes can inhibit pectate lyase activity by, e.g., targeting mRNA. Strategies for designing ribozymes and selecting the pectate lyase-specific antisense sequence for targeting are well described in the scientific and patent literature, and the skilled artisan can design such ribozymes using the novel reagents of the disclosure. Ribozymes act by binding to a target RNA through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA that cleaves the target RNA. Thus, the ribozyme recognizes and binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cleave and inactivate the target RNA. Cleavage of a target RNA in such a manner will destroy its ability to direct synthesis of an encoded protein if the cleavage occurs in the coding sequence. After a ribozyme has bound and cleaved its RNA target, it can be released from that RNA to bind and cleave new targets repeatedly.

[0227] In some circumstances, the enzymatic nature of a ribozyme can be advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its transcription, translation or association with another molecule) as the effective concentration of ribozyme necessary to effect a therapeutic treatment can be lower than that of an antisense oligonucleotide. This potential advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, a ribozyme is typically a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ratio of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, the specificity of action of a ribozyme can be greater than that of antisense oligonucleotide binding the same RNA site.

[0228] The ribozyme of the disclosure, e.g., an enzymatic ribozyme RNA molecule, can be formed in a hammerhead motif, a hairpin motif, as a hepatitis delta virus motif, a group I intron motif and/or an RNaseP-like RNA in association with an RNA guide sequence. Examples of hammerhead motifs are described by, e.g., Rossi (1992) *Aids Research and Human Retroviruses* 8:183; hairpin motifs by Hampel (1989) *Biochemistry* 28:4929, and Hampel (1990) *Nuc. Acids Res.* 18:299; the hepatitis delta virus motif by Perrotta (1992) *Biochemistry* 31:16; the RNaseP motif by Guerrier-Takada (1983) *Cell* 35:849; and the group I intron by Cech U.S. Pat. No. 4,987,071. The recitation of these specific motifs is not intended to be limiting. Those skilled in the art will recognize that such a ribozyme, e.g., an enzymatic RNA molecule, can have a specific substrate binding site complementary to one or more of the target gene RNA regions. A ribozyme can have a nucleotide sequence within or surrounding that substrate binding site which imparts an RNA cleaving activity to the molecule.

RNA interference (RNAi)

[0229] An RNA inhibitory molecule, a so-called "RNAi" molecule, comprises a pectate lyase sequence of the disclosure. The RNAi molecule comprises a double-stranded RNA (dsRNA) molecule. The RNAi can inhibit expression of a pectate lyase gene. In one aspect, the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length. The RNAi may enter a cell and cause the degradation of a single-stranded RNA (ssRNA) of similar or identical sequences, including endogenous mRNAs. When a cell is exposed to double-stranded RNA (dsRNA), mRNA from the homologous gene is selectively degraded by a process called RNA interference (RNAi). A possible basic mechanism behind RNAi is the breaking of a double-stranded RNA (dsRNA) matching a specific gene sequence into short pieces called short interfering RNA, which trigger the degradation of mRNA that matches its sequence. In one aspect, the RNAi's are used in gene-silencing therapeutics, see, e.g., Shuey (2002) Drug Discov. Today 7:1040-1046. This provides methods to selectively degrade RNA using the RNAi's. The process may be practiced *in vitro*, *ex vivo* or *in vivo*. In one aspect, the RNAi molecules can be used to generate a loss-of-function mutation in a cell, an organ or an animal. Methods for making and using RNAi molecules for selectively degrade RNA are well known in the art, see, e.g., U.S. Patent No. 6,506,559; 6,511,824; 6,515,109; 6,489,127.

Modification of Nucleic Acids

[0230] Methods may generate variants of the nucleic acids of the disclosure, e.g., those encoding a pectate lyase, and the disclosure provides the variant nucleic acids generated by these methods (e.g., SEQ ID NO:133) and polypeptides encoded by them (e.g., SEQ ID NO:134, as discussed below). These methods can be repeated or used in various combinations to generate pectate lyases having an altered or different activity or an altered or different stability from that of a pectate lyase encoded by the template nucleic acid. These methods also can be repeated or used in various combinations, e.g., to generate variations in gene/ message expression, message translation or message stability. In another aspect, the genetic composition of a cell is altered by, e.g., modification of a homologous gene *in vitro*, *in vivo* or *ex vivo*, followed by its reinsertion into the cell.

[0231] A nucleic acid can be altered by any means. For example, random or stochastic methods, or, non-stochastic, or "directed evolution," methods, see, e.g., U.S. Patent No. 6,361,974. Methods for random mutation of genes are well known in the art, see, e.g., U.S. Patent No. 5,830,696. For example, mutagens can be used to randomly mutate a gene. Mutagens include, e.g., ultraviolet light or gamma irradiation, or a chemical mutagen, e.g., mitomycin, nitrous acid, photoactivated psoralens, alone or in combination, to induce DNA breaks amenable to repair by recombination. Other chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other mutagens are analogues of nucleotide precursors, e.g., nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. These agents can be added to a PCR reaction in place of the nucleotide precursor, thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can be used.

[0232] Any technique in molecular biology can be used, e.g., random PCR mutagenesis, see, e.g., Rice (1992) Proc. Natl. Acad. Sci. USA 89:5467-5471; or, combinatorial multiple cassette mutagenesis, see, e.g., Cramer (1995) Biotechniques 18:194-196. Alternatively, nucleic acids, e.g., genes, can be reassembled after random, or "stochastic," fragmentation, see, e.g., U.S. Patent Nos. 6,291,242; 6,287,862; 6,287,861; 5,955,358; 5,830,721; 5,824,514; 5,811,238; 5,605,793. In alternative aspects, modifications, additions or deletions are introduced by error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis (GSSM™), synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, and/or a combination of these and other methods.

[0233] The following publications describe a variety of recursive recombination procedures and/or methods which can be incorporated into the methods of the disclosure: Stemmer (1999) "Molecular breeding of viruses for targeting and other clinical properties" Tumor Targeting 4:1-4; Ness (1999) Nature Biotechnology 17:893-896; Chang (1999) "Evolution of a cytokine using DNA family shuffling" Nature Biotechnology 17:793-797; Minshull (1999) "Protein evolution by molecular breeding" Current Opinion in Chemical Biology 3:284-290; Christians (1999) "Directed evolution of thymidine kinase for AZT phosphorylation using DNA family shuffling" Nature Biotechnology 17:259-264; Cramer (1998) "DNA shuffling of a family of genes from diverse species accelerates directed evolution" Nature 391:288-291; Cramer (1997) "Molecular evolution of an arsenate detoxification pathway by DNA shuffling," Nature Biotechnology 15:436-438; Zhang (1997) "Directed evolution of an effective fucosidase from a galactosidase by DNA shuffling and screening" Proc. Natl. Acad. Sci. USA 94:4504-4509; Patten et al. (1997) "Applications of DNA Shuffling to Pharmaceuticals and Vaccines"

Current Opinion in Biotechnology 8:724-733; Cramer, et al. (1996) "Construction and evolution of antibody-phage libraries by DNA shuffling" *Nature Medicine* 2:100-103; Gates et al. (1996) "Affinity selective isolation of ligands from peptide libraries through display on a lac repressor 'headpiece dimer'" *Journal of Molecular Biology* 255:373-386; Stemmer (1996) "Sexual PCR and Assembly PCR" In: *The Encyclopedia of Molecular Biology*. VCH Publishers, New York. pp.447-457; Cramer and Stemmer (1995) "Combinatorial multiple cassette mutagenesis creates all the permutations of mutant and wildtype cassettes" *BioTechniques* 18:194-195; Stemmer et al. (1995) "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides" *Gene*, 164:49-53; Stemmer (1995) "The Evolution of Molecular Computation" *Science* 270: 1510; Stemmer (1995) "Searching Sequence Space" *Bio/Technology* 13:549-553; Stemmer (1994) "Rapid evolution of a protein in vitro by DNA shuffling" *Nature* 370:389-391; and Stemmer (1994) "DNA shuffling by random fragmentation and reassembly: In vitro recombination for molecular evolution." *Proc. Natl. Acad. Sci. USA* 91:10747-10751.

[0234] Mutational methods of generating diversity include, for example, site-directed mutagenesis (Ling et al. (1997) "Approaches to DNA mutagenesis: an overview" *Anal Biochem.* 254(2): 157-178; Dale et al. (1996) "Oligonucleotide-directed random mutagenesis using the phosphorothioate method" *Methods Mol. Biol.* 57:369-374; Smith (1985) "In vitro mutagenesis" *Ann. Rev. Genet.* 19:423-462; Botstein & Shortle (1985) "Strategies and applications of in vitro mutagenesis" *Science* 229:1193-1201; Carter (1986) "Site-directed mutagenesis" *Biochem. J.* 237:1-7; and Kunkel (1987) "The efficiency of oligonucleotide directed mutagenesis" in *Nucleic Acids & Molecular Biology* (Eckstein, F. and Lilley, D. M. J. eds., Springer Verlag, Berlin)); mutagenesis using uracil containing templates (Kunkel (1985) "Rapid and efficient site-specific mutagenesis without phenotypic selection" *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel et al. (1987) "Rapid and efficient site-specific mutagenesis without phenotypic selection" *Methods in Enzymol.* 154, 367-382; and Bass et al. (1988) "Mutant T₄ polymerases with new DNA-binding specificities" *Science* 242:240-245); oligonucleotide-directed mutagenesis (*Methods in Enzymol.* 100: 468-500 (1983); *Methods in Enzymol.* 154: 329-350 (1987); Zoller & Smith (1982) "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment" *Nucleic Acids Res.* 10:6487-6500; Zoller & Smith (1983) "Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors" *Methods in Enzymol.* 100:468-500; and Zoller & Smith (1987) "Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template" *Methods in Enzymol.* 154:329-350); phosphorothioate-modified DNA mutagenesis (Taylor et al. (1985) "The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA" *Nucl. Acids Res.* 13: 8749-8764; Taylor et al. (1985) "The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA" *Nucl. Acids Res.* 13: 8765-8787 (1985); Nakamaye (1986) "Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis" *Nucl. Acids Res.* 14: 9679-9698; Sayers et al. (1988) "Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis" *Nucl. Acids Res.* 16:791-802; and Sayers et al. (1988) "Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide" *Nucl. Acids Res.* 16: 803-814); mutagenesis using gapped duplex DNA (Kramer et al. (1984) "The gapped duplex DNA approach to oligonucleotide-directed mutation construction" *Nucl. Acids Res.* 12: 9441-9456; Kramer & Fritz (1987) *Methods in Enzymol.* "Oligonucleotide-directed construction of mutations via gapped duplex DNA" 154:350-367; Kramer et al. (1988) "Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations" *Nucl. Acids Res.* 16: 7207; and Fritz et al. (1988) "Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro" *Nucl. Acids Res.* 16: 6987-6999).

[0235] Additional protocols that can be used to practice the methods include point mismatch repair (Kramer (1984) "Point Mismatch Repair" *Cell* 38:879-887), mutagenesis using repair-deficient host strains (Carter et al. (1985) "Improved oligonucleotide site-directed mutagenesis using M13 vectors" *Nucl. Acids Res.* 13: 4431-4443; and Carter (1987) "Improved oligonucleotide-directed mutagenesis using M13 vectors" *Methods in Enzymol.* 154: 382-403), deletion mutagenesis (Eghtedarzadeh (1986) "Use of oligonucleotides to generate large deletions" *Nucl. Acids Res.* 14: 5115), restriction-selection and restriction-selection and restriction-purification (Wells et al. (1986) "Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin" *Phil. Trans. R. Soc. Lond. A* 317: 415-423), mutagenesis by total gene synthesis (Nambiar et al. (1984) "Total synthesis and cloning of a gene coding for the ribonuclease S protein" *Science* 223: 1299-1301; Sakamar and Khorana (1988) "Total synthesis and expression of a gene for the α -subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin)" *Nucl. Acids Res.* 14: 6361-6372; Wells et al. (1985) "Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites" *Gene* 34:315-323; and Grundstrom et al. (1985) "Oligonucleotide-directed mutagenesis by microscale 'shot-gun' gene synthesis" *Nucl. Acids Res.* 13: 3305-3316), double-strand break repair (Mandecki (1986); Arnold (1993) "Protein engineering for unusual environments" *Current Opinion in Biotechnology* 4:450-455. "Oligonucleotide-directed double-strand break repair in plasmids of *Escherichia coli*: a method for site-specific mutagenesis" *Proc. Natl. Acad. Sci. USA*, 83:7177-7181). Additional details on many of the above methods can be found in *Methods in Enzymology* Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis methods.

[0236] Protocols that can be used are described, e.g., in U.S. Patent Nos. 5,605,793 to Stemmer (Feb. 25, 1997), "Methods for In Vitro Recombination;" U.S. Pat. No. 5,811,238 to Stemmer et al. (Sep. 22, 1998) "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" U.S. Pat. No. 5,830,721 to Stemmer et al. (Nov. 3, 1998), "DNA Mutagenesis by Random Fragmentation and Reassembly;" U.S. Pat. No. 5,834,252 to Stemmer, et al. (Nov. 10, 1998) "End-Complementary Polymerase Reaction;" U.S. Pat. No. 5,837,458 to Minshull, et al. (Nov. 17, 1998), "Methods and Compositions for Cellular and Metabolic Engineering;" WO 95/22625, Stemmer and Cramer, "Mutagenesis by Random Fragmentation and Reassembly;" WO 96/33207 by Stemmer and Lipschutz "End Complementary Polymerase Chain Reaction;" WO 97/20078 by Stemmer and Cramer, "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" WO 97/35966 by Minshull and Stemmer, "Methods and Compositions for Cellular and Metabolic Engineering;" WO 99/41402 by Punnonen et al. "Targeting of Genetic Vaccine Vectors;" WO 99/41383 by Punnonen et al. "Antigen Library Immunization;" WO 99/41369 by Punnonen et al. "Genetic Vaccine Vector Engineering;" WO 99/41368 by Punnonen et al. "Optimization of Immunomodulatory Properties of Genetic Vaccines;" EP 752008 by Stemmer and Cramer, "DNA Mutagenesis by Random Fragmentation and Reassembly;" EP 0932670 by Stemmer "Evolving Cellular DNA Uptake by Recursive Sequence Recombination;" WO 99/23107 by Stemmer et al., "Modification of Virus Tropism and Host Range by Viral Genome Shuffling;" WO 99/21979 by Apt et al., "Human Papillomavirus Vectors;" WO 98/31837 by del Cardayre et al. "Evolution of Whole Cells and Organisms by Recursive Sequence Recombination;" WO 98/27230 by Patten and Stemmer, "Methods and Compositions for Polypeptide Engineering;" WO 98/27230 by Stemmer et al., "Methods for Optimization of Gene Therapy by Recursive Sequence Shuffling and Selection;" WO 00/00632, "Methods for Generating Highly Diverse Libraries;" WO 00/09679, "Methods for Obtaining In Vitro Recombined Polynucleotide Sequence Banks and Resulting Sequences;" WO 98/42832 by Arnold et al., "Recombination of Polynucleotide Sequences Using Random or Defined Primers;" WO 99/29902 by Arnold et al., "Method for Creating Polynucleotide and Polypeptide Sequences;" WO 98/41653 by Vind, "An in Vitro Method for Construction of a DNA Library;" WO 98/41622 by Borchert et al., "Method for Constructing a Library Using DNA Shuffling," and WO 98/42727 by Pati and Zarling, "Sequence Alterations using Homologous Recombination."

[0237] Protocols that can be used (providing details regarding various diversity generating methods) are described, e.g., in U.S. Patent application serial no. (USSN) 09/407,800, "SHUFFLING OF CODON ALTERED GENES" by Patten et al. filed Sep. 28, 1999; "EVOLUTION OF WHOLE CELLS AND ORGANISMS BY RECURSIVE SEQUENCE RECOMBINATION" by del Cardayre et al., United States Patent No. 6,379,964; "OLIGONUCLEOTIDE MEDIATED NUCLEIC ACID RECOMBINATION" by Cramer et al., United States Patent Nos. 6,319,714; 6,368,861; 6,376,246; 6,423,542; 6,426,224 and PCT/US00/01203; "USE OF CODON-VARIED OLIGONUCLEOTIDE SYNTHESIS FOR SYNTHETIC SHUFFLING" by Welch et al., United States Patent No. 6,436,675; "METHODS FOR MAKING CHARACTER STRINGS, POLYNUCLEOTIDES & POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jan. 18, 2000, (PCT/US00/01202) and, e.g. "METHODS FOR MAKING CHARACTER STRINGS, POLYNUCLEOTIDES & POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jul. 18, 2000 (U.S. Ser. No. 09/618,579); "METHODS OF POPULATING DATA STRUCTURES FOR USE IN EVOLUTIONARY SIMULATIONS" by Selifonov and Stemmer, filed Jan. 18, 2000 (PCT/US00/01138); and "SINGLE-STRANDED NUCLEIC ACID TEMPLATE-MEDIATED RECOMBINATION AND NUCLEIC ACID FRAGMENT ISOLATION" by Affholter, filed Sep. 6, 2000 (U.S. Ser. No. 09/656,549); and United States Patent Nos. 6,177,263; 6,153,410.

[0238] Non-stochastic, or "directed evolution," methods include, e.g., saturation mutagenesis (GSSM™), synthetic ligation reassembly (SLR), or a combination thereof are used to modify the nucleic acids to generate pectate lyases with new or altered properties (e.g., activity under highly acidic or alkaline conditions, high temperatures, and the like). Polypeptides encoded by the modified nucleic acids can be screened for an activity before testing for proteolytic or other activity. Any testing modality or protocol can be used, e.g., using a capillary array platform. See, e.g., U.S. Patent Nos. 6,361,974; 6,280,926; 5,939,250.

Saturation mutagenesis, or, GSSM™

[0239] In one aspect, codon primers containing a degenerate N,N,G/T sequence are used to introduce point mutations into a polynucleotide, e.g., a pectate lyase or an antibody of the disclosure, so as to generate a set of progeny polypeptides in which a full range of single amino acid substitutions is represented at each amino acid position, e.g., an amino acid residue in an enzyme active site or ligand binding site targeted to be modified. These oligonucleotides can comprise a contiguous first homologous sequence, a degenerate N,N,G/T sequence, and, optionally, a second homologous sequence. The downstream progeny translational products from the use of such oligonucleotides include all possible amino acid changes at each amino acid site along the polypeptide, because the degeneracy of the N,N,G/T sequence includes codons for all 20 amino acids. In one aspect, one such degenerate oligonucleotide (comprised of, e.g., one degenerate N,N,G/T cassette) is used for subjecting each original codon in a parental polynucleotide template to a full range of codon substitutions. In another aspect, at least two degenerate cassettes are used - either in the same oligonucleotide

or not, for subjecting at least two original codons in a parental polynucleotide template to a full range of codon substitutions. For example, more than one N,N,G/T sequence can be contained in one oligonucleotide to introduce amino acid mutations at more than one site. This plurality of N,N,G/T sequences can be directly contiguous, or separated by one or more additional nucleotide sequence(s). In another aspect, oligonucleotides serviceable for introducing additions and deletions can be used either alone or in combination with the codons containing an N,N,G/T sequence, to introduce any combination or permutation of amino acid additions, deletions, and/or substitutions.

[0240] In one aspect, simultaneous mutagenesis of two or more contiguous amino acid positions is done using an oligonucleotide that contains contiguous N,N,G/T triplets, i.e. a degenerate (N,N,G/T)_n sequence. In another aspect, degenerate cassettes having less degeneracy than the N,N,G/T sequence are used. For example, it may be desirable in some instances to use (e.g. in an oligonucleotide) a degenerate triplet sequence comprised of only one N, where said N can be in the first second or third position of the triplet. Any other bases including any combinations and permutations thereof can be used in the remaining two positions of the triplet. Alternatively, it may be desirable in some instances to use (e.g. in an oligo) a degenerate N,N,N triplet sequence.

[0241] In one aspect, use of degenerate triplets (e.g., N,N,G/T triplets) allows for systematic and easy generation of a full range of possible natural amino acids (for a total of 20 amino acids) into each and every amino acid position in a polypeptide (in alternative aspects, the methods also include generation of less than all possible substitutions per amino acid residue, or codon, position). For example, for a 100 amino acid polypeptide, 2000 distinct species (i.e. 20 possible amino acids per position X 100 amino acid positions) can be generated. Through the use of an oligonucleotide or set of oligonucleotides containing a degenerate N,N,G/T triplet, 32 individual sequences can code for all 20 possible natural amino acids. Thus, in a reaction vessel in which a parental polynucleotide sequence is subjected to saturation mutagenesis using at least one such oligonucleotide, there are generated 32 distinct progeny polynucleotides encoding 20 distinct polypeptides. In contrast, the use of a non-degenerate oligonucleotide in site-directed mutagenesis leads to only one progeny polypeptide product per reaction vessel. Nondegenerate oligonucleotides can optionally be used in combination with degenerate primers disclosed; for example, nondegenerate oligonucleotides can be used to generate specific point mutations in a working polynucleotide. This provides one means to generate specific silent point mutations, point mutations leading to corresponding amino acid changes, and point mutations that cause the generation of stop codons and the corresponding expression of polypeptide fragments.

[0242] In one aspect, each saturation mutagenesis reaction vessel contains polynucleotides encoding at least 20 progeny polypeptide (e.g., pectate lyases) molecules such that all 20 natural amino acids are represented at the one specific amino acid position corresponding to the codon position mutagenized in the parental polynucleotide (other aspects use less than all 20 natural combinations). The 32-fold degenerate progeny polypeptides generated from each saturation mutagenesis reaction vessel can be subjected to clonal amplification (e.g. cloned into a suitable host, e.g., *E. coli* host, using, e.g., an expression vector) and subjected to expression screening. When an individual progeny polypeptide is identified by screening to display a favorable change in property (when compared to the parental polypeptide, such as increased proteolytic activity under alkaline or acidic conditions), it can be sequenced to identify the correspondingly favorable amino acid substitution contained therein.

[0243] In one aspect, upon mutagenizing each and every amino acid position in a parental polypeptide using saturation mutagenesis as disclosed herein, favorable amino acid changes may be identified at more than one amino acid position. One or more new progeny molecules can be generated that contain a combination of all or part of these favorable amino acid substitutions. For example, if 2 specific favorable amino acid changes are identified in each of 3 amino acid positions in a polypeptide, the permutations include 3 possibilities at each position (no change from the original amino acid, and each of two favorable changes) and 3 positions. Thus, there are 3 x 3 x 3 or 27 total possibilities, including 7 that were previously examined - 6 single point mutations (i.e. 2 at each of three positions) and no change at any position.

[0244] In another aspect, site-saturation mutagenesis can be used together with another stochastic or non-stochastic means to vary sequence, e.g., synthetic ligation reassembly (see below), shuffling, chimerization, recombination and other mutagenizing processes and mutagenizing agents. This provides for the use of any mutagenizing process(es), including saturation mutagenesis, in an iterative manner.

Synthetic Ligation Reassembly (SLR)

[0245] A non-stochastic gene modification system termed "synthetic ligation reassembly," or simply "SLR," a "directed evolution process," may be used to generate polypeptides, e.g., pectate lyases or antibodies, with new or altered properties. SLR is a method of ligating oligonucleotide fragments together non-stochastically. This method differs from stochastic oligonucleotide shuffling in that the nucleic acid building blocks are not shuffled, concatenated or chimerized randomly, but rather are assembled non-stochastically. See, e.g., U.S. Patent Application Serial No. (USSN) 09/332,835 entitled "Synthetic Ligation Reassembly in Directed Evolution" and filed on June 14, 1999 ("USSN 09/332,835"). In one aspect, SLR comprises the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises sequence encoding a homologous gene; (b) providing a plurality of building block polynucleotides, wherein

the building block polynucleotides are designed to cross-over reassemble with the template polynucleotide at a predetermined sequence, and a building block polynucleotide comprises a sequence that is a variant of the homologous gene and a sequence homologous to the template polynucleotide flanking the variant sequence; (c) combining a building block polynucleotide with a template polynucleotide such that the building block polynucleotide cross-over reassembles with the template polynucleotide to generate polynucleotides comprising homologous gene sequence variations.

[0246] SLR does not depend on the presence of high levels of homology between polynucleotides to be rearranged. Thus, this method can be used to non-stochastically generate libraries (or sets) of progeny molecules comprised of over 10100 different chimeras. SLR can be used to generate libraries comprised of over 101000 different progeny chimeras. Thus, aspects include non-stochastic methods of producing a set of finalized chimeric nucleic acid molecule having an overall assembly order that is chosen by design. This method includes the steps of generating by design a plurality of specific nucleic acid building blocks having serviceable mutually compatible ligatable ends, and assembling these nucleic acid building blocks, such that a designed overall assembly order is achieved.

[0247] The mutually compatible ligatable ends of the nucleic acid building blocks to be assembled are considered to be "serviceable" for this type of ordered assembly if they enable the building blocks to be coupled in predetermined orders. Thus, the overall assembly order in which the nucleic acid building blocks can be coupled is specified by the design of the ligatable ends. If more than one assembly step is to be used, then the overall assembly order in which the nucleic acid building blocks can be coupled is also specified by the sequential order of the assembly step(s). In one aspect, the annealed building pieces are treated with an enzyme, such as a ligase (e.g. T4 DNA ligase), to achieve covalent bonding of the building pieces.

[0248] In one aspect, the design of the oligonucleotide building blocks is obtained by analyzing a set of progenitor nucleic acid sequence templates that serve as a basis for producing a progeny set of finalized chimeric polynucleotides. These parental oligonucleotide templates thus serve as a source of sequence information that aids in the design of the nucleic acid building blocks that are to be mutagenized, e.g., chimerized or shuffled. In one aspect of this method, the sequences of a plurality of parental nucleic acid templates are aligned in order to select one or more demarcation points. The demarcation points can be located at an area of homology, and are comprised of one or more nucleotides. These demarcation points are preferably shared by at least two of the progenitor templates. The demarcation points can thereby be used to delineate the boundaries of oligonucleotide building blocks to be generated in order to rearrange the parental polynucleotides. The demarcation points identified and selected in the progenitor molecules serve as potential chimerization points in the assembly of the final chimeric progeny molecules. A demarcation point can be an area of homology (comprised of at least one homologous nucleotide base) shared by at least two parental polynucleotide sequences. Alternatively, a demarcation point can be an area of homology that is shared by at least half of the parental polynucleotide sequences, or, it can be an area of homology that is shared by at least two thirds of the parental polynucleotide sequences. Even more preferably a serviceable demarcation points is an area of homology that is shared by at least three fourths of the parental polynucleotide sequences, or, it can be shared by at almost all of the parental polynucleotide sequences. In one aspect, a demarcation point is an area of homology that is shared by all of the parental polynucleotide sequences.

[0249] In one aspect, a ligation reassembly process is performed exhaustively in order to generate an exhaustive library of progeny chimeric polynucleotides. In other words, all possible ordered combinations of the nucleic acid building blocks are represented in the set of finalized chimeric nucleic acid molecules. At the same time, in another aspect, the assembly order (i.e. the order of assembly of each building block in the 5' to 3' sequence of each finalized chimeric nucleic acid) in each combination is by design (or non-stochastic) as described above. Because of the non-stochastic nature of this invention, the possibility of unwanted side products is greatly reduced.

[0250] In another aspect, the ligation reassembly method is performed systematically. For example, the method is performed in order to generate a systematically compartmentalized library of progeny molecules, with compartments that can be screened systematically, e.g. one by one. In other words this invention provides that, through the selective and judicious use of specific nucleic acid building blocks, coupled with the selective and judicious use of sequentially stepped assembly reactions, a design can be achieved where specific sets of progeny products are made in each of several reaction vessels. This allows a systematic examination and screening procedure to be performed. Thus, these methods allow a potentially very large number of progeny molecules to be examined systematically in smaller groups. Because of its ability to perform chimerizations in a manner that is highly flexible yet exhaustive and systematic as well, particularly when there is a low level of homology among the progenitor molecules, these methods provide for the generation of a library (or set) comprised of a large number of progeny molecules. Because of the non-stochastic nature of the instant ligation reassembly, the progeny molecules generated preferably comprise a library of finalized chimeric nucleic acid molecules having an overall assembly order that is chosen by design. The saturation mutagenesis and optimized directed evolution methods also can be used to generate different progeny molecular species. It is appreciated that this provides freedom of choice and control regarding the selection of demarcation points, the size and number of the nucleic acid building blocks, and the size and design of the couplings. It is appreciated, furthermore, that the requirement for intermolecular homology is highly relaxed for the operability. In fact, demarcation points can even be chosen in areas of little or no intermolecular homology. For example, because of codon wobble, i.e. the degeneracy of codons,

nucleotide substitutions can be introduced into nucleic acid building blocks without altering the amino acid originally encoded in the corresponding progenitor template. Alternatively, a codon can be altered such that the coding for an originally amino acid is altered. This provides that such substitutions can be introduced into the nucleic acid building block in order to increase the incidence of intermolecular homologous demarcation points and thus to allow an increased number of couplings to be achieved among the building blocks, which in turn allows a greater number of progeny chimeric molecules to be generated.

[0251] In another aspect, the synthetic nature of the step in which the building blocks are generated allows the design and introduction of nucleotides (e.g., one or more nucleotides, which may be, for example, codons or introns or regulatory sequences) that can later be optionally removed in an in vitro process (e.g. by mutagenesis) or in an in vivo process (e.g. by utilizing the gene splicing ability of a host organism). It is appreciated that in many instances the introduction of these nucleotides may also be desirable for many other reasons in addition to the potential benefit of creating a serviceable demarcation point.

[0252] In one aspect, a nucleic acid building block is used to introduce an intron. Thus, functional introns are introduced into a man-made gene manufactured according to the methods described herein. The artificially introduced intron(s) can be functional in a host cells for gene splicing much in the way that naturally-occurring introns serve functionally in gene splicing.

Optimized Directed Evolution System

[0253] A non-stochastic gene modification system termed "optimized directed evolution system" may generate polypeptides, e.g., pectate lyases or antibodies, with new or altered properties. Optimized directed evolution is directed to the use of repeated cycles of reductive reassortment, recombination and selection that allow for the directed molecular evolution of nucleic acids through recombination. Optimized directed evolution allows generation of a large population of evolved chimeric sequences, wherein the generated population is significantly enriched for sequences that have a predetermined number of crossover events.

[0254] A crossover event is a point in a chimeric sequence where a shift in sequence occurs from one parental variant to another parental variant. Such a point is normally at the juncture of where oligonucleotides from two parents are ligated together to form a single sequence. This method allows calculation of the correct concentrations of oligonucleotide sequences so that the final chimeric population of sequences is enriched for the chosen number of crossover events. This provides more control over choosing chimeric variants having a predetermined number of crossover events.

[0255] In addition, this method provides a convenient means for exploring a tremendous amount of the possible protein variant space in comparison to other systems. Previously, if one generated, for example, 10^{13} chimeric molecules during a reaction, it would be extremely difficult to test such a high number of chimeric variants for a particular activity. Moreover, a significant portion of the progeny population would have a very high number of crossover events which resulted in proteins that were less likely to have increased levels of a particular activity. By using these methods, the population of chimeric molecules can be enriched for those variants that have a particular number of crossover events. Thus, although one can still generate 10^{13} chimeric molecules during a reaction, each of the molecules chosen for further analysis most likely has, for example, only three crossover events. Because the resulting progeny population can be skewed to have a predetermined number of crossover events, the boundaries on the functional variety between the chimeric molecules is reduced. This provides a more manageable number of variables when calculating which oligonucleotide from the original parental polynucleotides might be responsible for affecting a particular trait.

[0256] One method for creating a chimeric progeny polynucleotide sequence is to create oligonucleotides corresponding to fragments or portions of each parental sequence. Each oligonucleotide preferably includes a unique region of overlap so that mixing the oligonucleotides together results in a new variant that has each oligonucleotide fragment assembled in the correct order. Additional information can also be found, e.g., in USSN 09/332,835; U.S. Patent No. 6,361,974.

[0257] The number of oligonucleotides generated for each parental variant bears a relationship to the total number of resulting crossovers in the chimeric molecule that is ultimately created. For example, three parental nucleotide sequence variants might be provided to undergo a ligation reaction in order to find a chimeric variant having, for example, greater activity at high temperature. As one example, a set of 50 oligonucleotide sequences can be generated corresponding to each portions of each parental variant. Accordingly, during the ligation reassembly process there could be up to 50 crossover events within each of the chimeric sequences. The probability that each of the generated Chimeric polynucleotides will contain oligonucleotides from each parental variant in alternating order is very low. If each oligonucleotide fragment is present in the ligation reaction in the same molar quantity it is likely that in some positions oligonucleotides from the same parental polynucleotide will ligate next to one another and thus not result in a crossover event. If the concentration of each oligonucleotide from each parent is kept constant during any ligation step in this example, there is a 1/3 chance (assuming 3 parents) that an oligonucleotide from the same parental variant will ligate within the chimeric sequence and produce no crossover.

[0258] Accordingly, a probability density function (PDF) can be determined to predict the population of crossover events that are likely to occur during each step in a ligation reaction given a set number of parental variants, a number of oligonucleotides corresponding to each variant, and the concentrations of each variant during each step in the ligation reaction. The statistics and mathematics behind determining the PDF is described below. By utilizing these methods, one can calculate such a probability density function, and thus enrich the chimeric progeny population for a predetermined number of crossover events resulting from a particular ligation reaction. Moreover, a target number of crossover events can be predetermined, and the system then programmed to calculate the starting quantities of each parental oligonucleotide during each step in the ligation reaction to result in a probability density function that centers on the predetermined number of crossover events. These methods are directed to the use of repeated cycles of reductive reassortment, recombination and selection that allow for the directed molecular evolution of a nucleic acid encoding a polypeptide through recombination. This system allows generation of a large population of evolved chimeric sequences, wherein the generated population is significantly enriched for sequences that have a predetermined number of crossover events. A crossover event is a point in a chimeric sequence where a shift in sequence occurs from one parental variant to another parental variant. Such a point is normally at the juncture of where oligonucleotides from two parents are ligated together to form a single sequence. The method allows calculation of the correct concentrations of oligonucleotide sequences so that the final chimeric population of sequences is enriched for the chosen number of crossover events. This provides more control over choosing chimeric variants having a predetermined number of crossover events.

[0259] In addition, these methods provide a convenient means for exploring a tremendous amount of the possible protein variant space in comparison to other systems. By using the methods described herein, the population of chimeric molecules can be enriched for those variants that have a particular number of crossover events. Thus, although one can still generate 10^{13} chimeric molecules during a reaction, each of the molecules chosen for further analysis most likely has, for example, only three crossover events. Because the resulting progeny population can be skewed to have a predetermined number of crossover events, the boundaries on the functional variety between the chimeric molecules is reduced. This provides a more manageable number of variables when calculating which oligonucleotide from the original parental polynucleotides might be responsible for affecting a particular trait.

[0260] In one aspect, the method creates a chimeric progeny polynucleotide sequence by creating oligonucleotides corresponding to fragments or portions of each parental sequence. Each oligonucleotide preferably includes a unique region of overlap so that mixing the oligonucleotides together results in a new variant that has each oligonucleotide fragment assembled in the correct order. See also USSN 09/332,835.

Determining Crossover Events

[0261] A system and software may receive a desired crossover probability density function (PDF), the number of parent genes to be reassembled, and the number of fragments in the reassembly as inputs. The output of this program is a "fragment PDF" that can be used to determine a recipe for producing reassembled genes, and the estimated crossover PDF of those genes. The processing described herein is preferably performed in MATLAB™ (The Mathworks, Natick, Massachusetts) a programming language and development environment for technical computing.

Iterative Processes

[0262] These processes can be iteratively repeated. For example, a nucleic acid (or, the nucleic acid) responsible for an altered or new pectate lyase phenotype is identified, re-isolated, again modified, re-tested for activity. This process can be iteratively repeated until a desired phenotype is engineered. For example, an entire biochemical anabolic or catabolic pathway can be engineered into a cell, including, e.g., epoxide hydrolysis activity.

[0263] Similarly, if it is determined that a particular oligonucleotide has no affect at all on the desired trait (e.g., a new pectate lyase phenotype), it can be removed as a variable by synthesizing larger parental oligonucleotides that include the sequence to be removed. Since incorporating the sequence within a larger sequence prevents any crossover events, there will no longer be any variation of this sequence in the progeny polynucleotides. This iterative practice of determining which oligonucleotides are most related to the desired trait, and which are unrelated, allows more efficient exploration all of the possible protein variants that might be provide a particular trait or activity.

In vivo shuffling

[0264] *In vivo* shuffling of molecules is use in methods that provide variants of polypeptides of the disclosure, e.g., antibodies, pectate lyases, and the like. *In vivo* shuffling can be performed utilizing the natural property of cells to recombine multimers. While recombination *in vivo* has provided the major natural route to molecular diversity, genetic recombination remains a relatively complex process that involves 1) the recognition of homologies; 2) strand cleavage, strand invasion, and metabolic steps leading to the production of recombinant chiasma; and finally 3) the resolution of

chiasma into discrete recombined molecules. The formation of the chiasma requires the recognition of homologous sequences.

[0265] In one aspect, this provides a method for producing a hybrid polynucleotide from at least a first polynucleotide (e.g., a pectate lyase of the disclosure) and a second polynucleotide (e.g., an enzyme, such as a pectate lyase of the disclosure or any other pectate lyase, or, a tag or an epitope). This can be used to produce a hybrid polynucleotide by introducing at least a first polynucleotide and a second polynucleotide which share at least one region of partial sequence homology into a suitable host cell. The regions of partial sequence homology promote processes which result in sequence reorganization producing a hybrid polynucleotide. The term "hybrid polynucleotide", as used herein, is any nucleotide sequence which results from the method and contains sequence from at least two original polynucleotide sequences. Such hybrid polynucleotides can result from intermolecular recombination events which promote sequence integration between DNA molecules. In addition, such hybrid polynucleotides can result from intramolecular reductive reassortment processes which utilize repeated sequences to alter a nucleotide sequence within a DNA molecule.

Producing sequence variants

[0266] The disclosure also provides additional methods for making sequence variants of the nucleic acid (e.g., pectate lyase) sequences. The disclosure also provides additional methods for isolating pectate lyases using the nucleic acids and polypeptides of the disclosure. In one aspect, this provides for variants of a pectate lyase coding sequence (e.g., a gene, cDNA or message), which can be altered by any means, including, e.g., random or stochastic methods, or, non-stochastic, or "directed evolution," methods, as described above.

[0267] The isolated variants may be naturally occurring. Variant can also be created *in vitro*. Variants may be created using genetic engineering techniques such as site directed mutagenesis, random chemical mutagenesis, Exonuclease III deletion procedures, and standard cloning techniques. Alternatively, such variants, fragments, analogs, or derivatives may be created using chemical synthesis or modification procedures. Other methods of making variants are also familiar to those skilled in the art. These include procedures in which nucleic acid sequences obtained from natural isolates are modified to generate nucleic acids which encode polypeptides having characteristics which enhance their value in industrial or laboratory applications. In such procedures, a large number of variant sequences having one or more nucleotide differences with respect to the sequence obtained from the natural isolate are generated and characterized. These nucleotide differences can result in amino acid changes with respect to the polypeptides encoded by the nucleic acids from the natural isolates.

[0268] For example, variants may be created using error prone PCR. In error prone PCR, PCR is performed under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. Error prone PCR is described, e.g., in Leung, D.W., et al., *Technique*, 1:11-15, 1989) and Caldwell, R. C. & Joyce G.F., *PCR Methods Applic.*, 2:28-33, 1992. Briefly, in such procedures, nucleic acids to be mutagenized are mixed with PCR primers, reaction buffer, $MgCl_2$, $MnCl_2$, Taq polymerase and an appropriate concentration of dNTPs for achieving a high rate of point mutation along the entire length of the PCR product. For example, the reaction may be performed using 20 fmoles of nucleic acid to be mutagenized, 30 pmole of each PCR primer, a reaction buffer comprising 50mM KCl, 10mM Tris HCl (pH 8.3) and 0.01% gelatin, 7mM $MgCl_2$, 0.5mM $MnCl_2$, 5 units of Taq polymerase, 0.2mM dGTP, 0.2mM dATP, 1mM dCTP, and 1mM dTTP. PCR may be performed for 30 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min. However, it will be appreciated that these parameters may be varied as appropriate. The mutagenized nucleic acids are cloned into an appropriate vector and the activities of the polypeptides encoded by the mutagenized nucleic acids is evaluated.

[0269] Variants may also be created using oligonucleotide directed mutagenesis to generate site-specific mutations in any cloned DNA of interest. Oligonucleotide mutagenesis is described, e.g., in Reidhaar-Olson (1988) *Science* 241:53-57. Briefly, in such procedures a plurality of double stranded oligonucleotides bearing one or more mutations to be introduced into the cloned DNA are synthesized and inserted into the cloned DNA to be mutagenized. Clones containing the mutagenized DNA are recovered and the activities of the polypeptides they encode are assessed.

[0270] Another method for generating variants is assembly PCR. Assembly PCR involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions occur in parallel in the same vial, with the products of one reaction priming the products of another reaction. Assembly PCR is described in, e.g., U.S. Patent No. 5,965,408.

[0271] Still another method of generating variants is sexual PCR mutagenesis. In sexual PCR mutagenesis, forced homologous recombination occurs between DNA molecules of different but highly related DNA sequence *in vitro*, as a result of random fragmentation of the DNA molecule based on sequence homology, followed by fixation of the crossover by primer extension in a PCR reaction. Sexual PCR mutagenesis is described, e.g., in Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751. Briefly, in such procedures a plurality of nucleic acids to be recombined are digested with DNase to generate fragments having an average size of 50-200 nucleotides. Fragments of the desired average size are purified and resuspended in a PCR mixture. PCR is conducted under conditions which facilitate recombination between

the nucleic acid fragments. For example, PCR may be performed by resuspending the purified fragments at a concentration of 10-30ng/μl in a solution of 0.2mM of each dNTP, 2.2mM MgCl₂, 50mM KCL, 10mM Tris HCl, pH 9.0, and 0.1% Triton X-100. 2.5 units of Taq polymerase per 100μl of reaction mixture is added and PCR is performed using the following regime: 94°C for 60 seconds, 94°C for 30 seconds, 50-55°C for 30 seconds, 72°C for 30 seconds (30-45 times) and 72°C for 5 minutes. However, it will be appreciated that these parameters may be varied as appropriate. In some aspects, oligonucleotides may be included in the PCR reactions. In other aspects, the Klenow fragment of DNA polymerase I may be used in a first set of PCR reactions and Taq polymerase may be used in a subsequent set of PCR reactions. Recombinant sequences are isolated and the activities of the polypeptides they encode are assessed.

[0272] Variants may also be created by *in vivo* mutagenesis. In some aspects, random mutations in a sequence of interest are generated by propagating the sequence of interest in a bacterial strain, such as an *E. coli* strain, which carries mutations in one or more of the DNA repair pathways. Such "mutator" strains have a higher random mutation rate than that of a wild-type parent. Propagating the DNA in one of these strains will eventually generate random mutations within the DNA. Mutator strains suitable for use for *in vivo* mutagenesis are described, e.g., in PCT Publication No. WO 91/16427.

[0273] Variants may also be generated using cassette mutagenesis. In cassette mutagenesis a small region of a double stranded DNA molecule is replaced with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains completely and/or partially randomized native sequence.

[0274] Recursive ensemble mutagenesis may also be used to generate variants. Recursive ensemble mutagenesis is an algorithm for protein engineering (protein mutagenesis) developed to produce diverse populations of phenotypically related mutants whose members differ in amino acid sequence. This method uses a feedback mechanism to control successive rounds of combinatorial cassette mutagenesis. Recursive ensemble mutagenesis is described, e.g., in Arkin (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815.

[0275] In some aspects, variants are created using exponential ensemble mutagenesis. Exponential ensemble mutagenesis is a process for generating combinatorial libraries with a high percentage of unique and functional mutants, wherein small groups of residues are randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. Exponential ensemble mutagenesis is described, e.g., in Delegrave (1993) Biotechnology Res. 11:1548-1552. Random and site-directed mutagenesis are described, e.g., in Arnold (1993) Current Opinion in Biotechnology 4:450-455.

[0276] In some aspects, the variants are created using shuffling procedures wherein portions of a plurality of nucleic acids which encode distinct polypeptides are fused together to create chimeric nucleic acid sequences which encode chimeric polypeptides as described in, e.g., U.S. Patent Nos. 5,965,408; 5,939,250 (see also discussion, above).

[0277] Variants of polypeptides of the disclosure (e.g., pectate lyases) may comprise sequences in which one or more of the amino acid residues (e.g., of an exemplary polypeptide) are substituted with a conserved or non-conserved amino acid residue (e.g., a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Conservative substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Thus, polypeptides of the disclosure include those with conservative substitutions of sequences, e.g., the exemplary polypeptides of the disclosure, including but not limited to the following replacements: replacements of an aliphatic amino acid such as Alanine, Valine, Leucine and Isoleucine with another aliphatic amino acid; replacement of a Serine with a Threonine or vice versa; replacement of an acidic residue such as Aspartic acid and Glutamic acid with another acidic residue; replacement of a residue bearing an amide group, such as Asparagine and Glutamine, with another residue bearing an amide group; exchange of a basic residue such as Lysine and Arginine with another basic residue; and replacement of an aromatic residue such as Phenylalanine, Tyrosine with another aromatic residue. Other variants are those in which one or more of the amino acid residues of the polypeptides includes a substituent group.

[0278] Other variants within the desired scope are those in which the polypeptide is associated with another compound, such as a compound to increase the half-life of the polypeptide, for example, polyethylene glycol.

[0279] Additional variants are those in which additional amino acids are fused to the polypeptide, such as a leader sequence, a secretory sequence, a proprotein sequence or a sequence which facilitates purification, enrichment, or stabilization of the polypeptide.

[0280] In some aspects, the variants, fragments, derivatives and analogs of the polypeptides retain the same biological function or activity as the exemplary polypeptides, e.g., pectate lyase activity, as described herein. In other aspects, the variant, fragment, derivatives, or analog includes a proprotein, such that the variant, fragment, derivative, or analog can be activated by cleavage of the proprotein portion to produce an active polypeptide.

Optimizing codons to achieve high levels of protein expression in host cells

[0281] Methods may modify pectate lyase-encoding nucleic acids to modify codon usage. In one aspect, this provides methods for modifying codons in a nucleic acid encoding a pectate lyase to increase or decrease its expression in a

host cell. This also provides nucleic acids encoding a pectate lyase modified to increase its expression in a host cell, pectate lyase so modified, and methods of making the modified pectate lyases. The method comprises identifying a "non-preferred" or a "less preferred" codon in pectate lyase-encoding nucleic acid and replacing one or more of these non-preferred or less preferred codons with a "preferred codon" encoding the same amino acid as the replaced codon and at least one non-preferred or less preferred codon in the nucleic acid has been replaced by a preferred codon encoding the same amino acid. A preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell.

[0282] Host cells for expressing the nucleic acids, expression cassettes and vectors include bacteria, yeast, fungi, plant cells, insect cells and mammalian cells. Thus, this provides methods for optimizing codon usage in all of these cells, codon-altered nucleic acids and polypeptides made by the codon-altered nucleic acids. Exemplary host cells include gram negative bacteria, such as *Escherichia coli*; gram positive bacteria, such as any *Streptomyces*, *Lactobacillus gasser*, *Lactococcus lactis*, *Lactococcus cremoris*, any *Bacillus*, e.g., *Bacillus subtilis*, *Bacillus cereus*. Exemplary host cells also include eukaryotic organisms, e.g., various yeast, such as *Saccharomyces* sp., including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Pichia pastoris*, and *Kluyveromyces lactis*, *Hansenula polymorpha*, *Aspergillus niger*, and mammalian cells and cell lines and insect cells and cell lines. Thus, this also includes nucleic acids and polypeptides optimized for expression in these organisms and species.

[0283] For example, the codons of a nucleic acid encoding a pectate lyase isolated from a bacterial cell are modified such that the nucleic acid is optimally expressed in a bacterial cell different from the bacteria from which the pectate lyase was derived, a yeast, a plant cell, an insect cell or a mammalian cell. Methods for optimizing codons are well known in the art, see, e.g., U.S. Patent No. 5,795,737; Baca (2000) Int. J. Parasitol. 30:113-118; Hale (1998) Protein Expr. Purif. 12:185-188; Narum (2001) Infect. Immun. 69:7250-7253. See also Narum (2001) Infect. Immun. 69:7250-7253, describing optimizing codons in mouse systems; Outchkourov (2002) Protein Expr. Purif. 24:18-24, describing optimizing codons in yeast; Feng (2000) Biochemistry 39:15399-15409, describing optimizing codons in *E. coli*; Humphreys (2000) Protein Expr. Purif. 20:252-264, describing optimizing codon usage that affects secretion in *E. coli*.

Synthetic gene reassembly

[0284] A non-stochastic method termed synthetic gene reassembly (e.g., GeneReassembly™, see, e.g., U.S. Patent No. 6,537,776) may be for, e.g., modifying pectate lyases of the disclosure or building new pectate lyases within the scope of the disclosure. GeneReassembly™ differs from stochastic shuffling in that the nucleic acid building blocks are not shuffled or concatenated or chimerized randomly, but rather are assembled non-stochastically.

[0285] The synthetic gene reassembly method does not depend on the presence of a high level of homology between polynucleotides to be shuffled. This can be used to non-stochastically generate libraries (or sets) of progeny molecules comprised of over 10¹⁰⁰ different chimeras. Conceivably, synthetic gene reassembly can even be used to generate libraries comprised of over 10¹⁰⁰⁰ different progeny chimeras.

[0286] Thus, this provides a non-stochastic method of producing a set of finalized chimeric nucleic acid molecules having an overall assembly order that is chosen by design, which method is comprised of the steps of generating by design a plurality of specific nucleic acid building blocks having serviceable mutually compatible ligatable ends and assembling these nucleic acid building blocks, such that a designed overall assembly order is achieved.

[0287] In one aspect, synthetic gene reassembly comprises a method of: 1) preparing a progeny generation of molecule(s) (including a molecule comprising a polynucleotide sequence, e.g., a molecule comprising a polypeptide coding sequence), that is mutagenized to achieve at least one point mutation, addition, deletion, and/or chimerization, from one or more ancestral or parental generation template(s); 2) screening the progeny generation molecule(s), e.g., using a high throughput method, for at least one property of interest (such as an improvement in an enzyme activity); 3) optionally obtaining and/or cataloguing structural and/or functional information regarding the parental and/or progeny generation molecules; and 4) optionally repeating any of steps 1) to 3). In one aspect, there is generated (e.g., from a parent polynucleotide template), in what is termed "codon site-saturation mutagenesis," a progeny generation of polynucleotides, each having at least one set of up to three contiguous point mutations (i.e. different bases comprising a new codon), such that every codon (or every family of degenerate codons encoding the same amino acid) is represented at each codon position. Corresponding to, and encoded by, this progeny generation of polynucleotides, there is also generated a set of progeny polypeptides, each having at least one single amino acid point mutation. In a one aspect, there is generated, in what is termed "amino acid site-saturation mutagenesis", one such mutant polypeptide for each of the 19 naturally encoded polypeptide-forming alpha-amino acid substitutions at each and every amino acid position along the polypeptide. This yields, for each and every amino acid position along the parental polypeptide, a total of 20 distinct progeny polypeptides including the original amino acid, or potentially more than 21 distinct progeny polypeptides if additional amino acids are used either instead of or in addition to the 20 naturally encoded amino acids

[0288] Thus, in another aspect, this approach is also serviceable for generating mutants containing, in addition to

and/or in combination with the 20 naturally encoded polypeptide-forming alpha-amino acids, other rare and/or not naturally-encoded amino acids and amino acid derivatives. In yet another aspect, this approach is also serviceable for generating mutants by the use of, in addition to and/or in combination with natural or unaltered codon recognition systems of suitable hosts, altered, mutagenized, and/or designer codon recognition systems (such as in a host cell with one or more altered tRNA molecules.

[0289] In yet another aspect, this relates to recombination and more specifically to a method for preparing polynucleotides encoding a polypeptide by a method of *in vivo* reassortment of polynucleotide sequences containing regions of partial homology, assembling the polynucleotides to form at least one polynucleotide and screening the polynucleotides for the production of polypeptide(s) having a useful property.

[0290] In yet another aspect, this is serviceable for analyzing and cataloguing, with respect to any molecular property (e.g. an enzymatic activity) or combination of properties allowed by current technology, the effects of any mutational change achieved (including particularly saturation mutagenesis). Thus, a comprehensive method is provided for determining the effect of changing each amino acid in a parental polypeptide into each of at least 19 possible substitutions. This allows each amino acid in a parental polypeptide to be characterized and catalogued according to its spectrum of potential effects on a measurable property of the polypeptide.

[0291] In one aspect, an intron may be introduced into a chimeric progeny molecule by way of a nucleic acid building block. Introns often have consensus sequences at both termini in order to render them operational. In addition to enabling gene splicing, introns may serve an additional purpose by providing sites of homology to other nucleic acids to enable homologous recombination. For this purpose, and potentially others, it may be sometimes desirable to generate a large nucleic acid building block for introducing an intron. If the size is overly large easily generating by direct chemical synthesis of two single stranded oligos, such a specialized nucleic acid building block may also be generated by direct chemical synthesis of more than two single stranded oligos or by using a polymerase-based amplification reaction

[0292] The mutually compatible ligatable ends of the nucleic acid building blocks to be assembled are considered to be "serviceable" for this type of ordered assembly if they enable the building blocks to be coupled in predetermined orders. Thus, in one aspect, the overall assembly order in which the nucleic acid building blocks can be coupled is specified by the design of the ligatable ends and, if more than one assembly step is to be used, then the overall assembly order in which the nucleic acid building blocks can be coupled is also specified by the sequential order of the assembly step(s). In a one aspect, the annealed building pieces are treated with an enzyme, such as a ligase (e.g., T4 DNA ligase) to achieve covalent bonding of the building pieces.

[0293] Coupling can occur in a manner that does not make use of every nucleotide in a participating overhang. The coupling is particularly lively to survive (e.g. in a transformed host) if the coupling reinforced by treatment with a ligase enzyme to form what may be referred to as a "gap ligation" or a "gapped ligation". This type of coupling can contribute to generation of unwanted background product(s), but it can also be used advantageously increase the diversity of the progeny library generated by the designed ligation reassembly. Certain overhangs are able to undergo self-coupling to form a palindromic coupling. A coupling is strengthened substantially if it is reinforced by treatment with a ligase enzyme. Lack of 5' phosphates on these overhangs can be used advantageously to prevent this type of palindromic self-ligation. Accordingly, this provides that nucleic acid building blocks can be chemically made (or ordered) that lack a 5' phosphate group. Alternatively, they can be removed, e.g. by treatment with a phosphatase enzyme, such as a calf intestinal alkaline phosphatase (CIAP), in order to prevent palindromic self-ligations in ligation reassembly processes.

Transgenic non-human animals

[0294] Transgenic non-human animals may comprise a nucleic acid, a polypeptide (e.g., a pectate lyase), an expression cassette or vector or a transfected or transformed cell of the disclosure. The disclosure also provides methods of making and using these transgenic non-human animals.

[0295] The transgenic non-human animals can be, e.g., goats, rabbits, sheep, pigs, cows, rats and mice, comprising the nucleic acids of the disclosure. These animals can be used, e.g., as *in vivo* models to study pectate lyase activity, or, as models to screen for agents that change the pectate lyase activity *in vivo*. The coding sequences for the polypeptides to be expressed in the transgenic non-human animals can be designed to be constitutive, or, under the control of tissue-specific, developmental-specific or inducible transcriptional regulatory factors. Transgenic non-human animals can be designed and generated using any method known in the art; see, e.g., U.S. Patent Nos. 6,211,428; 6,187,992; 6,156,952; 6,118,044; 6,111,166; 6,107,541; 5,959,171; 5,922,854; 5,892,070; 5,880,327; 5,891,698; 5,639,940; 5,573,933; 5,387,742; 5,087,571, describing making and using transformed cells and eggs and transgenic mice, rats, rabbits, sheep, pigs and cows. See also, e.g., Pollock (1999) J. Immunol. Methods 231:147-157, describing the production of recombinant proteins in the milk of transgenic dairy animals; Baguisi (1999) Nat. Biotechnol. 17:456-461, demonstrating the production of transgenic goats. U.S. Patent No. 6,211,428, describes making and using transgenic non-human mammals which express in their brains a nucleic acid construct comprising a DNA sequence. U.S. Patent No. 5,387,742, describes injecting cloned recombinant or synthetic DNA sequences into fertilized mouse eggs, implanting the injected eggs in

pseudo-pregnant females, and growing to term transgenic mice whose cells express proteins related to the pathology of Alzheimer's disease. U.S. Patent No. 6,187,992, describes making and using a transgenic mouse whose genome comprises a disruption of the gene encoding amyloid precursor protein (APP).

[0296] "Knockout animals" can also be used to practice the methods of the disclosure. For example, in one aspect, the transgenic or modified animals comprise a "knockout animal," e.g., a "knockout mouse," engineered not to express an endogenous gene, which is replaced with a gene expressing a pectate lyase of the disclosure, or, a fusion protein comprising a pectate lyase of the disclosure.

Transgenic Plants and Seeds

[0297] The disclosure provides transgenic plants and seeds comprising a nucleic acid, a polypeptide (e.g., a pectate lyase), an expression cassette or vector or a transfected or transformed cell of the disclosure. The disclosure also provides plant products, e.g., oils, seeds, leaves, extracts and the like, comprising a nucleic acid and/or a polypeptide (e.g., a pectate lyase) of the disclosure. The transgenic plant can be dicotyledonous (a dicot) or monocotyledonous (a monocot). The disclosure also provides methods of making and using these transgenic plants and seeds. The transgenic plant or plant cell expressing a polypeptide of the present disclosure may be constructed in accordance with any method known in the art. See, for example, U.S. Patent No. 6,309,872.

[0298] Nucleic acids and expression constructs of the disclosure can be introduced into a plant cell by any means. For example, nucleic acids or expression constructs can be introduced into the genome of a desired plant host, or, the nucleic acids or expression constructs can be episomes. Introduction into the genome of a desired plant can be such that the host's pectate lyase production is regulated by endogenous transcriptional or translational control elements. This also provides "knockout plants" where insertion of gene sequence by, e.g., homologous recombination, has disrupted the expression of the endogenous gene. Means to generate "knockout" plants are well-known in the art, see, e.g., Strepp (1998) Proc Natl. Acad. Sci. USA 95:4368-4373; Miao (1995) Plant J 7:359-365. See discussion on transgenic plants, below.

[0299] The nucleic acids of the disclosure can be used to confer desired traits on essentially any plant, e.g., on starch-producing plants, such as potato, wheat, rice, barley, and the like. Nucleic acids of the disclosure can be used to manipulate metabolic pathways of a plant in order to optimize or alter host's expression of pectate lyase. The can change pectate lyase activity in a plant. Alternatively, a pectate lyase of the disclosure can be used in production of a transgenic plant to produce a compound not naturally produced by that plant. This can lower production costs or create a novel product.

[0300] In one aspect, the first step in production of a transgenic plant involves making an expression construct for expression in a plant cell. These techniques are well known in the art. They can include selecting and cloning a promoter, a coding sequence for facilitating efficient binding of ribosomes to mRNA and selecting the appropriate gene terminator sequences. One exemplary constitutive promoter is CaMV35S, from the cauliflower mosaic virus, which generally results in a high degree of expression in plants. Other promoters are more specific and respond to cues in the plant's internal or external environment. An exemplary light-inducible promoter is the promoter from the cab gene, encoding the major chlorophyll a/b binding protein.

[0301] In one aspect, the nucleic acid is modified to achieve greater expression in a plant cell. For example, a sequence of the disclosure is likely to have a higher percentage of A-T nucleotide pairs compared to that seen in a plant, some of which prefer G-C nucleotide pairs. Therefore, A-T nucleotides in the coding sequence can be substituted with G-C nucleotides without significantly changing the amino acid sequence to enhance production of the gene product in plant cells.

[0302] Selectable marker gene can be added to the gene construct in order to identify plant cells or tissues that have successfully integrated the transgene. This may be necessary because achieving incorporation and expression of genes in plant cells is a rare event, occurring in just a few percent of the targeted tissues or cells. Selectable marker genes encode proteins that provide resistance to agents that are normally toxic to plants, such as antibiotics or herbicides. Only plant cells that have integrated the selectable marker gene will survive when grown on a medium containing the appropriate antibiotic or herbicide. As for other inserted genes, marker genes also require promoter and termination sequences for proper function.

[0303] In one aspect, making transgenic plants or seeds comprises incorporating sequences of the disclosure and, optionally, marker genes into a target expression construct (e.g., a plasmid), along with positioning of the promoter and the terminator sequences. This can involve transferring the modified gene into the plant through a suitable method. For example, a construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. For example, see, e.g., Christou (1997) Plant Mol. Biol. 35:197-203; Pawlowski (1996) Mol. Biotechnol. 6:17-30; Klein (1987) Nature 327:70-73; Takumi (1997) Genes Genet. Syst. 72:63-69, discussing use of particle bombardment to introduce transgenes into wheat; and Adam (1997) supra,

for use of particle bombardment to introduce YACs into plant cells. For example, Rinehart (1997) supra, used particle bombardment to generate transgenic cotton plants. Apparatus for accelerating particles is described U.S. Pat. No. 5,015,580; and, the commercially available BioRad (Biolistics) PDS-2000 particle acceleration instrument; see also, John, U.S. Patent No. 5,608,148; and Ellis, U.S. Patent No. 5,681,730, describing particle-mediated transformation of gymnosperms.

[0304] In one aspect, protoplasts can be immobilized and injected with a nucleic acids, e.g., an expression construct. Although plant regeneration from protoplasts is not easy with cereals, plant regeneration is possible in legumes using somatic embryogenesis from protoplast derived callus. Organized tissues can be transformed with naked DNA using gene gun technique, where DNA is coated on tungsten microprojectiles, shot 1/100th the size of cells, which carry the DNA deep into cells and organelles. Transformed tissue is then induced to regenerate, usually by somatic embryogenesis. This technique has been successful in several cereal species including maize and rice.

[0305] Nucleic acids, e.g., expression constructs, can also be introduced in to plant cells using recombinant viruses. Plant cells can be transformed using viral vectors, such as, e.g., tobacco mosaic virus derived vectors (Rouwendal (1997) Plant Mol. Biol. 33:989-999), see Porta (1996) "Use of viral replicons for the expression of genes in plants," Mol. Biotechnol. 5:209-221.

[0306] Alternatively, nucleic acids, e.g., an expression construct, can be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. *Agrobacterium tumefaciens*-mediated transformation techniques, including disarming and use of binary vectors, are well described in the scientific literature. See, e.g., Horsch (1984) Science 233:496-498; Fraley (1983) Proc. Natl. Acad. Sci. USA 80:4803 (1983); Gene Transfer to Plants, Potrykus, ed. (Springer-Verlag, Berlin 1995). The DNA in an *A. tumefaciens* cell is contained in the bacterial chromosome as well as in another structure known as a Ti (tumor-inducing) plasmid. The Ti plasmid contains a stretch of DNA termed T-DNA (~20 kb long) that is transferred to the plant cell in the infection process and a series of vir (virulence) genes that direct the infection process. *A. tumefaciens* can only infect a plant through wounds: when a plant root or stem is wounded it gives off certain chemical signals, in response to which, the vir genes of *A. tumefaciens* become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant's chromosome. The T-DNA then enters the plant cell through the wound. One speculation is that the T-DNA waits until the plant DNA is being replicated or transcribed, then inserts itself into the exposed plant DNA. In order to use *A. tumefaciens* as a transgene vector, the tumor-inducing section of T-DNA have to be removed, while retaining the T-DNA border regions and the vir genes. The transgene is then inserted between the T-DNA border regions, where it is transferred to the plant cell and becomes integrated into the plant's chromosomes.

[0307] The disclosure provides for the transformation of monocotyledonous plants using the nucleic acids of the disclosure, including important cereals, see Hiei (1997) Plant Mol. Biol. 35:205-218. See also, e.g., Horsch, Science (1984) 233:496; Fraley (1983) Proc. Natl. Acad. Sci. USA 80:4803; Thykjaer (1997) supra; Park (1996) Plant Mol. Biol. 32:1135-1148, discussing T-DNA integration into genomic DNA. See also D'Halluin, U.S. Patent No. 5,712,135, describing a process for the stable integration of a DNA comprising a gene that is functional in a cell of a cereal, or other monocotyledonous plant.

[0308] In one aspect, the third step can involve selection and regeneration of whole plants capable of transmitting the incorporated target gene to the next generation. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker that has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp. 124-176, MacMillan Publishing Company, New York, 1983; and Binding, Regeneration of Plants, Plant Protoplasts, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee (1987) Ann. Rev. of Plant Phys. 38:467-486. To obtain whole plants from transgenic tissues such as immature embryos, they can be grown under controlled environmental conditions in a series of media containing nutrients and hormones, a process known as tissue culture. Once whole plants are generated and produce seed, evaluation of the progeny begins.

[0309] After the expression cassette is stably incorporated in transgenic plants, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed. Since transgenic expression of the nucleic acids leads to phenotypic changes, plants comprising the recombinant nucleic acids of the disclosure can be sexually crossed with a second plant to obtain a final product. Thus, the seed can be derived from a cross between two transgenic plants, or a cross between a plant of the disclosure and another plant. The desired effects (e.g., expression of the polypeptides of the disclosure to produce a plant in which flowering behavior is altered) can be enhanced when both parental plants express the polypeptides (e.g., a pectate lyase) of the disclosure. The desired effects can be passed to future plant generations by standard propagation means.

[0310] The nucleic acids and polypeptides of the disclosure are expressed in or inserted in any plant or seed. Transgenic plants can be dicotyledonous or monocotyledonous. Examples of monocot transgenic plants are grasses, such as

meadow grass (blue grass, *Poa*), forage grass such as festuca, lolium, temperate grass, such as *Agrostis*, and cereals, e.g., wheat, oats, rye, barley, rice, sorghum, and maize (corn). Examples of dicot transgenic plants are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous plants (family *Brassicaceae*), such as cauliflower, rape seed, and the closely related model organism *Arabidopsis thaliana*. Thus, the transgenic plants and seeds include a broad range of plants, including, but not limited to, species from the genera *Anacardium*, *Arachis*, *Asparagus*, *Atropa*, *Avena*, *Brassica*, *Citrus*, *Citrullus*, *Capsicum*, *Carthamus*, *Cocos*, *Coffea*, *Cucumis*, *Cucurbita*, *Daucus*, *Elaeis*, *Fragaria*, *Glycine*, *Gossypium*, *Helianthus*, *Heterocallis*, *Hordeum*, *Hyoscyamus*, *Lactuca*, *Linum*, *Lolium*, *Lupinus*, *Lycopersicon*, *Malus*, *Manihot*, *Majorana*, *Medicago*, *Nicotiana*, *Olea*, *Oryza*, *Panieum*, *Pannisetum*, *Persea*, *Phaseolus*, *Pistachia*, *Pisum*, *Pyrus*, *Prunus*, *Raphanus*, *Ricinus*, *Secale*, *Senecio*, *Sinapis*, *Solanum*, *Sorghum*, *Theobromus*, *Trigonella*, *Triticum*, *Vicia*, *Vitis*, *Vigna*, and *Zea*.

[0311] In alternative embodiments, the nucleic acids are expressed in plants which contain fiber cells, including, e.g., cotton, silk cotton tree (Kapok, *Ceiba pentandra*), desert willow, creosote bush, winterfat, balsa, ramie, kenaf, hemp, roselle, jute, sisal abaca and flax. In alternative embodiments, the transgenic plants can be members of the genus *Gossypium*, including members of any *Gossypium* species, such as *G. arboreum*, *G. herbaceum*, *G. barbadense*, and *G. hirsutum*.

[0312] The disclosure also provides for transgenic plants to be used for producing large amounts of the polypeptides (e.g., a pectate lyase or antibody) of the disclosure. For example, see Palmgren (1997) Trends Genet. 13:348; Chong (1997) Transgenic Res. 6:289-296 (producing human milk protein beta-casein in transgenic potato plants using an auxin-inducible, bidirectional mannopine synthase (*mas1',2'*) promoter with *Agrobacterium tumefaciens*-mediated leaf disc transformation methods).

[0313] Using known procedures, one of skill can screen for plants of the disclosure by detecting the increase or decrease of transgene mRNA or protein in transgenic plants. Means for detecting and quantitation of mRNAs or proteins are well known in the art.

Polypeptides and peptides

[0314] In one aspect, the disclosure provides isolated or recombinant polypeptides having a sequence identity (e.g., at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity) to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134. In one aspect, the polypeptide has a pectate lyase (e.g., pectinase) activity.

[0315] The identity can be over the full length of the polypeptide, or, the identity can be over a region of at least about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700 or more residues. Polypeptides can also be shorter than the full length of exemplary polypeptides. In alternative aspects, this provides polypeptides (peptides, fragments) ranging in size between about 5 and the full length of a polypeptide, e.g., an enzyme, such as a pectate lyase; exemplary sizes being of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, or more residues, e.g., contiguous residues of an exemplary pectate lyase. Peptides can be useful as, e.g., labeling probes, antigens, toleragens, motifs, pectate lyase active sites, carbohydrate binding domains, and the like. Polypeptides also include antibodies capable of binding to an enzyme of the disclosure.

[0316] The polypeptides include pectate lyases in an active or inactive form. For example, the polypeptides include proproteins before "maturation" or processing of prepro sequences, e.g., by a proprotein-processing enzyme, such as a proprotein convertase to generate an "active" mature protein. The polypeptides include pectate lyases inactive for other reasons, e.g., before "activation" by a post-translational processing event, e.g., an endo- or exo-peptidase or proteinase action, a phosphorylation event, an amidation, a glycosylation or a sulfation, a dimerization event, and the like. Methods for identifying "prepro" domain sequences and signal sequences are well known in the art, see, e.g., Van de Ven (1993) Crit. Rev. Oncog. 4(2):115-136. For example, to identify a prepro sequence, the protein is purified from the extracellular space and the N-terminal protein sequence is determined and compared to the unprocessed form.

[0317] The polypeptides include all active forms, including active subsequences, e.g., catalytic domains or active sites,

of an enzyme of the disclosure. In one aspect, this provides catalytic domains or active sites as set forth below. In one aspect, a peptide or polypeptide comprises or consists of an active site domain as predicted through use of a database such as Pfam (which is a large collection of multiple sequence alignments and hidden Markov models covering many common protein families, The Pfam protein families database, A. Bateman, E. Birney, L. Cerruti, R. Durbin, L. Ewinger, S.R. Eddy, S. Griffiths-Jones, K.L. Howe, M. Marshall, and E.L.L. Sonnhammer, Nucleic Acids Research, 30(1):276-280, 2002) or equivalent.

[0318] The disclosure includes polypeptides with or without a signal sequence and/or a prepro sequence. The disclosure includes polypeptides with heterologous signal sequences and/or prepro sequences. The prepro sequence (including a sequence used as a heterologous prepro domain) can be located on the amino terminal or the carboxy terminal end of the protein. The disclosure also includes isolated or recombinant signal sequences, prepro sequences and catalytic domains (e.g., "active sites") comprising sequences of the disclosure.

[0319] Polypeptides and peptides can be isolated from natural sources, be synthetic, or be recombinantly generated polypeptides. Peptides and proteins can be recombinantly expressed *in vitro* or *in vivo*. The peptides and polypeptides can be made and isolated using any method known in the art. Polypeptide and peptides can also be synthesized, whole or in part, using chemical methods well known in the art. See e.g., Caruthers (1980) Nucleic Acids Res. Symp. Ser. 215-223; Horn (1980) Nucleic Acids Res. Symp. Ser. 225-232; Banga, A.K., Therapeutic Peptides and Proteins, Formulation, Processing and Delivery Systems (1995) Technomic Publishing Co., Lancaster, PA. For example, peptide synthesis can be performed using various solid-phase techniques (see e.g., Roberge (1995) Science 269:202; Merrifield (1997) Methods Enzymol. 289:3-13) and automated synthesis may be achieved, e.g., using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

[0320] The peptides and polypeptides can also be glycosylated. The glycosylation can be added post-translationally either chemically or by cellular biosynthetic mechanisms, wherein the later incorporates the use of known glycosylation motifs, which can be native to the sequence or can be added as a peptide or added in the nucleic acid coding sequence. The glycosylation can be O-linked or N-linked.

[0321] The peptides and polypeptides, as defined above, include all "mimetic" and "peptidomimetic" forms. The terms "mimetic" and "peptidomimetic" refer to a synthetic chemical compound which has substantially the same structural and/or functional characteristics of the polypeptides of the disclosure. The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. As with polypeptides of the disclosure which are conservative variants, routine experimentation will determine whether a mimetic is within the desired scope, i.e., that its structure and/or function is not substantially altered. Thus, in one aspect, a mimetic composition is within the desired scope if it has a pectate lyase activity.

[0322] Polypeptide mimetic compositions can contain any combination of non-natural structural components. In alternative aspect, mimetic compositions include one or all of the following three structural groups: a) residue linkage groups other than the natural amide bond ("peptide bond") linkages; b) non-natural residues in place of naturally occurring amino acid residues; or c) residues which induce secondary structural mimicry, i.e., to induce or stabilize a secondary structure, e.g., a beta turn, gamma turn, beta sheet, alpha helix conformation, and the like. For example, a polypeptide can be characterized as a mimetic when all or some of its residues are joined by chemical means other than natural peptide bonds. Individual peptidomimetic residues can be joined by peptide bonds, other chemical bonds or coupling means, such as, e.g., glutaraldehyde, N-hydroxysuccinimide esters, bifunctional maleimides, N,N'-dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIC). Linking groups that can be an alternative to the traditional amide bond ("peptide bond") linkages include, e.g., ketomethylene (e.g., -C(=O)-CH₂- for -C(=O)-NH-), aminomethylene (CH₂-NH), ethylene, olefin (CH=CH), ether (CH₂-O), thioether (CH₂-S), tetrazole (CN₄-), thiazole, retroamide, thioamide, or ester (see, e.g., Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, "Peptide Backbone Modifications," Marcell Dekker, NY).

[0323] A polypeptide can also be characterized as a mimetic by containing all or some non-natural residues in place of naturally occurring amino acid residues. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L- naphthylalanine; D- or L- phenylglycine; D- or L-2 thienylalanine; D- or L-1, -2, 3-, or 4- pyrenylalanine; D- or L-3 thienylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine; D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; D- or L-p-methoxy-biphenylphenylalanine; D- or L-2-indole(alkyl)alanines; and, D- or L-alkylalanines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

[0324] Mimetics of acidic amino acids can be generated by substitution by, e.g., non-carboxylate amino acids while

maintaining a negative charge; (phosphono)alanine; sulfated threonine. Carboxyl side groups (e.g., aspartyl or glutamyl) can also be selectively modified by reaction with carbodiimides (R'-N-C-N-R') such as, e.g., 1-cyclohexyl-3(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3(4-azonia-4,4- dimethylpentyl) carbodiimide. Aspartyl or glutamyl can also be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions. Mimetics of basic amino acids can be generated by substitution with, e.g., (in addition to lysine and arginine) the amino acids ornithine, citrulline, or (guanidino)-acetic acid, or (guanidino)alkyl-acetic acid, where alkyl is defined above. Nitrile derivative (e.g., containing the CN-moiety in place of COOH) can be substituted for asparagine or glutamine. Asparaginyl and glutaminyl residues can be deaminated to the corresponding aspartyl or glutamyl residues. Arginine residue mimetics can be generated by reacting arginyl with, e.g., one or more conventional reagents, including, e.g., phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, or ninhydrin, preferably under alkaline conditions. Tyrosine residue mimetics can be generated by reacting tyrosyl with, e.g., aromatic diazonium compounds or tetranitromethane. N-acetylimidizol and tetranitromethane can be used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Cysteine residue mimetics can be generated by reacting cysteinyl residues with, e.g., alpha-haloacetates such as 2-chloroacetic acid or chloroacetamide and corresponding amines; to give carboxymethyl or carboxyamidomethyl derivatives. Cysteine residue mimetics can also be generated by reacting cysteinyl residues with, e.g., bromo-trifluoroacetone, alpha-bromo-beta-(5-imidazolyl) propionic acid; chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide; methyl 2-pyridyl disulfide; p-chloromercuribenzoate; 2-chloromercuri-4 nitrophenol; or, chloro-7-nitrobenzo-oxa-1,3-diazole. Lysine mimetics can be generated (and amino terminal residues can be altered) by reacting lysinyl with, e.g., succinic or other carboxylic acid anhydrides. Lysine and other alpha-amino-containing residue mimetics can also be generated by reaction with imidoesters, such as methyl picolinimate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitro-benzenesulfonic acid, O-methylisourea, 2,4, pentanedione, and transamidase-catalyzed reactions with glyoxylate. Mimetics of methionine can be generated by reaction with, e.g., methionine sulfoxide. Mimetics of proline include, e.g., pipecolic acid, thiazolidine carboxylic acid, 3- or 4- hydroxy proline, dehydropyrolidine, 3- or 4-methylproline, or 3,3,-dimethylproline. Histidine residue mimetics can be generated by reacting histidyl with, e.g., diethylprocarbonate or para-bromophenacyl bromide. Other mimetics include, e.g., those generated by hydroxylation of proline and lysine; phosphorylation of the hydroxyl groups of seryl or threonyl residues; methylation of the alpha-amino groups of lysine, arginine and histidine; acetylation of the N-terminal amine; methylation of main chain amide residues or substitution with N-methyl amino acids; or amidation of C-terminal carboxyl groups.

[0325] A residue, e.g., an amino acid, of a polypeptide of the disclosures can also be replaced by an amino acid (or peptidomimetic residue) of the opposite chirality. Thus, any amino acid naturally occurring in the L-configuration (which can also be referred to as the R or S, depending upon the structure of the chemical entity) can be replaced with the amino acid of the same chemical structural type or a peptidomimetic, but of the opposite chirality, referred to as the D-amino acid, but also can be referred to as the R- or S- form.

[0326] The disclosure also provides methods for modifying the polypeptides by either natural processes, such as post-translational processing (e.g., phosphorylation, acylation, etc), or by chemical modification techniques, and the resulting modified polypeptides. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also a given polypeptide may have many types of modifications. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphatidylinositol, cross-linking cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. See, e.g., Creighton, T.E., *Proteins - Structure and Molecular Properties* 2nd Ed., W.H. Freeman and Company, New York (1993); *Posttranslational Covalent Modification of Proteins*, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983).

[0327] Solid-phase chemical peptide synthesis methods can also be used to synthesize the polypeptide or fragments. Such method have been known in the art since the early 1960's (Merrifield, R. B., *J. Am. Chem. Soc.*, 85:2149-2154, 1963) (See also Stewart, J. M. and Young, J. D., *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, Ill., pp. 11-12) and have recently been employed in commercially available laboratory peptide design and synthesis kits (Cambridge Research Biochemicals). Such commercially available laboratory kits have generally utilized the teachings of H. M. Geysen et al, *Proc. Natl. Acad. Sci., USA*, 81:3998 (1984) and provide for synthesizing peptides upon the tips of a multitude of "rods" or "pins" all of which are connected to a single plate. When such a system is utilized, a plate of rods or pins is inverted and inserted into a second plate of corresponding wells or reservoirs, which contain solutions for attaching or anchoring an appropriate amino acid to the pin's or rod's tips. By repeating such a process step, i.e., inverting and inserting the rod's and pin's tips into appropriate solutions, amino acids are built into desired peptides. In addition, a number of available Fmoc peptide synthesis systems are available. For example, assembly of a polypeptide

or fragment can be carried out on a solid support using an Applied Biosystems, Inc. Model 431A™ automated peptide synthesizer. Such equipment provides ready access to the peptides, either by direct synthesis or by synthesis of a series of fragments that can be coupled using other known techniques.

[0328] The disclosure includes pectate lyases with and without signal. The polypeptide comprising a signal sequence can be a pectate lyase of the disclosure or another pectate lyase or another enzyme or other polypeptide.

[0329] The disclosure includes immobilized pectate lyases, anti-pectate lyase antibodies and fragments thereof. The disclosure provides methods for inhibiting pectate lyase activity, e.g., using dominant negative mutants or anti-pectate lyase antibodies of the disclosure. The disclosure includes heterocomplexes, e.g., fusion proteins, heterodimers, etc., comprising the pectate lyases of the disclosure.

[0330] Polypeptides can have a pectate lyase activity under various conditions, e.g., extremes in pH and/or temperature, oxidizing agents, and the like. The disclosure provides methods leading to alternative pectate lyase preparations with different catalytic efficiencies and stabilities, e.g., towards temperature, oxidizing agents and changing wash conditions. In one aspect, pectate lyase variants can be produced using techniques of site-directed mutagenesis and/or random mutagenesis. In one aspect, directed evolution can be used to produce a great variety of pectate lyase variants with alternative specificities and stability.

[0331] The proteins of the disclosure are also useful as research reagents to identify pectate lyase modulators, e.g., activators or inhibitors of pectate lyase activity. Briefly, test samples (compounds, broths, extracts, and the like) are added to pectate lyase assays to determine their ability to inhibit substrate cleavage. Inhibitors identified in this way can be used in industry and research to reduce or prevent undesired proteolysis. As with pectate lyases, inhibitors can be combined to increase the spectrum of activity.

[0332] There are also provided methods of discovering new pectate lyases using the nucleic acids, polypeptides and antibodies of the disclosure. In one aspect, lambda phage libraries are screened for expression-based discovery of pectate lyases. In one aspect, this uses lambda phage libraries in screening to allow detection of toxic clones; improved access to substrate; reduced need for engineering a host, bypassing the potential for any bias resulting from mass excision of the library; and, faster growth at low clone densities. Screening of lambda phage libraries can be in liquid phase or in solid phase. In one aspect, this provides screening in liquid phase. This gives a greater flexibility in assay conditions; additional substrate flexibility; higher sensitivity for weak clones; and ease of automation over solid phase screening.

[0333] The disclosure provides screening methods using the proteins and nucleic acids of the disclosure and robotic automation to enable the execution of many thousands of biocatalytic reactions and screening assays in a short period of time, e.g., per day, as well as ensuring a high level of accuracy and reproducibility (see discussion of arrays, below). As a result, a library of derivative compounds can be produced in a matter of weeks. For further teachings on modification of molecules, including small molecules, see PCT/US94/09174.

[0334] The disclosure includes pectate lyase enzymes which are non-naturally occurring carbonyl hydrolase variants (e.g., pectate lyase variants) having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Specifically, such pectate lyase variants have an amino acid sequence not found in nature, which is derived by substitution of a plurality of amino acid residues of a precursor pectate lyase with different amino acids. The precursor pectate lyase may be a naturally-occurring pectate lyase or a recombinant pectate lyase. The useful pectate lyase variants encompass the substitution of any of the naturally occurring L-amino acids at the designated amino acid residue positions.

Gene site saturation mutagenesis (GSSM™) variants

[0335] The disclosure provides pectate lyase variants and the nucleic acids that encode them. In one aspect, this provides SEQ ID NO:134, encoded by SEQ ID NO:133, respectively. SEQ ID NO:133 is a nucleic acid variant generated by gene site saturation mutagenesis (GSSM™) of SEQ ID NO:131 (which encodes SEQ ID NO:132). SEQ ID NO:131 and SEQ ID NO:132 are truncated variations of the nucleic acid as set forth in SEQ ID NO:77, encoding SEQ ID NO:78, respectively. The following Table 1 summarizes the amino acid changes resulting from the GSSM™-generated variations in their respective encoding nucleic acids (the full length SEQ ID NO:78 encoded by SEQ ID NO:77, and the truncated "parent" SEQ ID NO:132 encoded by SEQ ID NO:131:

Table 1			
Mutation - including amino acid position in SEQ ID NOS:131, 132	Nucleotide position in truncated wild-type gene (SEQ ID NOS:131, 132)	Nucleotide position in full length wild-type gene (SEQ ID NOS:77, 78)	Amino acid position in full length wild-type gene (SEQ ID NOS:77, 78)
A118H	352-354	1423-1425	475

(continued)

Table 1			
Mutation - including amino acid position in SEQ ID NOS:131, 132	Nucleotide position in truncated wild-type gene (SEQ ID NOS:131, 132)	Nucleotide position in full length wild-type gene (SEQ ID NOS:77, 78)	Amino acid position in full length wild-type gene (SEQ ID NOS:77, 78)
A182V	544-546	1615-1617	539
T190L	568-570	1639-1641	547
A197G	589-591	1660-1662	554
S208K	622-624	1693-1695	565
T219M	655-657	1726-1728	576
T223E	667-669	1738-1740	580
S255R	763-765	1834-1836	612
S263K	787-789	1858-1860	620
N275Y	823-825	1894-1896	632
Y309W	925-927	1996-1998	666
S312V	934-936	2005-2007	669

[0336] Figure 6 is a table summarizing exemplary sequence changes in pectate lyase polypeptides, characterized as "upmutants." The upmutants identified as A-S are combinatorial upmutants (each have several GSSM™-generated changes). The upmutants identified as AA-LL are single upmutants (one GSSM™-generated change each).

[0337] Figure 7 is a table summarizing exemplary melting temperatures and specific activities (SA) of exemplary enzymes at various temperatures. Specific activity (U/mg pure enzyme) was measured at different temperatures at pH 9.5 in 25 mM Glycine NaOH 25 mM TrisHCl buffer. One unit of enzymatic activity was defined as the amount of enzyme that produced 1 μmol of unsaturated oligogalacturonides equivalent to 1 μmol of unsaturated digalacturonide per minute. Protein concentrations of the pure enzyme preparations were measured at A280 using a molar extinction coefficient of 73800 M⁻¹cm⁻¹ (1 A280 eq. to 0.50 mg/mL). Melting temperatures were determined with a differential scanning calorimeter.

[0338] In these Figures, mutant "N" has a sequence as set forth in SEQ ID NO:134, encoded by SEQ ID NO:133.

Pectate lyase signal sequences, pectin methyl esterase domains and catalytic domains, carbohydrate binding modules and prepro domains

[0339] The disclosure provides signal sequences (e.g., signal peptides (SPs)), prepro domains and catalytic domains (CDs). The SPs, prepro domains and/or CDs can be isolated or recombinant peptides or can be part of a fusion protein, e.g., as a heterologous domain in a chimeric protein. The disclosure provides nucleic acids encoding these catalytic domains (CDs), prepro domains and signal sequences (SPs, e.g., a peptide having a sequence comprising/ consisting of amino terminal residues of a polypeptide of the disclosure).

[0340] The disclosure provides pectate lyase signal sequences (e.g., signal peptides (SPs)) and nucleic acids encoding these signal sequences, e.g., a peptide having a sequence comprising/ consisting of amino terminal residues of a polypeptide of the disclosure, e.g., signal peptides (SPs) as set forth in Table 2, below. In one aspect, this provides a signal sequence comprising a peptide comprising/ consisting of a sequence as set forth in residues 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 39, 1 to 40, 1 to 41, 1 to 42, 1 to 43, 1 to 44 of a polypeptide, e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ

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ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134.

[0341] This also provides pectate lyase pectin methyl esterase domains (PEDs) and catalytic domains (CDs) as set forth in Table 2, below.

[0342] The pectate lyase signal sequences (SPs), CDs, and/or prepro sequences can be isolated peptides, or, sequences joined to another hydrolase or a non-pectate lyase polypeptide, e.g., as a fusion (chimeric) protein. In one aspect, this provides polypeptides comprising the pectate lyase signal sequences. In one aspect, polypeptides comprising pectate lyase signal sequences SPs, CDs, and/or prepro of the disclosure comprise sequences heterologous to pectate lyases of the disclosure (e.g., a fusion protein comprising an SP, CD, and/or prepro and sequences from another pectate lyase or a non-pectate lyase protein). In one aspect, this provides pectate lyases of the disclosure with heterologous SPs, CDs, and/or prepro sequences, e.g., sequences with a yeast signal sequence. An pectate lyase of the disclosure can comprise a heterologous SP and/or prepro in a vector, e.g., a pPIC series vector (Invitrogen, Carlsbad, CA).

[0343] Table 2 summarizes signal sequences (i.e., signal peptides in their isolated form), catalytic domains, carbohydrate binding modules and pectin methyl esterase domains. For example, Table 2 describes: in row 1, a signal peptide (SP) at residues 1 to 28 of SEQ ID NO:102 (encoded by SEQ ID NO:101) and a catalytic domain (CD) at residues 78-459 of SEQ ID NO:102; in row 2, a signal peptide (SP) at residues 1 to 21 of SEQ ID NO:2 (encoded by SEQ ID NO:1), a pectin methyl esterase domain (PED) at residues 28-308, and a catalytic domain (CD) at residues;309-638; at row 3, etc.

Table 2

SEQ ID NO:	Modules (SP =signal peptide, CD=catalytic domain, CBM=carbohydrate binding module, PED=pectin methyl esterase domain)
101, 102	SP;1-28, CD;78-459
1, 2	SP;1-21, PED;28-308, CD;309-638
103, 104	SP;1-26, CD;27-366
105, 106	SP;1-43, CD;44-400
107, 108	SP;1-31, CD;32-357
109, 110	SP;1-21, PED;28-308, CD309-637
11, 12	CD;1-388
111, 112	SP;1-27, CD;82-461
113, 114	SP;1-18, CD;19-388
115, 116	CD;1-331
117, 118	SP;1-24, CD;25-574
119, 120	CBM;1-61, CBM;134-257, CD;258-615
121, 122	SP;1-29, CD;30-348
123, 124	SP;1-21, CD;22-390
125, 126	CD;24-325
127, 128	SP;1-24, CD;125-482
129, 130	CD;38-326
13, 14	SP;1-22, CD;23-354
15, 16	SP;1-33, CD;34-359
17, 18	CD;1-348
19, 20	CD;1-373
21, 22	SP;1-23, CD;24-422
23, 24	SP;1-18, CD;19-393
25, 26	SP;1-15, CD;16-397
27, 28	SP;1-21, PED;28-308, CD;309-638
29, 30	SP;1-27, CD;77-459
3, 4	SP;1-28, CD;81-476
31, 32	CD;1-348
33, 34	SP;1-18, CD;19-346
35, 36	CD;1-356
37, 38	SP;1-35, CD;36-387
39, 40	SP;1-32, CD; 33-358
41, 42	SP;1-21, CD;22-359

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(continued)

	SEQ ID NO:	Modules (SP =signal peptide, CD=catalytic domain, CBM=carbohydrate binding module, PED=pectin methyl esterase domain)
5	43, 44	CBM;4-89, CBM;152-275, CD;277-633
	45, 46	SP;1-20, CD;21-328
	47, 48	SP;1-21, CD;22-358
	49, 50	SP;1-16, CD;17-340
	5, 6	CD;1-358
10	51, 52	CD;1-376
	53, 54	SP;1-31, CBM;32-124, CBM;180-303, CD;304-658
	55, 56	CD;1-374
	57, 58	CD;1-389
15	59, 60	SP;1-24, CD;25-359
	61, 62	CD;90-407
	63, 64	SP;1-16, CD;17-340
	65, 66	SP;1-28, CD;29-436
	67, 68	SP;1-32, CBM;33-126, CBM;184-307, CD;308-664
20	69, 70	SP;1-22, CD;23-344
	7, 8	CD;1-374
	71, 72	SP;1-20, CD;21-345
	73, 74	SP;1-22, CD;23-406
25	75, 76	SP;1-34, CD;110-555
	77, 78	SP;1-33, CBM;34-126, CBM;199-322, CD;323-680
	79, 80	SP;1-28, CD;81-458
	81, 82	SP;1-30, CD;31-354
	83, 84	PED;268-556, CD;782-1164
30	85, 86	CD;1-383
	87, 88	SP;1-32, CD; 33-375
	89, 90	SP;1-31, CD;32-459
	9, 10	SP;1-29, CD;30-371
35	91, 92	CD;1-374
	93, 94	CD;1-353
	95, 96	SP;1-31, CD;32-357
	97, 98	PED;45-333, CD;336-698
40	99, 100	SP;1-35, CD;36-593

[0344] In one aspect, SPs, CDs, and/or prepro sequences are identified following identification of novel pectate lyase polypeptides. The pathways by which proteins are sorted and transported to their proper cellular location are often referred to as protein targeting pathways. One of the most important elements in all of these targeting systems is a short amino acid sequence at the amino terminus of a newly synthesized polypeptide called the signal sequence. This signal sequence directs a protein to its appropriate location in the cell and is removed during transport or when the protein reaches its final destination. Most lysosomal, membrane, or secreted proteins have an amino-terminal signal sequence that marks them for translocation into the lumen of the endoplasmic reticulum. The signal sequences can vary in length from 13 to 45 or more amino acid residues. Various methods of recognition of signal sequences are known to those of skill in the art. For example, in one aspect, novel pectate lyase signal peptides are identified by a method referred to as SignalP. SignalP uses a combined neural network which recognizes both signal peptides and their cleavage sites. (Nielsen, et al., "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites." Protein Engineering, vol. 10, no. 1, p. 1-6 (1997).

[0345] In some aspects pectate lyases do not have SPs and/or prepro sequences, and/or catalytic domains (CDs). In one aspect, this provides polypeptides (e.g., pectate lyases) lacking all or part of an SP, a CD and/or a prepro domain. In one aspect, this provides a nucleic acid sequence encoding a signal sequence (SP), a CD, and/or prepro from one pectate lyase operably linked to a nucleic acid sequence of a different pectate lyase or, optionally, a signal sequence (SPs) and/or prepro domain from a non-pectate lyase protein may be desired.

[0346] The disclosure also provides isolated or recombinant polypeptides comprising signal sequences (SPs), prepro domains, pectin methyl esterase domains (PEDs) and catalytic domains (CDs) and heterologous sequences. The heterologous sequences are sequences not naturally associated (e.g., to a pectate lyase) with an SP, prepro domain, PED, and/or CD. The sequence to which the SP, prepro domains, PED and/or CD are not naturally associated can be on the SP's, prepro domain's, PED's, and/or CD's amino terminal end, carboxy terminal end, and/or on both ends of the SP, prepro domain, PED and/or CD. In one aspect, this provides an isolated or recombinant polypeptide comprising (or consisting of) a polypeptide comprising a signal sequence (SP), prepro domain, pectin methyl esterase domain (PED) and/or catalytic domain (CD) with the proviso that it is not associated with any sequence to which it is naturally associated (e.g., a pectate lyase sequence). Similarly in one aspect, this provides isolated or recombinant nucleic acids encoding these polypeptides. Thus, in one aspect, the isolated or recombinant nucleic acid comprises coding sequence for a signal sequence (SP), prepro domain, pectin methyl esterase domain (PED) and/or catalytic domain (CD) and a heterologous sequence (i.e., a sequence not naturally associated with the a signal sequence (SP), prepro domain, pectin methyl esterase domain (PED) and/or catalytic domain (CD)). The heterologous sequence can be on the 3' terminal end, 5' terminal end, and/or on both ends of the SP, prepro domain, PED and/or CD coding sequence.

Glycosylation

[0347] The peptides and polypeptides (e.g., pectate lyases, antibodies) can also be glycosylated, for example, in one aspect, comprising at least one glycosylation site, e.g., an N-linked or O-linked glycosylation. In one aspect, the polypeptide can be glycosylated after being expressed in a *P. pastoris* or a *S. pombe*. The glycosylation can be added post-translationally either chemically or by cellular biosynthetic mechanisms, wherein the later incorporates the use of known glycosylation motifs, which can be native to the sequence or can be added as a peptide or added in the nucleic acid coding sequence.

Hybrid (chimeric) pectate lyases and peptide libraries

[0348] In one aspect, the disclosure provides hybrid pectate lyases and fusion proteins, including peptide libraries, comprising sequences of the disclosure. The peptide libraries can be used to isolate peptide modulators (e.g., activators or inhibitors) of targets, such as pectate lyase substrates, receptors, enzymes. The peptide libraries can be used to identify formal binding partners of targets, such as ligands, e.g., cytokines, hormones and the like. In one aspect, this provides Chimeric proteins comprising a signal sequence (SP), pectin methyl esterase domain (PED) and/or catalytic domain (CD) and a heterologous sequence (see above).

[0349] In one aspect, the fusion proteins (e.g., the peptide moiety) are conformationally stabilized (relative to linear peptides) to allow a higher binding affinity for targets. This provides fusions of the pectate lyases and other peptides, including known and random peptides. They can be fused in such a manner that the structure of the pectate lyases is not significantly perturbed and the peptide is metabolically or structurally conformationally stabilized. This allows the creation of a peptide library that is easily monitored both for its presence within cells and its quantity.

[0350] Amino acid sequence variants can be characterized by a predetermined nature of the variation, a feature that sets them apart from a naturally occurring form, e.g., an allelic or interspecies variation of a pectate lyase sequence. In one aspect, the variants exhibit the same qualitative biological activity as the naturally occurring analogue. Alternatively, the variants can be selected for having modified characteristics. In one aspect, while the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed pectate lyase variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, as discussed herein for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants can be done using assays of proteolytic activities. In alternative aspects, amino acid substitutions can be single residues; insertions can be on the order of from about 1 to 20 amino acids, although considerably larger insertions can be done. Deletions can range from about 1 to about 20, 30, 40, 50, 60, 70 residues or more. To obtain a final derivative with the optimal properties, substitutions, deletions, insertions or any combination thereof may be used. Generally, these changes are done on a few amino acids to minimize the alteration of the molecule. However, larger changes may be tolerated in certain circumstances.

[0351] The disclosure provides pectate lyases where the structure of the polypeptide backbone, the secondary or the tertiary structure, e.g., an alpha-helical or beta-sheet structure, has been modified. In one aspect, the charge or hydrophobicity has been modified. In one aspect, the bulk of a side chain has been modified. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative. For example, substitutions can be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example a alpha-helical or a beta-sheet structure; a charge or a hydrophobic site of the molecule, which can be at an

active site; or a side chain. This provides substitutions in polypeptide of the disclosure where (a) a hydrophilic residues, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g. lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g. glycine. The variants can exhibit the same qualitative biological activity (i.e. pectate lyase activity) although variants can be selected to modify the characteristics of the pectate lyases as needed.

[0352] In one aspect, pectate lyases comprise epitopes or purification tags, signal sequences or other fusion sequences, etc. In one aspect, the pectate lyases can be fused to a random peptide to form a fusion polypeptide. By "fused" or "operably linked" herein is meant that the random peptide and the pectate lyase are linked together, in such a manner as to minimize the disruption to the stability of the pectate lyase structure, e.g., it retains pectate lyase activity. The fusion polypeptide (or fusion polynucleotide encoding the fusion polypeptide) can comprise further components as well, including multiple peptides at multiple loops.

[0353] In one aspect, the peptides and nucleic acids encoding them are randomized, either fully randomized or they are biased in their randomization, e.g. in nucleotide/residue frequency generally or per position. "Randomized" means that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. In one aspect, the nucleic acids which give rise to the peptides can be chemically synthesized, and thus may incorporate any nucleotide at any position. Thus, when the nucleic acids are expressed to form peptides, any amino acid residue may be incorporated at any position. The synthetic process can be designed to generate randomized nucleic acids, to allow the formation of all or most of the possible combinations over the length of the nucleic acid, thus forming a library of randomized nucleic acids. The library can provide a sufficiently structurally diverse population of randomized expression products to affect a probabilistically sufficient range of cellular responses to provide one or more cells exhibiting a desired response. Thus, this provides an interaction library large enough so that at least one of its members will have a structure that gives it affinity for some molecule, protein, or other factor.

Screening Methodologies and "On-line" Monitoring Devices

[0354] In practicing the methods, a variety of apparatus and methodologies can be used to in conjunction with the polypeptides and nucleic acids of the disclosure, e.g., to screen polypeptides for pectate lyase activity, to screen compounds as potential modulators, e.g., activators or inhibitors, of a pectate lyase activity, for antibodies that bind to a polypeptide of the disclosure, for nucleic acids that hybridize to a nucleic acid of the disclosure, to screen for cells expressing a polypeptide of the disclosure and the like.

Capillary Arrays

[0355] Capillary arrays, such as the GIGAMATRIX™, Diversa Corporation, San Diego, CA, can be used to in the methods. Nucleic acids or polypeptides can be immobilized to or applied to an array, including capillary arrays. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide. Capillary arrays provide another system for holding and screening samples. For example, a sample screening apparatus can include a plurality of capillaries formed into an array of adjacent capillaries, wherein each capillary comprises at least one wall defining a lumen for retaining a sample. The apparatus can further include interstitial material disposed between adjacent capillaries in the array, and one or more reference indicia formed within of the interstitial material. A capillary for screening a sample, wherein the capillary is adapted for being bound in an array of capillaries, can include a first wall defining a lumen for retaining the sample, and a second wall formed of a filtering material, for filtering excitation energy provided to the lumen to excite the sample.

[0356] A polypeptide or nucleic acid, e.g., a ligand, can be introduced into a first component into at least a portion of a capillary of a capillary array. Each capillary of the capillary array can comprise at least one wall defining a lumen for retaining the first component. An air bubble can be introduced into the capillary behind the first component. A second component can be introduced into the capillary, wherein the second component is separated from the first component by the air bubble. A sample of interest can be introduced as a first liquid labeled with a detectable particle into a capillary of a capillary array, wherein each capillary of the capillary array comprises at least one wall defining a lumen for retaining the first liquid and the detectable particle, and wherein the at least one wall is coated with a binding material for binding the detectable particle to the at least one wall. The method can further include removing the first liquid from the capillary tube, wherein the bound detectable particle is maintained within the capillary, and introducing a second liquid into the capillary tube.

[0357] The capillary array can include a plurality of individual capillaries comprising at least one outer wall defining a lumen. The outer wall of the capillary can be one or more walls fused together. Similarly, the wall can define a lumen

that is cylindrical, square, hexagonal or any other geometric shape so long as the walls form a lumen for retention of a liquid or sample. The capillaries of the capillary array can be held together in close proximity to form a planar structure. The capillaries can be bound together, by being fused (e.g., where the capillaries are made of glass), glued, bonded, or clamped side-by-side. The capillary array can be formed of any number of individual capillaries, for example, a range from 100 to 4,000,000 capillaries. A capillary array can form a micro titer plate having about 100,000 or more individual capillaries bound together.

Arrays, or "Biochips"

[0358] Nucleic acids or polypeptides can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the disclosure. For example, in one aspect a monitored parameter is transcript expression of a pectate lyase gene. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array, or "biochip." By using an "array" of nucleic acids on a microchip, some or all of the transcripts of a cell can be simultaneously quantified. Alternatively, arrays comprising genomic nucleic acid can also be used to determine the genotype of a newly engineered strain made by the methods of the disclosure. Polypeptide arrays" can also be used to simultaneously quantify a plurality of proteins. This can be practiced with any known "array," also referred to as a "microarray" or "nucleic acid array" or "polypeptide array" or "antibody array" or "biochip," or variation thereof. Arrays are generically a plurality of "spots" or "target elements," each target element comprising a defined amount of one or more biological molecules, e.g., oligonucleotides, immobilized onto a defined area of a substrate surface for specific binding to a sample molecule, e.g., mRNA transcripts.

[0359] In practicing the methods, any known array and/or method of making and using arrays can be incorporated in whole or in part, or variations thereof, as described, for example, in U.S. Patent Nos. 6,277,628; 6,277,489; 6,261,776; 6,258,606; 6,054,270; 6,048,695; 6,045,996; 6,022,963; 6,013,440; 5,965,452; 5,959,098; 5,856,174; 5,830,645; 5,770,456; 5,632,957; 5,556,752; 5,143,854; 5,807,522; 5,800,992; 5,744,305; 5,700,637; 5,556,752; 5,434,049; see also, e.g., WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; see also, e.g., Johnston (1998) Curr. Biol. 8:R171-R174; Schummer (1997) Biotechniques 23:1087-1092; Kern (1997) Biotechniques 23:120-124; Solinas-Toldo (1997) Genes, Chromosomes & Cancer 20:399-407; Bowtell (1999) Nature Genetics Supp. 21:25-32. See also published U.S. patent applications Nos. 20010018642; 20010019827; 20010016322; 20010014449; 20010014448; 20010012537; 20010008765.

Antibodies and Antibody-based screening methods

[0360] The disclosure provides isolated or recombinant antibodies that specifically bind to a pectate lyase of the disclosure. These antibodies can be used to isolate, identify or quantify the pectate lyases of the disclosure or related polypeptides. These antibodies can be used to isolate other polypeptides within the desired scope or other related pectate lyases. The antibodies can be designed to bind to an active site of a pectate lyase. Thus, this provides methods of inhibiting pectate lyases using the antibodies.

[0361] Fragments of the enzymes include immunogenic fragments of a polypeptide SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134. The immunogenic peptides (e.g., the immunogenic fragments of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124,

SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134) can further comprise adjuvants, carriers and the like.

[0362] The antibodies can be used in immunoprecipitation, staining, immunoaffinity columns, and the like. If desired, nucleic acid sequences encoding for specific antigens can be generated by immunization followed by isolation of polypeptide or nucleic acid, amplification or cloning and immobilization of polypeptide onto an array. Alternatively, the methods can be used to modify the structure of an antibody produced by a cell to be modified, e.g., an antibody's affinity can be increased or decreased. Furthermore, the ability to make or modify antibodies can be a phenotype engineered into a cell by the methods.

[0363] Methods of immunization, producing and isolating antibodies (polyclonal and monoclonal) are known to those of skill in the art and described in the scientific and patent literature, see, e.g., Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler (1975) Nature 256:495; Harlow (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York. Antibodies also can be generated *in vitro*, e.g., using recombinant antibody binding site expressing phage display libraries, in addition to the traditional *in vivo* methods using animals. See, e.g., Hoogenboom (1997) Trends Biotechnol. 15:62-70; Katz (1997) Annu. Rev. Biophys. Biomol. Struct. 26:27-45.

[0364] Polypeptides or peptides can be used to generate antibodies which bind specifically to the polypeptides, e.g., the pectate lyases. The resulting antibodies may be used in immunoaffinity chromatography procedures to isolate or purify the polypeptide or to determine whether the polypeptide is present in a biological sample. In such procedures, a protein preparation, such as an extract, or a biological sample is contacted with an antibody capable of specifically binding to one of the polypeptides of the disclosure.

[0365] In immunoaffinity procedures, the antibody is attached to a solid support, such as a bead or other column matrix. The protein preparation is placed in contact with the antibody under conditions in which the antibody specifically binds to one of the polypeptides of the disclosure. After a wash to remove non-specifically bound proteins, the specifically bound polypeptides are eluted.

[0366] The ability of proteins in a biological sample to bind to the antibody may be determined using any of a variety of procedures familiar to those skilled in the art. For example, binding may be determined by labeling the antibody with a detectable label such as a fluorescent agent, an enzymatic label, or a radioisotope. Alternatively, binding of the antibody to the sample may be detected using a secondary antibody having such a detectable label thereon. Particular assays include ELISA assays, sandwich assays, radioimmunoassays, and Western Blots.

[0367] Polyclonal antibodies generated against the polypeptides of the disclosure can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to a non-human animal. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies which may bind to the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from cells expressing that polypeptide.

[0368] For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique, the trioma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (see, e.g., Cole (1985) in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

[0369] Techniques described for the production of single chain antibodies (see, e.g., U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to the polypeptides of the invention. Alternatively, transgenic mice may be used to express humanized antibodies to these polypeptides or fragments thereof.

[0370] Antibodies generated against the polypeptides may be used in screening for similar polypeptides (e.g., pectate lyases) from other organisms and samples. In such techniques, polypeptides from the organism are contacted with the antibody and those polypeptides which specifically bind the antibody are detected. Any of the procedures described above may be used to detect antibody binding.

Kits

[0371] Kits may comprise the compositions, e.g., nucleic acids, expression cassettes, vectors, cells, transgenic seeds or plants or plant parts, polypeptides (e.g., pectate lyases) and/or antibodies of the disclosure. The kits also can contain instructional material teaching the methodologies and industrial uses, as described herein.

Measuring Metabolic Parameters

[0372] The methods provide whole cell evolution, or whole cell engineering, of a cell to develop a new cell strain having a new phenotype, e.g., a new or modified pectate lyase activity, by modifying the genetic composition of the cell. The genetic composition can be modified by addition to the cell of a nucleic acid of the disclosure. To detect the new phenotype,

at least one metabolic parameter of a modified cell is monitored in the cell in a "real time" or "on-line" time frame. In one aspect, a plurality of cells, such as a cell culture, is monitored in "real time" or "on-line." In one aspect, a plurality of metabolic parameters is monitored in "real time" or "on-line." Metabolic parameters can be monitored using the pectate lyases of the disclosure.

[0373] Metabolic flux analysis (MFA) is based on a known biochemistry framework. A linearly independent metabolic matrix is constructed based on the law of mass conservation and on the pseudo-steady state hypothesis (PSSH) on the intracellular metabolites. In practicing the methods, metabolic networks are established, including the:

- identity of all pathway substrates, products and intermediary metabolites
- identity of all the chemical reactions interconverting the pathway metabolites, the stoichiometry of the pathway reactions,
- identity of all the enzymes catalyzing the reactions, the enzyme reaction kinetics,
- the regulatory interactions between pathway components, e.g. allosteric interactions, enzyme-enzyme interactions etc,
- intracellular compartmentalization of enzymes or any other supramolecular organization of the enzymes, and,
- the presence of any concentration gradients of metabolites, enzymes or effector molecules or diffusion barriers to their movement.

[0374] Once the metabolic network for a given strain is built, mathematic presentation by matrix notion can be introduced to estimate the intracellular metabolic fluxes if the on-line metabolome data is available. Metabolic phenotype relies on the changes of the whole metabolic network within a cell. Metabolic phenotype relies on the change of pathway utilization with respect to environmental conditions, genetic regulation, developmental state and the genotype, etc. In one aspect, after the on-line MFA calculation, the dynamic behavior of the cells, their phenotype and other properties are analyzed by investigating the pathway utilization. For example, if the glucose supply is increased and the oxygen decreased during the yeast fermentation, the utilization of respiratory pathways will be reduced and/or stopped, and the utilization of the fermentative pathways will dominate. Control of physiological state of cell cultures will become possible after the pathway analysis. The methods can help determine how to manipulate the fermentation by determining how to change the substrate supply, temperature, use of inducers, etc. to control the physiological state of cells to move along desirable direction. In practicing the methods, the MFA results can also be compared with transcriptome and proteome data to design experiments and protocols for metabolic engineering or gene shuffling, etc.

[0375] In practicing the methods, any modified or new phenotype can be conferred and detected, including new or improved characteristics in the cell. Any aspect of metabolism or growth can be monitored.

Monitoring Expression of an mRNA transcript

[0376] In one aspect, the engineered phenotype comprises increasing or decreasing the expression of an mRNA transcript (e.g., a pectate lyase message) or generating new (e.g., pectate lyase) transcripts in a cell. This increased or decreased expression can be traced by testing for the presence of a pectate lyase of the disclosure or by pectate lyase activity assays. mRNA transcripts, or messages, also can be detected and quantified by any method known in the art, including, e.g., Northern blots, quantitative amplification reactions, hybridization to arrays, and the like. Quantitative amplification reactions include, e.g., quantitative PCR, including, e.g., quantitative reverse transcription polymerase chain reaction, or RT-PCR; quantitative real time RT-PCR, or "real-time kinetic RT-PCR" (see, e.g., Kreuzer (2001) Br. J. Haematol. 114:313-318; Xia (2001) Transplantation 72:907-914).

[0377] In one aspect, the engineered phenotype is generated by knocking out expression of a homologous gene. The gene's coding sequence or one or more transcriptional control elements can be knocked out, e.g., promoters or enhancers. Thus, the expression of a transcript can be completely ablated or only decreased.

[0378] In one aspect, the engineered phenotype comprises increasing the expression of a homologous gene. This can be effected by knocking out of a negative control element, including a transcriptional regulatory element acting in cis- or trans- , or, mutagenizing a positive control element. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array.

Monitoring Expression of a polypeptides, peptides and amino acids

[0379] In one aspect, the engineered phenotype comprises increasing or decreasing the expression of a polypeptide (e.g., a pectate lyase) or generating new polypeptides in a cell. This increased or decreased expression can be traced by determining the amount of pectate lyase present or by pectate lyase activity assays. Polypeptides, peptides and amino acids also can be detected and quantified by any method known in the art, including, e.g., nuclear magnetic

resonance (NMR), spectrophotometry, radiography (protein radiolabeling), electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, various immunological methods, e.g. immunoprecipitation, immunodiffusion, immuno-electrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, gel electrophoresis (e.g., SDS-PAGE), staining with antibodies, fluorescent activated cell sorter (FACS), pyrolysis mass spectrometry, Fourier-Transform Infrared Spectrometry, Raman spectrometry, GC-MS, and LC-Electrospray and cap-LC-tandem-electrospray mass spectrometries, and the like. Novel bioactivities can also be screened using methods, or variations thereof, described in U.S. Patent No. 6,057,103. Furthermore, as discussed below in detail, one or more, or, all the polypeptides of a cell can be measured using a protein array.

Industrial Applications

Detergent Compositions

[0380] Detergent compositions comprise one or more polypeptides (e.g., pectate lyases) of the disclosure, and the disclosure includes methods of making and using these compositions, see, e.g., U.S. Patent No. 6,413,928; 6,399,561; 6,365,561; 6,380,147. The detergent compositions can be a one and two part aqueous composition, a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel and/or a paste and a slurry form. The pectate lyases can also be used as a detergent additive product in a solid or a liquid form. Such additive products are intended to supplement or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process.

[0381] Also provided are methods capable of removing gross food soils, films of food residue and other minor food compositions using these detergent compositions. Pectate lyases can facilitate the removal of starchy stains by means of catalytic hydrolysis or trans-elimination of pectins, including the disruption of plant and bacterial cell walls. Pectate lyases can be used in dishwashing detergents in textile laundering detergents.

[0382] The actual active enzyme content depends upon the method of manufacture of a detergent composition and is not critical, assuming the detergent solution has the desired enzymatic activity. In one aspect, the amount of pectate lyase present in the final solution ranges from about 0.001 mg to 0.5 mg per gram of the detergent composition. The particular enzyme chosen for use in the process and products of this disclosure depends upon the conditions of final utility, including the physical product form, use pH, use temperature, and soil types to be degraded or altered. The enzyme can be chosen to provide optimum activity and stability for any given set of utility conditions. In one aspect, the pectate lyases of the present disclosure are active in the pH ranges of from about 4 to about 12 and in the temperature range of from about 20°C to about 95°C. The detergents can comprise cationic, semi-polar nonionic or zwitterionic surfactants; or, mixtures thereof.

[0383] Pectate lyases can be formulated into powdered and liquid detergents having pH between 4.0 and 12.0 at levels of about 0.01 to about 5% (preferably 0.1% to 0.5%) by weight. These detergent compositions can also include other enzymes such as proteases, cellulases, lipases or endoglycosidases, endo-beta.-1,4-glucanases, beta-glucanases, endo-beta-1,3(4)-glucanases, cutinases, peroxidases, laccases, amylases, glucoamylases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, arabinanases, hemicellulases, mannanases, xyloglucanases, xylanases, pectin acetyl esterases, rhamnogalacturonan acetyl esterases, polygalacturonases, rhamnogalacturonases, galactanases, pectin lyases, pectin methylesterases, cellobiohydrolases and/or transglutaminases. These detergent compositions can also include builders and stabilizers.

[0384] The addition of pectate lyases to conventional cleaning compositions does not create any special use limitation. In other words, any temperature and pH suitable for the detergent is also suitable for the compositions as long as the enzyme is active at or tolerant of the pH and/or temperature of the intended use. In addition, the pectate lyases can be used in a cleaning composition without detergents, again either alone or in combination with builders and stabilizers.

[0385] Cleaning compositions include detergent compositions for cleaning hard surfaces, detergent compositions for cleaning fabrics, dishwashing compositions, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning solutions.

[0386] A method for washing an object comprises contacting the object with a polypeptide of the disclosure under conditions sufficient for washing. A pectate lyase may be included as a detergent additive. The detergent composition may, for example, be formulated as a hand or machine laundry detergent composition comprising a polypeptide of the disclosure. A laundry additive suitable for pre-treatment of stained fabrics can comprise a polypeptide of the disclosure. A fabric softener composition can comprise a pectate lyase of the disclosure. Alternatively, a pectate lyase can be formulated as a detergent composition for use in general household hard surface cleaning operations. In alternative aspects, detergent additives and detergent compositions may comprise one or more other enzymes such as a protease, a lipase, a cutinase, another pectate lyase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g., a lactase, and/or a peroxidase (see also, above). The properties of the

enzyme(s) of the disclosure are chosen to be compatible with the selected detergent (i.e. pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.) and the enzyme(s) is present in effective amounts. In one aspect, the pectate lyase enzymes are used to remove malodorous materials from fabrics. Various detergent compositions and methods for making them are described in, e.g., U.S. Patent Nos. 6,333,301; 6,329,333; 6,326,341; 6,297,038; 6,309,871; 6,204,232; 6,197,070; 5,856,164.

[0387] When formulated as compositions suitable for use in a laundry machine washing method, the pectate lyases can comprise both a surfactant and a builder compound. They can additionally comprise one or more detergent components, e.g., organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional detergent components. Such compositions containing carbohydrase can provide fabric cleaning, stain removal, whiteness maintenance, softening, color appearance, dye transfer inhibition and sanitization when formulated as laundry detergent compositions.

[0388] The density of the laundry detergent compositions can range from about 200 to 1500 g/liter, or, about 400 to 1200 g/liter, or, about 500 to 950 g/liter, or, 600 to 800 g/liter, of composition; this can be measured at about 20°C.

[0389] The "compact" form of laundry detergent compositions is best reflected by density and, in terms of composition, by the amount of inorganic filler salt. Inorganic filler salts are conventional ingredients of detergent compositions in powder form. In conventional detergent compositions, the filler salts are present in substantial amounts, typically 17% to 35% by weight of the total composition. In one aspect of the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, or, not exceeding 10%, or, not exceeding 5% by weight of the composition. The inorganic filler salts can be selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides, e.g., sodium sulphate.

[0390] Liquid detergent compositions can also be in a "concentrated form." In one aspect, the liquid detergent compositions can contain a lower amount of water, compared to conventional liquid detergents. In alternative aspects, the water content of the concentrated liquid detergent is less than 40%, or, less than 30%, or, less than 20% by weight of the detergent composition. Detergent compounds can comprise formulations as described in WO 97/01629.

Treating fibers and textiles

[0391] Methods of treating fibers, fabrics or any pectate- or polygalacturonic acid-comprising material use one or more pectate lyases of the disclosure. The pectate lyases can be used in any fiber- or fabric-treating method, which are well known in the art, see, e.g., U.S. Patent No. 6,261,828; 6,077,316; 6,024,766; 6,021,536; 6,017,751; 5,980,581; US Patent Publication No. 20020142438 A1. For example, the pectate lyases can be used in fiber and/or fabric scouring. In one aspect, the feel and appearance of a fabric is improved by a method comprising contacting the fabric with a pectate lyase of the disclosure in a solution. In one aspect, the fabric is treated with the solution under pressure. For example, the pectate lyases can be used in the removal of stains.

[0392] In one aspect, the pectate lyases are applied during or after the weaving of textiles, or during the desizing stage, or during one or more additional fabric processing steps. During the weaving of textiles, the threads are exposed to considerable mechanical strain. Prior to weaving on mechanical looms, warp yarns are often coated with sizing starch or starch derivatives in order to increase their tensile strength and to prevent breaking. After the textiles have been woven, a fabric can proceed to a desizing stage. This can be followed by one or more additional fabric processing steps. Desizing is the act of removing "size" from textiles. After weaving, the size coating must be removed before further processing the fabric in order to ensure a homogeneous and wash-proof result.

[0393] The enzymes of the disclosure can be used to scour fabrics or any pectate- or polygalacturonic acid-comprising material, including cotton-containing fabrics, as detergent additives, e.g., in aqueous compositions. For the manufacture of clothes, the fabric can be cut and sewn into clothes or garments. These can be finished before or after the treatment. In particular, for the manufacture of denim jeans, different enzymatic finishing methods have been developed. The finishing of denim garment normally is initiated with an enzymatic desizing step, during which garments are subjected to the action of amylolytic enzymes in order to provide softness to the fabric and make the cotton more accessible to the subsequent enzymatic finishing steps. This provides methods of finishing denim garments, enzymatic desizing and providing softness to fabrics by using any combination of enzymes, such as amylases, endoglucanases, and a pectate lyase of the disclosure.

[0394] In one aspect, an alkaline and thermostable amylase and pectate lyase are combined in a single bath desizing and bioscouring. Among advantages of combining desizing and scouring in one step are cost reduction and lower environmental impact due to savings in energy and water usage and lower waste production. Application conditions for desizing and bioscouring can be between about pH 8.5 to pH 10.0 and temperatures at about 40°C and up. Low enzyme dosages (e.g., about 5 g per a ton of cotton) and short reaction times (e.g., about 15 minutes) can be used to obtain efficient desizing and scouring with out added calcium.

[0395] The pectate lyases can be used in combination with other carbohydrate degrading enzymes, e.g., cellulase,

arabinanase, xyloglucanase, pectinase, xylanase, and the like, for the preparation of fibers or for cleaning of fibers. Proteases can also be used in combination. These can be used in combination with detergents. In one aspect, the pectate lyases can be used in treatments to prevent the graying of a textile.

[0396] The pectate lyases can be used to treat any cellulosic material, including fibers (e.g., fibers from cotton, hemp, flax or linen), sewn and unsewn fabrics, e.g., knits, wovens, denims, yarns, and toweling, made from cotton, cotton blends or natural or manmade cellulose (e.g. originating from xylan-containing cellulose fibers such as from wood pulp) or blends thereof. Examples of blends are blends of cotton or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyvinylidene chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, hemp, flax/linen, jute, cellulose acetate fibers, lyocell).

[0397] The textile treating processes (for example, scouring using pectate lyases) can be used in conjunction with other textile treatments, e.g., desizing and bleaching. Scouring is the removal of non-cellulosic material from the cotton fiber, e.g., the cuticle (mainly consisting of waxes) and primary cell wall (mainly consisting of pectin, protein and xyloglucan). A proper wax removal is necessary for obtaining a high wettability. This is needed for dyeing. Removal of the primary cell walls by the processes improves wax removal and ensures a more even dyeing. Treating textiles with the processes can improve whiteness in the bleaching process. The main chemical used in scouring is sodium hydroxide in high concentrations and at high temperatures. Bleaching comprises oxidizing the textile. Bleaching typically involves use of hydrogen peroxide as the oxidizing agent in order to obtain either a fully bleached (white) fabric or to ensure a clean shade of the dye.

[0398] A single-bath process may be for desizing, scouring and bleaching of cellulosic materials. In one aspect, desizing, scouring and bleaching are carried in a single-bath by contacting the cellulosic materials simultaneously or sequentially in a container (a "single-bath") with an enzyme system and a bleaching system comprising hydrogen peroxide or at least one peroxy compound which can generate hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator. Cellulosic materials including crude fibers, yarn, or woven or knit textiles, made of cotton, linen, flax, ramie, rayon, hemp, jute, or blends of these fibers with each other or with other natural or synthetic fibers, can be treated by the processes.

[0399] This also provides alkaline pectinases (pectate lyases active under alkaline conditions). These have wide-ranging applications in textile processing, degumming of plant fibers (e.g., plant bast fibers), treatment of pectic wastewaters, paper making, and coffee and tea fermentations. See, e.g., Hoondal (2002) Applied Microbiology and Biotechnology 59:409-418.

Treating foods and food processing

[0400] The pectate lyases of the disclosure have numerous applications in food processing industry. For example, in one aspect, the pectate lyases are used to improve the extraction of oil from oil-rich plant material, e.g., oil-rich seeds, for example, soybean oil from soybeans, olive oil from olives, rapeseed oil from rapeseed and/or sunflower oil from sunflower seeds.

[0401] The pectate lyases can be used for separation of components of plant cell materials. For example, the pectate lyases can be used in the separation of pectin-rich material (e.g., cell walls), sugar or starch-rich plant material into components, e.g., sucrose from sugar beet or starch or sugars from potato, pulp or hull fractions. In one aspect, the pectate lyases can be used to separate protein-rich or oil-rich crops into valuable protein and oil and hull fractions. The separation process may be performed by use of methods known in the art.

[0402] The pectate lyases can be used in the preparation of fruit or vegetable juices, syrups, extracts and the like to increase yield. The pectate lyases can be used in the enzymatic treatment (e.g., hydrolysis of pectins and/or polygalacturonic acid, such as 1,4-linked alpha-D-galacturonic acid) of various plant cell wall-derived materials or waste materials, e.g. from wine or juice production, or agricultural residues such as vegetable hulls, bean hulls, sugar beet pulp, olive pulp, potato pulp, and the like. The pectate lyases can be used to modify the consistency and appearance of processed fruit or vegetables. For example, the pectate lyases can be used in the production of clear juices, e.g., from apples, pears or berries; to cloud stable juices, e.g., from apples, pears, bennies, citrus or tomatoes; and to treat purees, e.g., from carrots and tomatoes, and to treat date syrup (see, e.g., Sidhu (2002) Food Chemistry 79:215-220). In these processes, the pectate lyases can be used with other enzymes (e.g., cellulases, amylases, etc.) or other compositions. For example, in one aspect, pectinase and cellulase enzymes are used to improve juice yield, stability and quality from a fruit, e.g., prickly pear fruit. A pectinase of the disclosure can improve the yield, stability and color (color-assayed as release of anthocyanins or carotenoids) and clarify of a juice. In one aspect, a combination of pectinase and cellulase is used; pectinase at 0.50% v/w can produce a high yield, a sediment-free clear juice and high-quality juice. See, e.g., Essa, Hesham A., et. al., 2002, Nahrung, 46(4):245-250.

[0403] In one aspect, an enzyme or enzyme preparation of the disclosure is used for de-pectinization and viscosity reduction in vegetable and/or fruit juice, e.g., in apple or pear juices or other apple or pear food preparations (e.g.,

saucers). In one aspect, the fruit or vegetable juice is treated with an enzyme preparation of the disclosure in an amount effective for degrading pectin-containing material contained in the fruit or vegetable juice.

[0404] In one aspect, the enzyme or enzyme preparation is used in the treatment of mash from fruits and vegetables in order to improve the extractability or degradability of the mash. The enzyme preparation can be used in the treatment of mash from apples and pears for juice production, and in the mash treatment of grapes for wine production.

[0405] The pectate lyases can be used to treat plant material to facilitate processing of plant material, including foods, facilitate purification or extraction of plant components such as galactans, pectins and/or polygalacturonic acids. The pectate lyases can be used to purify pectins from citrus, improve feed value, decrease the water binding capacity, improve the degradability in waste water plants and/or improve the conversion of plant material to ensilage, and the like.

Animal feeds and food or feed additives

[0406] Methods for treating animal feeds and foods and food or feed additives use pectate lyases of the disclosure for animals including mammals (e.g., humans), birds, fish and the like. Animal feeds, foods, and additives comprise pectate lyases of the disclosure. In one aspect, treating animal feeds, foods and additives using the pectate lyases can help in the availability of nutrients, e.g., starch, in the animal feed or additive. This can result in release of readily digestible and easily absorbed nutrients and sugars.

[0407] Pectate lyases of the disclosure, in the modification of animal feed or a food, can process the food or feed either *in vitro* (by modifying components of the feed or food) or *in vivo*. Pectate lyases can be added to animal feed or food compositions containing high amounts of arabinogalactans or galactans, e.g. feed or food containing plant material from soy bean, rape seed, lupin and the like. When added to the feed or food the pectate lyase significantly improves the *in vivo* break-down of plant cell wall material, whereby a better utilization of the plant nutrients by the animal (e.g., human) is achieved. In one aspect, the growth rate and/or feed conversion ratio (i.e. the weight of ingested feed relative to weight gain) of the animal is improved. For example the indigestible galactan is degraded by the pectate lyase, e.g. in combination with beta-galactosidase, to galactose or galactooligomers. These enzyme digestion products are more digestible by the animal. Thus, they can contribute to the available energy of the feed. Also, by the degradation of galactan the pectate lyase can improve the digestibility and uptake of non-carbohydrate feed constituents such as protein, fat and minerals.

[0408] In another aspect, the pectate lyase can be supplied by expressing the enzymes directly in transgenic feed crops (as, e.g., transgenic plants, seeds and the like), such as corn, soy bean, rape seed, lupin and the like. As discussed above, this provides transgenic plants, plant parts and plant cells comprising a nucleic acid sequence encoding a polypeptide of the disclosure. In one aspect, the nucleic acid is expressed such that the pectate lyase is produced in recoverable quantities. The pectate lyase can be recovered from any plant or plant part. Alternatively, the plant or plant part containing the recombinant polypeptide can be used as such for improving the quality of a food or feed, e.g., improving nutritional value, palatability, and rheological properties, or to destroy an antinutritive factor.

Paper or pulp treatment

[0409] The pectate lyases can be used in paper or pulp treatment or paper deinking. For example, a paper treatment process uses pectate lyases of the disclosure. In one aspect, the pectate lyases can be used to modify pectin and/or polygalacturonic acid, such as 1,4-linked alpha-D-galacturonic acid. In another aspect, paper components of recycled photocopied paper during chemical and enzymatic deinking processes. In one aspect, the pectate lyases can be used in combination with cellulases. The paper can be treated by the following three processes: 1) disintegration in the presence of pectate lyases, 2) disintegration with a deinking chemical and pectate lyases, and/or 3) disintegration after soaking with pectate lyases. The recycled paper treated with pectate lyases can have a higher brightness due to removal of toner particles as compared to the paper treated with just cellulase. The effect of the pectate lyases may be due to its behavior as surface-active agents in pulp suspension.

[0410] Methods of treating paper and paper pulp use one or more pectate lyases of the disclosure. The pectate lyases can be used in any paper- or pulp-treating method, which are well known in the art, see, e.g., U.S. Patent No. 6,241,849; 6,066,233; 5,582,681. For example, a method for deinking and decolorizing a printed paper containing a dye comprises pulping a printed paper to obtain a pulp slurry, and dislodging an ink from the pulp slurry in the presence of the pectate lyases (other enzymes can also be added). In another aspect, a method for enhancing the freeness of pulp, e.g., pulp made from secondary fiber is by adding an enzymatic mixture comprising the pectate lyases (can also include other enzymes, e.g., cellulase, amylase or glucoamylase enzymes) to the pulp and treating under conditions to cause a reaction to produce an enzymatically treated pulp. The freeness of the enzymatically treated pulp is increased from the initial freeness of the secondary fiber pulp without a loss in brightness.

Repulping: treatment of lignocellulosic materials

[0411] A method for the treatment of lignocellulosic fibers, uses the pectate lyases of the disclosure, in an amount which is efficient for improving the fiber properties. The pectate lyases may also be used in the production of lignocellulosic materials such as pulp, paper and cardboard, from starch-reinforced waste paper and cardboard, especially where repulping occurs at pH above 7 and where pectate lyases can facilitate the disintegration of the waste material through degradation of cell walls. The pectate lyases can be useful in a process for producing a papermaking pulp from starch-coated printed paper. The process may be performed as described in, e.g., WO 95/14807.

[0412] An exemplary process comprises disintegrating the paper to produce a pulp, treating with a pectin-degrading enzyme before, during or after the disintegrating, and separating ink particles from the pulp after disintegrating and enzyme treatment. See also U.S. Patent No. 6,309,871 and other US patents cited herein. Thus, this includes a method for enzymatic deinking of recycled paper pulp, wherein pectate lyases are applied in an amount which is efficient for effective de-inking of the fiber surface.

Waste treatment

[0413] The pectate lyases can be used in a variety of other industrial applications, e.g., in waste treatment. For example, a solid waste digestion process uses pectate lyases of the disclosure. The methods can comprise reducing the mass and volume of substantially untreated solid waste. Solid waste can be treated with an enzymatic digestive process in the presence of an enzymatic solution (including the pectate lyases) at a controlled temperature. This results in a reaction without appreciable bacterial fermentation from added microorganisms. The solid waste is converted into a liquefied waste and any residual solid waste. The resulting liquefied waste can be separated from said any residual solidified waste. See e.g., U.S. Patent No. 5,709,796.

Oral care products

[0414] Oral care product may comprise pectate lyases of the disclosure. Exemplary oral care products include tooth-pastes, dental creams, gels or tooth powders, odontics, mouth washes, pre- or post brushing rinse formulations, chewing gums, lozenges, or candy. See, e.g., U.S. Patent No. 6,264,925.

Brewing and fermenting

[0415] Methods of brewing (e.g., fermenting) beer may comprise pectate lyases of the disclosure. In one exemplary process, starch-containing raw materials are disintegrated and processed to form a malt. The pectate lyase is used at any point in the fermentation process. For example, the pectate lyases can be used in the processing of barley malt. The major raw material of beer brewing is barley malt. This can be a three stage process. First, the barley grain can be steeped to increase water content, e.g., to around about 40%. Second, the grain can be germinated by incubation at 15 to 25°C for 3 to 6 days when enzyme synthesis is stimulated under the control of gibberellins. In one aspect, the pectate lyases are added at this (or any other) stage of the process. The action of pectate lyases results in an increase in fermentable reducing sugars. This can be expressed as the diastatic power, DP, which can rise from around 80 to 190 in 5 days at 12°C. The pectate lyases can be used in any beer or alcoholic beverage producing process, as described, e.g., in U.S. Patent No. 5,762,991; 5,536,650; 5,405,624; 5,021,246; 4,788,066.

Other industrial applications

[0416] The disclosure also provides a method of increasing the flow of production fluids from a subterranean formation by removing a viscous, pectin-containing, damaging fluid formed during production operations and found within the subterranean formation which surrounds a completed well bore comprising allowing production fluids to flow from the well bore; reducing the flow of production fluids from the formation below expected flow rates; formulating an enzyme treatment by blending together an aqueous fluid and a polypeptide of the disclosure; pumping the enzyme treatment to a desired location within the well bore; allowing the enzyme treatment to degrade the viscous, pectin-containing, damaging fluid, whereby the fluid can be removed from the subterranean formation to the well surface; and wherein the enzyme treatment is effective to attack the pectin in cell walls.

[0417] The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples.

EXAMPLESEXAMPLE 1: Pectate lyase activity assays

[0418] The following example describes exemplary pectate lyase activity assays to determine the catalytic activity of a pectate lyase. These exemplary assays can be used to determine if a polypeptide is within the scope of the invention.

APSU unit viscosity assay

[0419] APSU units: The APSU unit assay is a viscosity measurement using the substrate polygalacturonic acid with no added calcium.

[0420] The substrate 5% polygalacturonic acid sodium salt (Sigma P-1879) is solubilized in 0.1 M glycine buffer pH 10. The 4 ml substrate is preincubated for 5 min at 40°C. The enzyme is added (in a volume of 250 µl) and mixed for 10 sec on a mixer at maximum speed, it is then incubated for 20 min at 40°C. For a standard curve double determination of a dilution of enzyme concentration in the range of 5 APSU/ml to above 100 APSU/ml with minimum of 4 concentrations between 10 and 60 APSU per ml. The viscosity can be measured using a MIVI 600™ (Sofraser, Villemandeur, France). The viscosity can be measured as mV after 10 sec. The GRAFPAD PRISM™ Prism program, using a non linear fit with a one phase exponential decay with a plateau, can be used for calculations. The plateau plus span is the mV obtained without enzyme. See, e.g., U.S. Patent No. 6,368,843.

Beta-elimination assay

[0421] An exemplary lyase assay (at 235 nm) for the determination of the beta-elimination activity measures increases in absorbance at 235 nm. The substrate 0.1% polygalacturonic acid sodium salt (Sigma P-1879) is solubilized in 0.1 M Glycine buffer pH 10. For calculation of the catalytic rate an increase of 5.2 absorbency at 235 units per min corresponds to formation of 1 µmol of unsaturated product (see, e.g., Nasuna (1966) J. Biol. Chem. 241:5298-5306; Bartling (1995) Microbiology 141:873-881). Steady state condition is measured using a 0.5 ml cuvette with a 1 cm light path on a HP diode array spectrophotometer in a temperature controlled cuvette holder with continuous measurement of the absorbency at 235 nm. For steady state a linear increase for at least 200 sec can be used for calculation of the rate. It is used for converting pmol per min product. See, e.g., U.S. Patent No. 6,368,843.

Agar Assay

[0422] Pectate lyase activity can be measured by an agar assay. A test solution is applied to 4 mm holes punched out in agar plates (e.g., LB agar), containing 0.7% w/v sodium polygalacturonate (Sigma P 1879). The plates are then incubated for 6 h at a particular temperature (e.g., 75°C.). The plates are then soaked in either (i) 1M CaCl₂ for 0.5 h or (ii) 1% mixed alkyl trimethylammonium Br (MTAB, Sigma M-7635) for 1 h. Both of these procedures cause the precipitation of polygalacturonate within the agar. Pectate lyase activity can be detected by the appearance of clear zones within a background of precipitated polygalacturonate. Sensitivity of the assay is calibrated using dilution of a standard preparation of pectate lyase.

[0423] Endpoint Analysis--Trans-elimination at 235 nm for Pectate Lyases (High Calcium Method: 1 mM Calcium in the Final Incubation Mixture). In this method, the substrate and enzyme is incubated for 20 min at 37°C followed by measurement at 235 nm of the formation of double bonds. Finally, the rate of the degradation is calculated based on the molar extinction coefficient in terms of Trans Units.

[0424] Procedure: Mixing of 0.5 ml enzyme dilution with 0.5 ml substrate solution. Substrate: Polygalacturonic acid from Sigma P-1879 lot 77H3784. Buffer 2x 0.1M Glycine pH 10+, 2.0 mmol CaCl₂, Stop reagent: 0.02 M H₃ PO₄, Temperature of incubation 37°C, Reaction time 20 min. Extinction coefficient of the trans-elimination 0.0052 µmol cm⁻¹. Enzyme diluted in ion-free water to 0.5 to 5 APSU per ml. Main value in duplicate 0.5 ml. The 2% w/v substrate in 2x.buffer is mixed with 0.5 ml diluted enzyme. Both pre-incubated 5 min on water bath at 37°C. Incubate for 20 min. Stop using 5 ml stop reagent and mix. Blank mix enzyme and stop reagent first and then add substrate all in the same volume.

Enzyme	0.5 ml
Substrate	0.5 ml
Stop	5 ml
Total volume	6 ml

Measure the absorbency at 235 nm in a 1 cm cuvette. Calculate the formation of trans-elimination per min using the extinction coefficient of $0.0052 \mu\text{mol cm}^{-1}$. See, e.g., U.S. Patent No. 6,368,843.

EXAMPLE 2: Cotton Bio-Scouring Application Assay

[0425] The following example describes an exemplary Cotton Scouring Application Assay using the pectate lyase enzymes of the invention. Use of the pectate lyases of the invention to hydrolyze primary cell wall pectin ("bioscouring") can eliminate the need for caustics and high temperatures in cotton fiber scouring.

Materials/ Preparation :

[0426]

- Requires 50 mM Sodium-Bicarbonate buffer at optimum pH
- 1:10 dilution of Calloway 1663 surfactant
- 50 mM Phosphate buffer pH 6
Mix 43.3 mL of 1.0 M Na-P monobasic, 6.6 mL of 1.0 M Na-P dibasic, adjust volume to 1 L with D.I. water. Adjust pH to 6
- Ruthenium Red (R-2751 SIGMA)
Add 0.5g of Ruthenium Red to the 1L Phosphate Buffer producing a final concentration of 0.05%.
- NaOAc (5g/L pH 5)
- Cotton fabric 400R (Testfabrics Inc.) which is desized prior scouring

Scouring Procedure:

[0427]

1. Place 1.0 g of desized cotton fabric (into each Labomat beaker).
2. Each experiment should use a blank, untreated cotton (no enzyme added).
3. Add 50 mL of 50 mM Sodium-Bicarbonate buffer at pH 8.5-9 to each beaker and 2.5 mL of 1:10 dilution of Calloway Surfactant 1663.
4. Tighten the lids using an Allen wrench and install the beakers into the Labomat. making sure that the beakers are distributed evenly on the rotary rack. Connect beaker 1 with the temperature detecting cable to the connector in the middle of the rack.
5. Ramp up the heat to the desired temperature and hold for 10 minutes.
6. Add 50-200 uL of enzyme (e.g., a pectate lyase of the invention) at a concentration previously diluted to 0.1 ug/uL through septum in the beaker using a syringe. Total enzyme concentration used to scour 1 gram cotton fabric can be between 5-20 ug.
7. Run the reaction in the Labomat at temperature for 15 minutes.
8. Rinse the cotton fabric twice by pouring the cotton fabric into the hand and squeezing the cotton dry, place the cotton back into the beaker and filling the beaker with D.I. water and repeating this step again, finish with squeezing the excess water out of the cotton.
9. Soak the cotton fabric in NaOAc (5g/L pH 5) for 2 minutes.
10. Repeat the 2X rinse cycle in step 8.
11. Place the cotton fabric on weigh-boats and allow the fabric to dry overnight in the laminar flow biohoods.

Dyeing Procedure:

[0428]

1. Place the treated cotton fabric in the Labomat beakers.
2. Add 100 mL of 0.05% Ruthenium Red, Na-P buffer pH 6 to each beaker.
3. Tighten the lids using an Allen wrench and install the beakers into the Labomat making sure that the beakers are distributed evenly on the rotary rack. Connect beaker 1 with the temperature detecting cable to the connector in the middle of the rack.
4. Ramp up the heat to 50°C and hold for 30 minutes.
5. Rinse the fabric twice by pouring the cotton into the hand and squeezing the cotton dry, place the fabric back into the beaker and filling the beaker with D.I. water and repeating this step again, finish with squeezing the excess water out of the fabric.

6. Place the dyed fabric into the beaker and add 100 mL of D.I. water.
7. Tighten the lids using Allen wrench and install the beakers into the Labomat making sure that the beakers are distributed evenly on the rotary rack.
8. Ramp up the heat to 100°C and hold for 10 minutes; cool the beakers down to 60°C.
9. Repeat the 2X rinse cycle in step 5.
10. Place the dyed fabric on weigh-boats and allow the fabric to dry overnight in the laminar flow biohoods.

Enzyme Scouring Quantification:

[0429]

1. Calibrate the GretagMacbeth Color Eye 7000A by selecting the Color Eye Icon on the desk top of the computer.
2. Place the black lens over the orifice and hit enter when the program request the calibration of the negative threshold.
3. Place the white filter over the orifice and hit enter when the program request the calibration of the white balance.
4. Place the dry dyed fabric over the orifice and push F4 to read the fabric whiteness.
5. Record the L* number, turn the fabric over to read the other side and record the L* number. Compute the average L* number for each sample.
6. Graph the delta L for each cotton scoured sample by subtracting the samples L* number with the untreated fabric L*.

EXAMPLE 3: A single-bath process for desizing and scouring

[0430] The following example describes an exemplary single-bath process for desizing and scouring. The invention provides methods and compositions for desizing, scouring and bleaching of cellulosic materials by contacting the cellulosic materials simultaneously or sequentially in a single-bath process with an enzyme system comprising a pectate lyase of the invention. The single-bath process can further comprise a bleaching system comprising hydrogen peroxide or at least one peroxy compound which generates hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator.

[0431] Cellulosic materials including crude fibers, yarn, or woven or knit textiles, made of cotton, linen, flax, ramie, rayon, hemp, jute, or blends of these fibers with each other or with other natural or synthetic fibers, can be treated by this single-bath process of the invention. In one aspect, a fabric weighing is loaded into a container, which is subsequently filled with a buffer solution (e.g., 20 mM Na phosphate buffer, pH 9.2) comprising a pectate lyase of the invention (e.g., 3000 APSU/kg-fiber of pectate lyase), wetting agent (e.g., 0.5 g/L), H₂O₂ (e.g., 1.7 g/L) and stabilizer (e.g., 0.75 g/L). The fabric can be treated, e.g., at 55°C for about 15 min, after which temperature was raised at 5°C/min to 70°C for 1 h. The fabric is then washed thoroughly with water to remove the residual chemicals and dried at room temperature overnight.

EXAMPLE 4: Assay for detecting thermotolerant enzymes

[0432] The following example describes an exemplary assay for detecting thermotolerant enzymes that can be used to determine if an enzyme is within the scope of the invention. This example describes an absorbance based screening ("discovery") assay for detecting thermotolerant enzymes, which, in one aspect, can be characterized as "up-mutants" from a "parental" pectate lyase gene. This exemplary protocol can be used for variants, or mutants, generated by either the GSSM™ or combinatorial methods.

Materials and preparations

[0433]

- a. Polygalacturonic Acid (PGA), and 2% [Sigma P-3889]
- b. UV (friendly) 96 Well Flat Bottom Plates [Thomson Instrument 931801B]
- c. COSTAR 96 Well Plates
- d. Adhesive PCR Foil Seals [Marsh AB-0626]
- e. B-PER [PIERCE 78248]
- f. LBamp100 or LBcarb100
- g. TRIS pH 8.0 (250 mM, 10X)
- h. Glycine (250 mM, 10X)
- i. 0.2% Polygalacturonic Acid Substrate for enzyme activity detection: 100 mL of each of the following: 10X Tris,

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10X Glycine, and 2% PGA, plus 700 mL of holy water.

j. Plates: aliquot 200 μ L of medium, LBamp100 or LBcarb100, into the wells of both the COSTAR and the UV friendly 96 well flat bottom plates

Colony Picking and plate replication

[0434] GSSM™ or combinatorial mutant clones colonies were picked with an Autogen (Framingham, MA) colony picker and the cells were inoculated into LBamp100 medium. A total of 168 GSSM™ clones were screened per residue site or 13,000 clones from the combinatorial library 2328 were screened. The mutated clones were picked into rows A, B, C, E, F, G, and H of the 96 well plate. Wild Type (wt) clone (SEQ ID NO:132, encoded by SEQ ID NO:131) were picked into row D as a control. After completing the colony picking, the plates were incubated overnight (approximately 18 hrs) at 37°C, shaking at 150 RPM. These plates will be referred to as the master plates from now on.

[0435] Copies of each master plate were made into UV friendly plates (now called "assay plates") using the automated plate replicator. Once replication was complete, the assay plates were placed in a humidified 30°C incubator overnight.

Primary Assay

[0436] Cell densities in each well of all plates were determined at OD₆₀₀ using a SPECTRAMAX™ (Molecular Devices Corporation, Sunnyvale CA) system. All assay plates were then sealed with PCR Foil Seals and then spun at 2200 rpm in an Eppendorf centrifuge for 10 minutes. Using the PowerWasher system, the supernatant was then aspirated out of the assay plates leaving only the cells behind. 20 μ L of B-PER™ (Pierce Biotechnology, Rockford, IL) was then added to each well and the assay plates were resealed. The plates were then placed on a plate shaker for 10 minutes in order to ensure proper cell lyses. The plates were then placed in an incubator preheated to 50°C for 50 minutes for the GSSM™ assay or 70°C for 25 minutes for the combinatorial up-mutant assay. The assay plates were then removed from the incubator after the proper heat challenge time and quickly cooled to room temperature. The SPECTRAMAX™ was used to read kinetics at wavelength 235 nm over a 2 minute period. Any putative hit that performed better than wild type was broken out for a secondary assay. Figure 8 illustrates a residue with multiple positive hits. In Figure 8, Row D contains the residual activity of the wild type (wt), SEQ ID NO:132, and rows A, B, C, E, F, G, H are the GSSM™ clones of mutation site 182.

Secondary Assay

[0437] All wells that showed an improved enzymatic rate compared to the wild type performance were identified and the clones from the respective master plate were broken out. Using aseptic techniques, a sterile toothpick was used in the well of a putative hit from the master plate. Cells adhering to the toothpick were transferred to a new plate selecting a new well with 200 μ L LBamp100. Also, in the same manner, row D was filled with WT for each break out plate. The secondary master plates were placed in the 30°C humidified incubator overnight. The secondary master plates were then pin tooled into UV friendly 96 well plates. The secondary assay plates were then placed in a 30°C humidified incubator overnight. Cell densities in each well of all plates were determined at OD₆₀₀ using the SpectraMax systems. All assay plates were then sealed with PCR Foil Seals and then spun at 2200 rpm in an Eppendorf Centrifuge for 10 minutes. The remaining steps of the secondary assay were the same as indicated for the primary assay. Any confirmed hits that performed better than wild type were broken out and tested again in the tertiary assay.

Tertiary Assay

[0438] 5 μ L of culture from wells that confirmed improved thermotolerance activity from the wild type clone were aliquotted onto a small LBcarb100 petri dish to make streak plates. 5 μ L of one of the control "wild type" (wt) (SEQ ID NO:132, encoded by SEQ ID NO:131) wells was also used to make a streak plate. The streak plates were incubated at 37°C overnight. A small section of an individual colony was scraped and the cells were inoculated 5 mL of LBcarb100. The culture was allowed to grow overnight at 37°C at 200 RPM. The confirmed hit was then diluted to OD₆₀₀ = 0.2. 200 μ L of a confirmed clone was aliquotted into a well, filling an entire row on the 96 well UV friendly plate. The same was done for a wt control. All plates were then sealed and spun at 2000 rpm in the Eppendorf Centrifuge for 10 minutes. The remaining steps of the tertiary assay were the same as indicated for the primary assay. At the end, all putative hits that performed better than wild type were sent for sequencing. Glycerol stocks were also prepared.

EXAMPLE 5: Processes and formulations for enzymes of the invention

[0439] The following example describes exemplary processes (e.g., a bioscouring process) and formulations of the

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invention. Compositions and processes were tested using the pectate lyase having a sequence as set forth in SEQ ID NO:134, encoded by, e.g., SEQ ID NO:133 ("SEQ ID NO:134").

Definition of unit:

[0440] Pectate lyase activity (of SEQ ID NO:78) was routinely measured using 0.2% (w/v) polygalacturonic acid (Sigma, P3850) in 25mM TrisHCl-25mM Glycine NaOH buffer. One unit of enzyme activity was defined as the amount of protein that produced 1 μ mol of unsaturated oligogalacturonides per minute equivalent to 1 μ mol of unsaturated digalacturonide, using molecular extinction coefficient value of 4600 M⁻¹cm⁻¹ at 235 nm for dimer.

[0441] SpectraMax instrumentation in 96-well UV plates was used.

Formulation strength:

[0442] The enzymes of the invention can be formulated in any dosage to suit a particular need; assays for determining the optimal dosage for any particular formulation are known in the art, and several are described herein. In alternative aspects, formulations of the invention can have a low strength of between about 2000 to 4000 u/ml (where u = unit). This is comparable to the other products on the market. In one aspect, the formulation minimum is about 1000 u/ml, and, in another aspect, the formulation maximum is about 10,000 u/ml, e.g., the formulation can comprise an enzyme of the invention in an amount of between about 1000 u/ml and 10,000 u/ml.

[0443] Solubility studies with lyophilized product (SEQ ID NO: 134) resuspended in water indicated that the solubility of the enzyme can be as high as 25000 u/ml at 4°C. Therefore, in one aspect, the invention provides formulations having a level as high as about 25000 u/ml, or more. In one aspect the invention provides formulations comprising an enzyme of the invention in an amount of between about 100 u/ml and 25000 u/ml, 30000 u/ml, 35000 u/ml or 40000 u/ml, or more.

Formulation design:

[0444] The invention provides formulations comprising at least one enzyme of the invention, and, in alternative aspects, further comprising any additive(s). Formulations of the invention can be based on known additives in other, e.g., analogous, enzyme formulations. For example, formulations of the invention can comprise the additives and/or conditions set forth in Tables 3, 4, 5 and 6, below, or any variation thereof. For example, formulations of the invention can comprise glycerol, sucrose, sodium chloride, Dextrin, propylene glycol, sorbitol, sodium sulphate or TRIS, or an equivalent.

[0445] In one aspect, a formulation of the invention can be a water based formulation, or, an oil-based formulation.

[0446] Two phases of formulation stability studies were conducted; these studies used the exemplary enzyme SEQ ID NO:134:

Accelerated stability study at 37°C

[0447] Note: these are buffer based formulations.

- Screen various additives
- Test different pH values
- Formulations at approximately 2000 u/ml.
- SEQ ID NO:78 was the exemplary enzyme tested

Table 3 (SN = sample number)

SN	pH	Glycerol	Sucrose	Sodium chloride	Dextrin	Propylene glycol	sorbitol	Sodium sulphate	Effective TRIS Conc.
1	5								40mM
2	6								40mM
3	7								40mM
4	8								40mM
5	7.5	35%							20mM

(continued)

SN	pH	Glycerol	Sucrose	Sodium chloride	Dextrin	Propylene glycol	sorbitol	Sodium sulphate	Effective TRIS Conc.
6	7.5	50%							20mM
7	7.5		35%						20mM
8	7.5			20%					20mM
9	7.5				10%				20mM
10	7.5					30%			20mM
11	7.5							100mM	20mM
12	7.5						35%		20mM
13	5.5	35%							40mM
14	5.5	50%							40mM

[0448] Best performing formulations (using SEQ ID NO:134 as an exemplary enzyme of the invention) based on physical appearance and retention of greater than 80% activity:

Table 4

Formulation	Additive
1	pH 5.0, 40mM TRIS
3	pH 7.0, 40mM TRIS
4	pH 8.0, 40mM TRIS
6	pH 7.5, 50% glycerol
8	pH 7.5, 20% NaCl
10	pH 7.5, 30% propylene glycol
11	pH 7.5, 100mM sodium sulfate
13	pH 5.5, 35% glycerol

[0449] In alternative aspects, the formulations of the invention can be at approximately 10,000 u/ml, or in an amount of between about 100 u/ml, 200 u/ml, 300 u/ml, 400 u/ml or 500 u/ml and 10,000 u/ml, 15,000 u/ml, 20,000 u/ml, 25000 u/ml, 30000 u/ml, 35000 u/ml or 40000 u/ml, or more. In alternative aspects, the formulations of the invention can be at approximately 500 to 30,000 units/ml, 1000 to 25,000 units/ml, or, between about 1000 to 20,000 units/ml, 1000 to 15,000 units/ml, 1000 to 10000 units/ml, 1000 to 5000 units/ml, between about 2000 to 20000 units/ml, between about 2000 to 15000 units/ml, between about 2000 to 10000 units/ml, or between about 2000 to 4000 units/ml. In alternative aspects, the formulations of the invention can comprise a water-based formulation, e.g., when no buffer is feasible; any water-based buffer system can be used.

Table 5:

PECTATE LYASE FORMULATION STABILITY STUDY PHASE II					
Formulation No.	pH	Buffer	ADDITIVES		
1	pH 7.0	NA			0.1% sodium benzoate, 0.1 % potassium sorbate
2	pH 7.0	NA			300 ppm proxel
3	pH 7.0	NA	sodium chloride 15%		0.1% sodium benzoate, 0.1% potassium sorbate
4	pH 7.0	NA	sodium chloride 15%		300 ppm proxel

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(continued)

PECTATE LYASE FORMULATION STABILITY STUDY PHASE II

Formulation No.	pH	Buffer	ADDITIVES		
5	pH 7.0	NA		glycerol 35%	0.1% sodium benzoate, 0.1% potassium sorbate
6	pH 7.0	NA		glycerol 35%	300 ppm proxel
7	pH 7.0	NA	sodium chloride 10%	glycerol 25%	0.1% sodium benzoate, 0.1 % potassium sorbate
8	pH 7.0	NA	sodium chloride 10%	glycerol 25%	300 ppm proxel
9	pH 5.5	NA			0.1 % sodium benzoate, 0.1 % potassium sorbate
10	pH 5.5	NA		300 ppm proxel	
11	pH 5.5	NA	sodium chloride 15%		0.1% sodium benzoate, 0.1 % potassium sorbate
12	pH 5.5	NA	sodium chloride 15%		300 ppm proxel
13	pH 5.5	NA		glycerol 35%	0.1% sodium benzoate, 0.1 % potassium sorbate
14	pH 5.5	NA		glycerol 35%	300 ppm proxel
15	pH 5.5	NA	sodium chloride 10%	glycerol 25%	0.1% sodium benzoate, 0.1% potassium sorbate
16	pH 5.5	NA	sodium chloride 10%	glycerol 25%	300 ppm proxel
CONTROLS					
17	pH 7.0	TRIS		glycerol 35%	0.1% sodium benzoate, 0.1 % potassium sorbate
18	pH 5.5	TRIS		glycerol 35%	0.1% sodium benzoate, 0.1 % potassium sorbate
19	pH 7.0	Acetate		glycerol 35%	0.1% sodium benzoate, 0.1 % potassium sorbate
20	pH 5.5	Acetate		glycerol 35%	0.1% sodium benzoate, 0.1 % potassium sorbate

[0450] Additional buffers that can be used in a formulation of the invention: 20 mM MOPS, pH 7 or 25 mM MOPS, 50 mM NaCl, pH 7.5.

[0451] Best performing formulations (using SEQ ID NO:134 as an exemplary enzyme of the invention) based on physical appearance and retention of greater than 80% activity:

Table 6	
Formulation No	Details
5	pH 7, 35 % glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate
6	pH 7, 35% glycerol, 300 ppm proxel
7	pH 7, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate
8	pH 7, 10% sodium chloride, 25% glycerol, 300 ppm proxel

(continued)

Table 6	
Formulation No	Details
13	pH 5.5, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate
14	pH 5.5, 35% glycerol, 300 ppm proxel
15	pH 5.5, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate
20*	20mM acetate buffer, pH 5.5, 35% glycerol

[0452] For example, the invention provides formulations comprising at least one enzyme of the invention and comprising a buffer (formulation) of: pH 7, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 35% glycerol, 300 ppm proxel; pH 7, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 10% sodium chloride, 25% glycerol, 300 ppm proxel; pH 5.5, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 5.5, 35% glycerol, 300 ppm proxel; pH 5.5, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; or, 20mM acetate buffer, pH 5.5, 35% glycerol; 20 mM MOPS, pH 7 or 25 mM MOPS, 50 mM NaCl, pH 7.5; pH 5.0, 40mM TRIS; pH 7.0, 40mM TRIS; pH 8.0, 40mM TRIS; pH 7.5, 50% glycerol; pH 7.5, 20% NaCl; pH 7.5, 30% propylene glycol; pH 7.5, 100mM sodium sulfate; pH 5.5, 35% glycerol; or, any combination thereof, or, with equivalents thereof.

Exemplary Bioscouring Application

[0453]

- In one aspect, pH is pH 8.5 (bicarbonate buffer)
- Non-ionic wetting agent (1 g/L) [e.g.: Apollowet NFW]
- Liquor ratio in the enzyme bath: 10:1 to 50:1 (L liquor:kg fabric)
- Enzyme dose: 0.137 ml of the concentrated extract per kg of fabric
- Temperature range: between about 50°C to 70°C
- Treatment time about 20 min
- Chelants should be excluded from the enzyme bath, and should only be added after 20 minutes of enzyme treatment and retained for 10 minutes before discharging bath

[0454] Thus, the invention provides a bioscouring process using at least one enzyme of the invention comprising at least one, several or all of the following steps/ limitations: pH is pH 8.5, in bicarbonate buffer, comprising a non-ionic wetting agent (at, e.g., 1 g/L), where the liquor ratio in the enzyme bath is between about 10:1 to 50:1 (L liquor:kg fabric), where the enzyme dose is between about 0.1 and 0.2 ml, e.g., at about 0.137 ml of the concentrated extract per kg of fabric, at a temperature range: between about 50°C to 70°C; with a treatment time about 20 min; and, in one aspect, comprising chelants, which should be excluded from the enzyme bath and should only be added after 20 minutes of enzyme treatment and retained for 10 minutes before discharging bath.

[0455] The enzyme SEQ ID NO:134 performed well in the range of 5 to 25 grams of pure enzyme per ton of treated fabric.

SEQUENCE LISTING

[0456]

<110> verenum Corporation

<120> PECTATE LYASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

<130> P/50529.ep02

<140> Divisional of EP 04758802.5

<141> 2004-04-02

<150> 60/460,842

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<151> 2003-04-04

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<151> 2003-07-03

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tatgagatcg	gcactaatcg	tccgatcttc	gccgggctg	acggcgctcg	taagtatgat	1800
gtgaaacaga	tcgatgagga	acgacgaaag	aattacgcgt	ggtacgttga	cgacgcagcg	1860
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<210> 2

<211> 638

<212> PRT

<213> Unknown

<220>

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<223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(21)

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<221> DOMAIN
 <222> (28)...(308)
 <223> Pectin methyl esterase domain

<221> DOMAIN
 <222> (309)...(638)
 <223> Catalytic domain

<400> 2

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Met Ser Leu Phe Arg Lys Leu Ala Leu Leu Val Leu Cys Gly Leu Leu
 1      5      10      15
Leu Ser Val Gly Ala Glu Thr Arg Ala Ser Lys Arg Ile Val Val Ala
 20      25      30      35
Ala Asp Gly Ser Gly Asp Val Arg Thr Ile Gln Gln Ala Val Asp Gln
 40      45      50      55
Val Pro Lys Asp Asn Thr His Pro Val Leu Ile Gln Ile Lys Pro Gly
 60      65      70      75
Val Tyr Gln Glu Gln Val Arg Val Ala Ala Gly Lys Arg Phe Ile Thr
 80      85      90      95
Phe Arg Gly Asp Asp Ala Ser Lys Thr Val Ile Thr Tyr Arg Leu Ser
100      105      110      115
Ala Leu Gln Ala Gly Asn Thr Arg Leu Ala Phe Thr Thr Phe Val Asn
120      125      130      135
Ala Asp Asp Phe Arg Ala Glu Asn Leu Thr Phe Glu Asn Ser Phe Gly
140      145      150      155
Thr Gly Ser Gln Ala Val Ala Leu Phe Val Asp Ala Asp Arg Ala Thr
160      165      170      175
Phe Glu Asn Cys Arg Phe Leu Gly Trp Gln Asp Thr Leu Phe Val Asn
180      185      190      195
Gly Ser Arg His Phe Phe Lys Asp Cys Tyr Val Glu Gly His Val Asp
200      205      210      215
Phe Ile Phe Gly Thr Ala Ser Ala Val Phe Glu Asn Cys Thr Ile His
220      225      230      235
Ser Lys Gly Glu Gly Tyr Val Thr Ala His Tyr Arg Thr Ser Asp Glu
240      245      250      255
Met Asp Thr Gly Phe Val Phe His Arg Cys Arg Leu Thr Gly Arg Asp
260      265      270      275
Thr Gly Arg Gly Val Tyr Leu Gly Arg Pro Trp Arg Pro Tyr Ala Arg
280      285      290      295
Val Val Phe Ile Asp Cys Trp Leu Asp Ala His Ile Arg Pro Glu Gly
300      305      310      315
Trp Asp Asn Trp Arg Asp Pro Glu Arg Glu Lys Thr Ala Trp Phe Ala
320      325      330      335
Glu Tyr Lys Ser Lys Gly Pro Gly Ala Asn Pro Val Ala Arg Val Ala
340      345      350      355
Trp Ser Arg Gln Leu Thr Thr Glu Gln Ala Ala Glu Phe Ser Arg Glu
360      365      370      375
Arg Phe Phe Ser Arg Ala Val Arg Gly Leu Ser Gly Gln Ala Asn Gln
380      385      390      395
Ala Val Gly Thr Ile Ala Trp Asp Asp Ala Gln Lys Lys Pro Asn Glu
400      405      410      415
Trp Tyr Ala Ser Ala Glu Ala Leu Arg Ile Ala Asp Asn Val Val Leu
420      425      430      435
Tyr Gln Arg Asp Ser Gly Gly Trp Pro Lys Asn Ile Asp Met Gly Lys
440      445      450      455
Pro Leu Asp Glu Lys Gly Arg Ala Gly Leu Leu Arg Val Arg Lys Lys
460      465      470      475
Asn Asp Ser Thr Ile Asp Asn Gly Ala Thr Tyr Thr Gln Leu Ser Phe
480      485      490      495
Leu Ala Arg Val Tyr Thr Ala Gln Lys Gln Glu Arg His Arg Glu Ser
500      505      510      515

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	Phe	Leu	Lys	Gly	Leu	Asp	Tyr	Leu	Leu	Lys	Ala	Gln	Tyr	Pro	Asn	Gly	
				420					425					430			
	Gly	Trp	Pro	Gln	Phe	Tyr	Pro	Asn	Leu	Asn	Gly	Tyr	Tyr	Lys	His	Ile	
			435					440					445				
5	Thr	Phe	Asn	Asp	Asn	Ala	Met	Ile	Gly	Val	Met	Lys	Leu	Leu	Arg	Asp	
		450					455					460					
	Val	Ala	Thr	Ala	Lys	Pro	Ala	Tyr	Ala	Phe	Val	Asp	Glu	Ala	Arg	Arg	
	465					470				475					480		
	Thr	Ser	Ala	Ala	Lys	Ala	Val	Glu	Lys	Gly	Ile	Glu	Cys	Ile	Leu	Lys	
				485						490					495		
10	Thr	Gln	Val	Val	Val	Asn	Gly	Arg	Arg	Thr	Val	Trp	Cys	Ala	Gln	His	
				500					505					510			
	Asp	Glu	Val	Thr	Leu	Ala	Pro	Ala	Pro	Ala	Arg	Thr	Phe	Glu	Leu	Val	
			515					520					525				
	Ser	Leu	Ser	Gly	Gly	Glu	Ser	Val	Glu	Ile	Val	Arg	Phe	Leu	Met	Ser	
		530					535					540					
15	Ile	Lys	Asn	Pro	Ser	Pro	Ala	Val	Val	Glu	Ala	Ile	Glu	Ser	Ala	Val	
	545					550					555					560	
	Ala	Trp	Phe	Glu	Gln	Ser	Gln	Val	Lys	Asp	Pro	Ala	Gly	Lys	Pro	Ala	
				565						570					575		
	Trp	Ala	Arg	Phe	Tyr	Glu	Ile	Gly	Thr	Asn	Arg	Pro	Ile	Phe	Ala	Gly	
				580					585					590			
20	Arg	Asp	Gly	Val	Val	Lys	Tyr	Asp	Val	Lys	Gln	Ile	Asp	Glu	Glu	Arg	
			595					600					605				
	Arg	Lys	Asn	Tyr	Ala	Trp	Tyr	Val	Asp	Asp	Ala	Ala	Lys	Leu	Leu	Lys	
		610					615				620						
	Thr	Asp	Tyr	Pro	Glu	Trp	Lys	Glu	Lys	Asn	Ala	Lys	Asp	Gln			
25	625					630					635						

<210> 3

<211> 1416

<212> DNA

30 <213> Unknown

<220>

<223> Obtained from an environmental sample

35 <400> 3

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	atcgtgaaac	gcatcaagcg	acctcgtttc	ccgatgcgca	cgtttgatct	cacggagttt	180
40	ggagcgaag	gtgatggacg	aacagattgc	acgttggtct	tccgtcgcgc	gatcgatcga	240
	tgcacgaacg	ccggtggttg	gagagtagtt	gttccaccgg	gttcgtatct	cactggcgcc	300
	attcatttga	agagcaacgt	cgaccttcac	atctcagaag	gtactacggg	caagttcagc	360
	cagaaccgga	aagactacct	gcccgttggt	ttctcgcgtt	gggaaggcgt	cgagggtgtc	420
	aactactcgc	cttttatcta	cgcttctgaa	caaacgaaca	ttgcgatcac	tggcaagggc	480
	acgctcaacg	gtcaaagcga	caacgaacac	tgggtggcct	ggaacggacg	tgccgcgtac	540
45	ggctggaaag	aagggatgag	caatcagcgt	cccgatcgaa	atgcgctggt	tgcgatggcc	600
	gaaaaaggtg	tcccgtttca	ggagcgcatt	tttggtgagg	gccattactt	aaggccgcag	660
	ttcattcaac	cttatcgttg	tgagaacgtg	ctgatcgaag	gtgtcactat	tcgaaactcg	720
	ccgatgtggg	aaattcatcc	ggtgctctgc	cggaatgtca	tcgtccaaaa	tgtgatcatc	780
	aacagtcattg	gtccaaacaa	cgacgggtgt	aatcctgagt	cgtgcacgga	tgtgttgatt	840
	aaggattgtg	acttcgacac	tggtagcatg	tgtatcgaga	tcaagtcagg	ccgaaatgca	900
50	gatgggcggc	gactgaaggc	tcctactgaa	aacattatcg	tgactgggtg	tcgcatgaaa	960
	gatggtcacg	gcgggattac	ggtgggcagc	gagatttcgg	gtgggggtgc	aaatcttttc	1020
	gcatccaactg	gccggctcga	cagtcggaac	ctggaccatg	cattgcgggt	taagaataac	1080
	gctatgcgtg	gcgggctggt	ggagaatctg	cacttccgaa	atatcgacgt	cgggcaagtg	1140
	gcgcacgcgg	tgatcacgat	cgatttcaat	tatgaggaag	gcgcgaaggg	atcggttcacg	1200
	ccagtcgttc	gtgattacac	cgctgatggc	cttcgcagca	cgaaaagtaa	gtacgcgctc	1260
55	gatgtgcagg	gcttggcgac	ggcgccgatc	gtgaatctgc	gtctaaccac	ctgcattctc	1320
	gacaatgtcg	ctgaaggaaa	tgttgtgaag	aacgtaaagg	atgcaactat	cgagaatgtc	1380
	aaaatcaatg	gaaaaagcgt	tgatgcagtg	ccgtag			1416

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<210> 4
<211> 471
<212> PRT
<213> Unknown

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<220>
<223> Obtained from an environmental sample

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<221> SIGNAL
<222> (1)...(28)

15

<221> DOMAIN
<222> (81)...(476)
<223> Catalytic domain

<400> 4

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	Met	Ser	Ser	Arg	Arg	Glu	Phe	Ile	Arg	Asp	Leu	Leu	Thr	Gly	Gly	Ala
	1				5					10					15	
	Leu	Ile	Ala	Val	Ala	Pro	Arg	Leu	Ser	Ala	Phe	Ala	Ala	Glu	Glu	Asn
				20					25					30		
5	Pro	Trp	Glu	Thr	Val	Met	Pro	Ser	Ile	Val	Lys	Arg	Ile	Lys	Arg	Pro
			35					40					45			
	Arg	Phe	Pro	Met	Arg	Thr	Phe	Asp	Leu	Thr	Glu	Phe	Gly	Ala	Lys	Gly
		50					55					60				
	Asp	Gly	Arg	Thr	Asp	Cys	Thr	Leu	Ala	Phe	Arg	Arg	Ala	Ile	Asp	Arg
	65					70					75					80
10	Cys	Thr	Asn	Ala	Gly	Gly	Gly	Arg	Val	Val	Val	Pro	Pro	Gly	Ser	Tyr
					85					90					95	
	Leu	Thr	Gly	Ala	Ile	His	Leu	Lys	Ser	Asn	Val	Asp	Leu	His	Ile	Ser
				100					105					110		
	Glu	Gly	Thr	Thr	Val	Lys	Phe	Ser	Gln	Asn	Pro	Lys	Asp	Tyr	Leu	Pro
			115					120					125			
15	Val	Val	Phe	Ser	Arg	Trp	Glu	Gly	Val	Glu	Val	Phe	Asn	Tyr	Ser	Pro
		130					135					140				
	Phe	Ile	Tyr	Ala	Phe	Glu	Gln	Thr	Asn	Ile	Ala	Ile	Thr	Gly	Lys	Gly
	145					150					155					160
	Thr	Leu	Asn	Gly	Gln	Ser	Asp	Asn	Glu	His	Trp	Trp	Pro	Trp	Asn	Gly
					165					170					175	
20	Arg	Ala	Ala	Tyr	Gly	Trp	Lys	Glu	Gly	Met	Ser	Asn	Gln	Arg	Pro	Asp
				180					185					190		
	Arg	Asn	Ala	Leu	Phe	Ala	Met	Ala	Glu	Lys	Gly	Val	Pro	Val	Gln	Glu
			195					200					205			
	Arg	Ile	Phe	Gly	Glu	Gly	His	Tyr	Leu	Arg	Pro	Gln	Phe	Ile	Gln	Pro
		210					215					220				
25	Tyr	Arg	Cys	Glu	Asn	Val	Leu	Ile	Glu	Gly	Val	Thr	Ile	Arg	Asn	Ser
	225					230					235					240
	Pro	Met	Trp	Glu	Ile	His	Pro	Val	Leu	Cys	Arg	Asn	Val	Ile	Val	Gln
					245					250					255	
	Asn	Val	Ile	Ile	Asn	Ser	His	Gly	Pro	Asn	Asn	Asp	Gly	Cys	Asn	Pro
				260					265					270		
30	Glu	Ser	Cys	Thr	Asp	Val	Leu	Ile	Lys	Asp	Cys	Asp	Phe	Asp	Thr	Gly
			275					280					285			
	Asp	Asp	Cys	Ile	Ala	Ile	Lys	Ser	Gly	Arg	Asn	Ala	Asp	Gly	Arg	Arg
		290					295					300				
	Leu	Lys	Ala	Pro	Thr	Glu	Asn	Ile	Ile	Val	Thr	Gly	Cys	Arg	Met	Lys
						310					315					320
35	Asp	Gly	His	Gly	Gly	Ile	Thr	Val	Gly	Ser	Glu	Ile	Ser	Gly	Gly	Val
					325					330					335	
	Arg	Asn	Leu	Phe	Ala	Ser	Asn	Cys	Arg	Leu	Asp	Ser	Pro	Asn	Leu	Asp
				340					345					350		
	His	Ala	Leu	Arg	Val	Lys	Asn	Asn	Ala	Met	Arg	Gly	Gly	Leu	Leu	Glu
			355					360					365			
40	Asn	Leu	His	Phe	Arg	Asn	Ile	Asp	Val	Gly	Gln	Val	Ala	His	Ala	Val
							375					380				
	Ile	Thr	Ile	Asp	Phe	Asn	Tyr	Glu	Glu	Gly	Ala	Lys	Gly	Ser	Phe	Thr
						390					395					400
	Pro	Val	Val	Arg	Asp	Tyr	Thr	Val	Asp	Gly	Leu	Arg	Ser	Thr	Lys	Ser
					405					410					415	
45	Lys	Tyr	Ala	Leu	Asp	Val	Gln	Gly	Leu	Ala	Thr	Ala	Pro	Ile	Val	Asn
					420				425					430		
	Leu	Arg	Leu	Thr	Asn	Cys	Ile	Phe	Asp	Asn	Val	Ala	Glu	Gly	Asn	Val
			435					440					445			
	Val	Lys	Asn	Val	Lys	Asp	Ala	Thr	Ile	Glu	Asn	Val	Lys	Ile	Asn	Gly
50																

		450			455			460
	Lys	Ser	Val	Asp	Ala	Val	Pro	
	465					470		

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 <210> 5
 <211> 1077
 <212> DNA

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<213> Unknown

<220>

<223> Obtained from an environmental sample

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<400> 5

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10	gtgatccctt	atcaacgcga	caacggtggt	tggccgaaga	atatcgacat	ggccgccatg	180
	ctcatgcagg	cagaacgcga	aaaacttagt	cgcgagaaga	gcgagaccga	cacgacaatc	240
	gacaacggcg	cgacgacaac	ccagctcgcg	tatctggcga	aggatcatcac	ggccaagaat	300
	atcgaaagcc	atcgcgtcgc	gtttttcaaa	ggcctcgatt	ttcttttcgc	catgcagtac	360
	gggaatggcg	gcttcccgca	attttttcct	ctgcgtgacg	attatttcgcg	cgagattacg	420
	ttcaacgaca	acgcgatgat	aaatgtgctt	cggttgctcc	gcgacatagc	cgatcgaaag	480
15	aacgattatg	tgtttgtcga	tgaagagcgg	cgagcgaagg	ccgagcaggc	tgtaaggcgt	540
	gcatcccggt	tgatcctcag	cacgcaggtc	gtcgtcgatg	gaaagaaaac	cgtctgggct	600
	gcgcagtatg	atgagaagac	attgaagccg	gccgcggcgc	gaaagtctga	gccggcatca	660
	ttgaccgccg	gcgagagcgt	tggcatcgtc	cggtttttga	tgctagaaaa	accaacaccc	720
	gagatcatta	acgcgatcga	atccgccatc	gcttggtaca	aggcgaacaa	catctcggga	780
	cttaggtggg	agaggcgaaa	cggcgagaac	attgtgatca	aagacaagaa	cgcgccgccc	840
20	gtctgggccc	gcttttatca	gatcgaaacg	atgaggccga	tcttcgccgg	tcgcgatgcg	900
	gtcatcagat	acgatgtgat	gcagatcgag	tcggaacgtc	gaaacggata	tgcatggtac	960
	gtatccgaac	cgaatgagtt	gttgaatgaa	gattatccga	agtggaggac	aaggagtgcg	1020
	aagcgtgccc	agatctttca	acgtccgcct	cttggttcga	gatttcggac	cgtgtag	1077

25 <210> 6

<211> 358

<212> PRT

<213> Unknown

30 <220>

<223> Obtained from an environmental sample

<221> DOMAIN

<222> (1)...(358)

35 <223> Catalytic domain

<400> 6

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Met Asp Asp Lys Trp Ala Glu Arg Thr Ser Pro Asp Phe Asn Leu Val
1 5 10 15
Ser Trp Asn Glu Ile Leu Lys Gln Pro Lys Leu Trp Tyr Ala Val Asp
20 25 30
5 Glu Ala Thr Arg Ile Ala Asn Gln Val Ile Leu Tyr Gln Arg Asp Asn
35 40 45
Gly Gly Trp Pro Lys Asn Ile Asp Met Ala Ala Met Leu Met Gln Ala
50 55 60
Glu Arg Glu Lys Leu Ser Arg Glu Lys Ser Glu Thr Asp Thr Thr Ile
65 70 75 80
10 Asp Asn Gly Ala Thr Thr Thr Gln Leu Ala Tyr Leu Ala Lys Val Ile
85 90 95
Thr Ala Lys Asn Ile Glu Ser His Arg Val Ala Phe Phe Lys Gly Leu
100 105 110
Asp Phe Leu Phe Ala Met Gln Tyr Gly Asn Gly Gly Phe Pro Gln Phe
115 120 125
15 Phe Pro Leu Arg Asp Asp Tyr Ser Arg Glu Ile Thr Phe Asn Asp Asn
130 135 140
Ala Met Ile Asn Val Leu Arg Leu Leu Arg Asp Ile Ala Asp Arg Lys
145 150 155 160
Asn Asp Tyr Val Phe Val Asp Glu Glu Arg Arg Ala Lys Ala Glu Gln
165 170 175
20 Ala Val Arg Arg Ala Ile Pro Leu Ile Leu Ser Thr Gln Val Val Val

180 185 190
25 Asp Gly Lys Lys Thr Val Trp Ala Ala Gln Tyr Asp Glu Lys Thr Leu
195 200 205
Lys Pro Ala Ala Ala Arg Lys Phe Glu Pro Ala Ser Leu Thr Ala Gly
210 215 220
Glu Ser Val Gly Ile Val Arg Phe Leu Met Leu Glu Lys Pro Thr Pro
225 230 235 240
30 Glu Ile Ile Asn Ala Ile Glu Ser Ala Ile Ala Trp Tyr Lys Ala Asn
245 250 255
Asn Ile Ser Gly Leu Arg Trp Glu Arg Arg Asn Gly Glu Asn Ile Val
260 265 270
Ile Lys Asp Lys Asn Ala Pro Pro Val Trp Ala Arg Phe Tyr Gln Ile
275 280 285
35 Glu Thr Met Arg Pro Ile Phe Ala Gly Arg Asp Ala Val Ile Arg Tyr
290 295 300
Asp Val Met Gln Ile Glu Ser Glu Arg Arg Asn Gly Tyr Ala Trp Tyr
305 310 315 320
Val Ser Glu Pro Asn Glu Leu Leu Asn Glu Asp Tyr Pro Lys Trp Arg
325 330 335 340
40 Thr Arg Ser Ala Lys Arg Ala Gln Ile Phe Gln Arg Pro Pro Leu Gly
340 345 350
Ser Arg Phe Arg Thr Val
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45 <210> 7
<211> 1125
<212> DNA
<213> Unknown

50 <220>
<223> Obtained from an environmental sample

<400> 7

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	gcgcggctct	cccgaaccat	tccgtcctcg	ccggcgcgaga	gcgctgcggt	cagcgggggtt	120
	ccggcgggcga	tccgctgggg	agcggacgtc	ctgcggcgaga	agccggagtg	gtatgcctcg	180
	cgagaggcga	ggacgatcgc	cgacagcgtc	atccagtacc	aggcgggcgga	cggcgggtgg	240
5	cccaagaaca	ccgacctcgg	gactccgccc	acggctgaat	cacgcgccgg	cgcggcgggc	300
	gacgtgacgt	cgagcaccat	cgacaacaac	ggcacgacga	tgccgatgca	gttccttgcg	360
	ctggtggcgg	acgcgaccgg	cgaggctcgc	tatcgcgcg	cgttcctccg	cggcttcgac	420
	tacctgctcg	ccgcgcagta	tcccaacggc	ggctggccgc	agttccttcc	gctccgccgc	480
	gggtattaca	cccacatcac	cttcaacgac	aacgcgatgg	tcaacgtgct	gaccgtgctg	540
	cgcgatgccg	cggccgggtca	ggcgccatac	gccttcgtgg	acgagccccg	ccgcgcgaa	600
10	gcccgcgccg	ccgtgtccc	ggggatcgac	gtcatcctga	agacccaagt	gaaacagaa	660
	ggcaagctga	cggcgtgggt	cgcgcagcac	gacgagaaga	ccctcgcgcc	ggcgtggggc	720
	cgcgcttacg	agccgccatc	gctctccggc	agcgaaaccg	tcggcatcgt	ccgcttcctg	780
	atggagatcg	agaagccgtc	accggagatc	gtcgcccgca	tcgaaggggc	cgtcgccctg	840
	ctgaagtccg	tggcgattcc	ggggctgcgc	tacgagtcct	tcaccggcg	ggacggacag	900
	agggaccgcc	gcgtcgttcc	ggatccatcg	gccggactcc	tgtggggcgc	gttctacgag	960
15	ctcggcacca	accggccgat	cttcctcggc	cgcgactccg	tggttcgcgc	cgcgctcagt	1020
	gacatcgaac	gcgagcgccg	cgccggctac	gcctactacg	gtacgtggcc	ggcgagcctg	1080
	attgctgcgg	actaccgcg	ctggcgttcg	acgctccggc	gctga		1125

<210> 8
 <211> 374
 <212> PRT
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<221> DOMAIN
 <222> (1)...(374)
 <223> Catalytic domain

<400> 8

	Met	His	Ala	Gly	Ala	Lys	His	Val	Ser	Arg	Trp	Arg	Glu	Glu	Phe	Leu
	1				5					10					15	
35										-						

40

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Arg Asp Phe Ala Ala Arg Leu Ser Arg Thr Ile Pro Ser Ser Pro Ala
 Gln Ser Ala Ala Val Ser Gly Val Pro Ala Ala Ile Arg Trp Gly Ala
 Asp Val Leu Arg Gln Lys Pro Glu Trp Tyr Ala Ser Arg Glu Ala Arg
 Thr Ile Ala Asp Ser Val Ile Gln Tyr Gln Ala Ala Asp Gly Gly Trp
 Pro Lys Asn Thr Asp Leu Gly Thr Pro Pro Thr Ala Glu Ser Arg Ala
 Gly Ala Ala Ala Asp Val Thr Ser Ser Thr Ile Asp Asn Asn Gly Thr
 Thr Met Pro Met Gln Phe Leu Ala Leu Val Ala Asp Ala Thr Gly Glu
 Ala Arg Tyr Arg Ala Ser Phe Leu Arg Gly Phe Asp Tyr Leu Leu Ala
 Ala Gln Tyr Pro Asn Gly Gly Trp Pro Gln Phe Phe Pro Leu Arg Arg
 Gly Tyr Tyr Thr His Ile Thr Phe Asn Asp Asn Ala Met Val Asn Val
 Leu Thr Val Leu Arg Asp Ala Ala Ala Gly Gln Ala Pro Tyr Ala Phe
 Val Asp Glu Pro Arg Arg Ala Lys Ala Arg Ala Ala Val Ser Arg Gly
 Ile Asp Val Ile Leu Lys Thr Gln Val Lys Gln Asn Gly Lys Leu Thr
 Ala Trp Cys Ala Gln His Asp Glu Lys Thr Leu Ala Pro Ala Trp Ala
 Arg Ala Tyr Glu Pro Pro Ser Leu Ser Gly Ser Glu Thr Val Gly Ile
 Val Arg Phe Leu Met Glu Ile Glu Lys Pro Ser Pro Glu Ile Val Ala
 Ala Ile Glu Gly Ala Val Ala Trp Leu Lys Ser Val Ala Ile Pro Gly
 Leu Arg Tyr Glu Ser Phe Thr Gly Ala Asp Gly Gln Arg Asp Arg Arg
 Val Val Pro Asp Pro Ser Ala Gly Leu Leu Trp Ala Arg Phe Tyr Glu
 Leu Gly Thr Asn Arg Pro Ile Phe Leu Gly Arg Asp Ser Val Val Arg
 Ala Ala Leu Ser Asp Ile Glu Arg Glu Arg Arg Ala Gly Tyr Ala Tyr
 Tyr Gly Thr Trp Pro Ala Ser Leu Ile Ala Ala Asp Tyr Pro Arg Trp
 Arg Ser Thr Leu Arg Arg

<210> 9

<211> 1116

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 9

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	ctgctctttg	cggcagccac	acaggggaacc	aagccgcccg	aagtgcggtg	gaatgagtg	120
	ctaaaccaa	aacctgcctg	gtacggcagc	cgggaagcgg	tgcgcattgc	tgacaacctg	180
	ttgctttacc	aacgcgacca	cggcggctgg	cacaagaata	tcgaaatggc	tgcggtcttg	240
5	accgaacagc	aacaggcaga	gttgaaagcg	caaaaggcaa	ccgacgattc	gacgattgat	300
	aacggcgcg	cctataccca	ggtgatttat	ctggcgcgcg	tcttcaatgc	gacgaagcag	360
	gagcgattca	aaaccgcggt	tctcaaagga	ttcgattatc	tgctcaaggc	tcagtatgcg	420
	aacggcggtg	ggccgcagta	ttaccgcggt	ttgcagggtt	attacaaaca	catcacgttc	480
	aacgatgacg	cgatggtcgg	cgtgcttgat	cttctgcgcg	atggtgcgcg	cggcgattcc	540
	ggttatcggg	tcgtggacag	cgaccggcgc	gcccgcgcca	gccaggccgt	gcaaaaagga	600
10	attgagtgc	tcttgaaatg	ccagatcgtg	gtcgccggga	aaaagaccgc	ctggtgcgcg	660
	caacacgatg	aagtgcattt	cgcccccgcg	cgggcacgca	cctacgagaa	aatttcgctg	720

	agcggcagcg	aatcggttgg	cctgatccgc	ttcctgatgg	gāattgaaca	accggacgcg	780
	cgtgtagttg	aggcgattga	gtccgcccgt	gcctggctca	agcaagccaa	gctgaccggc	840
15	atcaaagtgg	ttcagaaggc	ggatgcttcg	aagcccaatg	gcttcgaccg	ggtcgtcgtt	900
	gaagatgcac	aagccggggc	attgtgggcg	cgcttttacg	agatcggtac	gggcccggcg	960
	atcttttccg	gacgtgacgg	catcgtcaaa	tacagcttgg	cggaaatcga	acacgaacgg	1020
	cgcacgggct	acggctggta	cacgaatgcg	cccgcgaaat	tgctggaaca	agattatccg	1080
	gcctggcaaa	tcaaacgcgg	gggcaagaaa	aagtaa			1116

20
<210> 10
<211> 371
<212> PRT
<213> Unknown

25
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<223> Obtained from an environmental sample

30
<221> SIGNAL
<222> (1)...(29)

35
<221> DOMAIN
<222> (30)...(371)
<223> Catalytic domain

40
<400> 10

45

50

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	1				5					10				15		
	Leu	Ser	Cys	Ala	Leu	Leu	Phe	Ala	Ala	Ala	Thr	Gln	Gly	Thr	Lys	Pro
				20					25					30		
5	Pro	Glu	Val	Arg	Trp	Asn	Glu	Cys	Leu	Asn	Gln	Lys	Pro	Ala	Trp	Tyr
			35					40					45			
	Gly	Ser	Pro	Glu	Ala	Val	Arg	Ile	Ala	Asp	Asn	Leu	Leu	Leu	Tyr	Gln
		50					55					60				
	Arg	Asp	His	Gly	Gly	Trp	His	Lys	Asn	Ile	Glu	Met	Ala	Ala	Val	Leu
	65					70					75				80	
10	Thr	Glu	Gln	Gln	Gln	Ala	Glu	Leu	Lys	Ala	Gln	Lys	Ala	Thr	Asp	Asp
					85					90					95	
	Ser	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Tyr	Thr	Gln	Val	Ile	Tyr	Leu	Ala
				100					105					110		
	Arg	Val	Phe	Asn	Ala	Thr	Lys	Gln	Glu	Arg	Phe	Lys	Thr	Ala	Phe	Leu
			115					120					125			
15	Lys	Gly	Phe	Asp	Tyr	Leu	Leu	Lys	Ala	Gln	Tyr	Ala	Asn	Gly	Gly	Trp
		130					135					140				
	Pro	Gln	Tyr	Tyr	Pro	Arg	Leu	Gln	Gly	Tyr	Tyr	Lys	His	Ile	Thr	Phe
	145					150					155				160	
	Asn	Asp	Asp	Ala	Met	Val	Gly	Val	Leu	Asp	Leu	Leu	Arg	Asp	Val	Ala
					165					170					175	
20	Arg	Gly	Asp	Ser	Gly	Tyr	Arg	Phe	Val	Asp	Ser	Asp	Arg	Arg	Ala	Arg
				180					185					190		
	Ala	Ser	Gln	Ala	Val	Gln	Lys	Gly	Ile	Glu	Cys	Ile	Leu	Lys	Cys	Gln
			195					200					205			
	Ile	Val	Val	Ala	Gly	Lys	Lys	Thr	Ala	Trp	Cys	Ala	Gln	His	Asp	Glu
		210					215					220				
25	Val	Thr	Phe	Ala	Pro	Ala	Pro	Ala	Arg	Thr	Tyr	Glu	Lys	Ile	Ser	Leu
	225					230					235				240	
	Ser	Gly	Ser	Glu	Ser	Val	Gly	Leu	Ile	Arg	Phe	Leu	Met	Gly	Ile	Glu
					245					250				255		
	Gln	Pro	Asp	Ala	Arg	Val	Val	Glu	Ala	Ile	Glu	Ser	Ala	Val	Ala	Trp
				260					265					270		
30	Leu	Lys	Gln	Ala	Lys	Leu	Thr	Gly	Ile	Lys	Val	Val	Gln	Lys	Ala	Asp
			275					280					285			
	Ala	Ser	Lys	Pro	Asn	Gly	Phe	Asp	Arg	Val	Val	Val	Glu	Asp	Ala	Gln
		290					295					300				
	Ala	Gly	Pro	Leu	Trp	Ala	Arg	Phe	Tyr	Glu	Ile	Gly	Thr	Gly	Arg	Pro
	305					310					315				320	
35	Ile	Phe	Ser	Gly	Arg	Asp	Gly	Ile	Val	Lys	Tyr	Ser	Leu	Ala	Glu	Ile
					325					330				335		
	Glu	His	Glu	Arg	Arg	Thr	Gly	Tyr	Gly	Trp	Tyr	Thr	Asn	Ala	Pro	Ala
				340					345					350		

40	Lys	Leu	Leu	Glu	Gln	Asp	Tyr	Pro	Ala	Trp	Gln	Ile	Lys	Arg	Gly	Gly
			355					360					365			
	Lys	Lys	Lys													
			370													

45 <210> 11
 <211> 1167
 <212> DNA
 <213> Unknown

50 <220>
 <223> Obtained from an environmental sample

<400> 11

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	ggtgttgagg	caaagttggt	cgatcgcgag	cggttcttca	gcctcgctgc	ggaacgaacc	120
	tctaagaaga	atgaccagca	agtcggcgcc	atcgcggtga	aagatgcaca	cggaaaggca	180
5	gatgagtgg	atgcgagcgt	tgaggcactt	cgtatagccg	ataacgtcgt	tttctatcaa	240
	cgtgactcag	gtggctggcc	caagaatata	gagatggcga	agacgttgag	cgatcgtgag	300
	aaggctgcga	ttctccgcga	gaagaaaaag	aatgactcaa	caatcgacaa	tggcgcgact	360
	cacactcagt	tatcttttct	ggcgcgcgct	tatacagcac	aacagcagga	gcgacatcgc	420
	gagtcatttt	taaaaggact	ggattactta	ctgaaggcgc	agtattcaaa	tgggtggctgg	480
	ccacagttct	atccaaactt	gaatggctac	tacaaacgga	tcacgtacaa	cgatggcgcg	540
	atgatcgggt	tgatgaagct	tctgcgtgat	gttgcggcag	cgaacactga	atacgcgttt	600
10	gtcgaatgaaa	ctcggcgctgc	gaaggctgcg	aacgcgggtg	aaaaaggcat	cgtgtgcatt	660
	ttgaaaacgc	aggtggttgt	tgatggcgct	cgcactgttt	ggtgtgcaca	acacgacgaa	720
	gtgacgtttg	cgcccgcgcc	tgcaagaaag	tttgagttag	cttcgttgag	cggcggtgag	780
	agcgtcgata	ttgttcgatt	tctaattgtcg	ataaaggatc	catcgcgtaa	cgtggttgaa	840
	tcgattgaat	cggcagttaa	atggttttag	cagtcggagc	taaaaggcgt	taagtgggtc	900
	aagaaaaccg	acgctactca	acctaattggg	ttcgattgtg	tcgttgttaa	agatccggag	960
15	agctctgttt	ggcgcgctt	ttacgagatt	ggcacgaacc	gcccgatctt	tgccgggctg	1020
	gatggagtgc	ctaagtatga	cgtcgcgcag	atcgaacacg	agcgacgaac	gggttacgaa	1080
	tggtacgttg	atgaggcagc	aaaactgctg	aaaaaagatt	atccggcgctg	gaagaaacga	1140
	catgtcgtca	cgacgcgagt	tcattag				1167

20 <210> 12
 <211> 388
 <212> PRT
 <213> Unknown

25 <220>
 <223> Obtained from an environmental sample

<221> DOMAIN
 <222> (1)...(388)
 30 <223> Catalytic domain

<400> 12

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			20						25					30		
	Phe	Ser	Leu	Ala	Ala	Glu	Arg	Thr	Ser	Lys	Lys	Asn	Asp	Gln	Gln	Val
			35					40					45			
40	Gly	Ala	Ile	Ala	Trp	Lys	Asp	Ala	His	Gly	Lys	Ala	Asp	Glu	Trp	Tyr
		50					55					60				
	Ala	Ser	Val	Glu	Ala	Leu	Arg	Ile	Ala	Asp	Asn	Val	Val	Phe	Tyr	Gln
	65					70				75						80
	Arg	Asp	Ser	Gly	Gly	Trp	Pro	Lys	Asn	Ile	Glu	Met	Ala	Lys	Thr	Leu
				85					90					95		
45	Ser	Asp	Arg	Glu	Lys	Ala	Ala	Ile	Leu	Arg	Glu	Lys	Lys	Lys	Asn	Asp
			100						105					110		
	Ser	Thr	Ile	Asp	Asn	Gly	Ala	Thr	His	Thr	Gln	Leu	Ser	Phe	Leu	Ala
			115					120					125			
	Arg	Val	Tyr	Thr	Ala	Gln	Gln	Gln	Glu	Arg	His	Arg	Glu	Ser	Phe	Leu
		130				135						140				
50	Lys	Gly	Leu	Asp	Tyr	Leu	Leu	Lys	Ala	Gln	Tyr	Ser	Asn	Gly	Gly	Trp
	145					150					155					160

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5 Pro Gln Phe Tyr 165 Asn Leu Asn Gly Tyr Tyr Lys Arg Ile Thr Tyr
 Asn Asp Gly Ala 180 Met Ile Gly Val Met Lys Leu Leu Arg Asp Val Ala
 Ala Ala Lys 195 Pro Glu Tyr Ala Phe Val Asp Glu Thr Arg Arg Ala Lys
 Ala Ala Asn 210 Ala Val Glu Lys 215 Gly Ile Val Cys Ile Leu Lys Thr Gln
 Val Val Val Asp Gly Arg Arg Thr Val Trp Cys Ala Gln His Asp Glu
 10 Val Thr Phe Ala 245 Pro Ala Pro Ala Arg Lys Phe Glu Leu Ala Ser Leu
 Ser Gly Gly Glu 260 Ser Val Asp Ile Val Arg Phe Leu Met Ser Ile Lys
 Asp Pro Ser Arg Asn Val Val Glu Ser Ile Glu Ser Ala Val Lys Trp
 15 Phe Glu Gln Ser Glu Leu Lys 295 Gly Val Lys Trp Val Lys Lys Thr Asp
 Ala Thr Gln Pro Asn Gly 310 Phe Asp Cys Val Val Lys Asp Pro Glu
 20 Ser Ser Val Trp Ala Arg Phe Tyr Glu Ile Gly Thr Asn Arg Pro Ile
 Phe Ala Gly Arg 340 Asp Gly Val Pro Lys Tyr Asp Val Ala Gln Ile Glu
 His Glu Arg Arg Thr Gly Tyr Glu Trp Tyr Val Asp Glu Ala Ala Lys
 25 Leu Leu Lys Lys Asp Tyr Pro Ala Trp Lys Lys Arg His Val Val Thr
 Thr Arg Val His 385

30 <210> 13
 <211> 1065
 <212> DNA
 <213> Unknown

35 <220>
 <223> Obtained from an environmental sample

<400> 13

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 gcggcacgct ggaacgaatt cgcccagaag gcggatgatt ggtatcgagg tgacgaaggc 120
 aggcgcgttg cttcgaatat tctttctcac caatcactgc aaggaagctg gcccaagaat 180
 accgatacca ccgcgagatt cttcaatgga gatctagcga agattcaggg cacgttcgac 240
 aacggtgcca cgacggacga gttgcgtttc ctggcccgcg cgtttgtcgc cacgaaagaa 300
 aaaaactacg agtcagcgtt ccgaaaaggc ttcgaacaca ttctcgcggc gcaatacgcg 360
 45 aacggcggat ggccgcaata ttccgcccgc cccaaaagtt accaccgaca cattaccttc 420
 aacgataatt cgatggtgcg gctgatgatt ttctttcgcg aggtcacgac ttcgaatctc 480
 tactcgttcg tcgaagcgcc gctgcgaaca caagcccgcg aaagtttcga tcgcggtgtg 540
 cggtgcattc ttaagtgcc aatcgctcgtg aacgggcaca agaccgcgtg gtgcgcgcaa 600
 catgatgaaa cggatttcag ccccgatcc gcgcgtagtt acgaactgcc ttcgctgagc 660
 ggttctgaat cagtcggcat tgtgcgcttg ctgatgagcc tcgatcagcc gagccgcgga 720
 50 gtgatcgatg ccatcaccaa cgccgtagcg tggttcgaat cggcgaagct gcccgggatc 780
 aaaaccgttc aagagaccga tccgaattcg cccaaaggct ggaatcgcgt cgctcgtaaaa 840
 gatgaaagtg cccgaccgat gtgggcgcgt ttctacgaca tcaacaccaa caaacggttc 900
 ttttgtgatc gcgatggtgt gccaaagccg agtcttgccg agatcgggta tgaacggcgg 960
 aacggttatg cgtggctcgg atactggcct gaagacttgc tcgcaagaga gtatccagcg 1020
 55 tggaagatga agtggctgaa gcccaaagag cgcccagcat tttga 1065

<210> 14
 <211> 354
 <212> PRT

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<213> Unknown

<220>

<223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(22)

<221> DOMAIN

<222> (23)...(354)

<223> Catalytic domain

<400> 14

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 20      25      30
Asp Trp Tyr Arg Gly Asp Glu Gly Arg Arg Val Ala Ser Asn Ile Leu
 35      40      45
Ser His Gln Ser Leu Gln Gly Ser Trp Pro Lys Asn Thr Asp Thr Thr
 50      55      60
Ala Arg Phe Phe Asn Gly Asp Leu Ala Lys Ile Gln Gly Thr Phe Asp
 65      70      75      80
Asn Gly Ala Thr Thr Asp Glu Leu Arg Phe Leu Ala Arg Ala Phe Val
 85      90      95
Ala Thr Lys Glu Lys Asn Tyr Glu Ser Ala Phe Arg Lys Gly Phe Glu
100      105      110
His Ile Leu Ala Ala Gln Tyr Ala Asn Gly Gly Trp Pro Gln Tyr Ser
115      120      125
Pro Pro Pro Lys Ser Tyr His Arg His Ile Thr Phe Asn Asp Asn Ser
130      135      140
Met Val Arg Leu Met Ile Phe Leu Arg Glu Val Thr Thr Ser Asn Leu
145      150      155      160
Tyr Ser Phe Val Glu Ala Pro Leu Arg Thr Gln Ala Arg Glu Ser Phe
165      170      175
Asp Arg Gly Val Arg Cys Ile Leu Lys Cys Gln Ile Val Val Asn Gly
180      185      190
His Lys Thr Ala Trp Cys Ala Gln His Asp Glu Thr Asp Phe Ser Pro
195      200      205
Arg Ser Ala Arg Ser Tyr Glu Leu Pro Ser Leu Ser Gly Ser Glu Ser
210      215      220
Val Gly Ile Val Arg Leu Leu Met Ser Leu Asp Gln Pro Ser Arg Gly
225      230      235      240
Val Ile Asp Ala Ile Thr Asn Ala Val Ala Trp Phe Glu Ser Ala Lys
245      250      255
Leu Pro Gly Ile Lys Thr Val Gln Glu Thr Asp Pro Asn Ser Pro Lys
260      265      270
Gly Trp Asn Arg Val Val Val Lys Asp Glu Ser Ala Arg Pro Met Trp
275      280      285
Ala Arg Phe Tyr Asp Ile Asn Thr Asn Lys Pro Phe Phe Cys Asp Arg
290      295      300
Asp Gly Val Pro Lys Pro Ser Leu Ala Glu Ile Gly Tyr Glu Arg Arg
305      310      315      320
Asn Gly Tyr Ala Trp Leu Gly Tyr Trp Pro Glu Asp Leu Leu Ala Arg
325      330      335
Glu Tyr Pro Ala Trp Lys Met Lys Trp Leu Lys Pro Lys Glu Arg Pro
340      345      350
Ala Phe

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<210> 15

<211> 1575

<212> DNA

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<213> Bacteria

<400> 15

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	tacgccggcc	agaacggcgg	caccaccggc	ggtgccggcg	gccagaccgt	acggggccacc	180
	acgggcaccg	ccatccacgc	ggccctgtgc	ggacggggcca	gcagcagcac	cccgatcacg	240
	atcgaggctg	agggaaacgat	caaccacgcc	aacaccgccca	aggtgtccgg	ccccagctgc	300
10	aacaccggcg	ccggagtgat	cgagctgaag	cagatcagca	acgtcacgct	cgtcgggggtc	360
	ggctccggcg	ccgtcttcga	ccaactcggc	atccacatcc	gcgagtccag	caacatcatc	420
	atccagaacg	tgacgggtccg	gaacgtcaag	aagtcggggt	cgccgctgtc	caacggcggc	480

sequence listing.txt

15	gacgccatcg	gcatggagag	cgacgtccgc	aacgtctggg	tcgaccactc	caccctggag	540
	gcctcggggc	gcgagtccga	gggctacgac	ggcctcttcg	acatgaagga	caacaccggg	600
	tacgtgacct	tgctgtacag	catcctgcgc	aaatccgggc	gcggcgccct	cgtgggggtcc	660
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	ccggcggggc	cggaccgcga	gtccaacacc	accgtctcca	tcccctacgc	cttcagcctc	1020
	gacccggcca	cctgcgtgcc	ggacgtcgtg	agccgaacgg	cgggtgccgg	caagggactt	1080
	caggtgtcga	acggcagctg	ctccccgcag	acaccacgcg	ccacgccgac	gggcacgccg	1140
25	accacaccgg	cggcgacgac	tcccaccccc	agcccgacgc	cctccacgcc	cggaccgacc	1200
	cagcccgggc	ggacgaacct	cagcatcggg	gccgggtccg	acggttcgag	caaggccgac	1260
	ggcaccagct	acggcaacgt	ccgggacggg	gacctcgcca	cccactgggt	tccggccggg	1320
	tcgaccggct	ccgtgtcgat	caagtggggc	agcgccacca	cgggtctccc	catcgtcatc	1380
	cgcgaggcgg	cgggcgcgac	gggcgtcatc	ggctcctggc	tcgtcctgaa	cggcgacacc	1440
	ggcgccgtgc	tgacctccgg	cagcggggcg	gggacgatct	ccgtcccccg	gacggccctg	1500
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<210> 16

<211> 524

35 <212> PRT

<213> Bacteria

<220>

<221> SIGNAL

40 <222> (1)...(33)

<221> DOMAIN

<222> (34)...(359)

<223> Catalytic domain

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<400> 16

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	Ala	Leu	Ala	Ala	Ala	Thr	Gly	Val	Val	Leu	Ser	Ile	Pro	Gln	Ala	Ser
				20					25					30		
5	Ala	Ala	Ala	Gly	Gly	Ala	Thr	Gly	Tyr	Ala	Gly	Gln	Asn	Gly	Gly	Thr
			35					40					45			
	Thr	Gly	Gly	Ala	Gly	Gly	Gln	Thr	Val	Arg	Ala	Thr	Thr	Gly	Thr	Ala
		50					55					60				
	Ile	His	Ala	Ala	Leu	Cys	Gly	Arg	Ala	Ser	Ser	Ser	Thr	Pro	Ile	Thr
	65					70					75					80
10	Ile	Glu	Val	Glu	Gly	Thr	Ile	Asn	His	Ala	Asn	Thr	Ala	Lys	Val	Ser
				85						90					95	
	Gly	Pro	Ser	Cys	Asn	Thr	Ala	Ala	Gly	Val	Ile	Glu	Leu	Lys	Gln	Ile
				100					105					110		
	Ser	Asn	Val	Thr	Leu	Val	Gly	Val	Gly	Ser	Gly	Ala	Val	Phe	Asp	Gln
			115					120					125			
15	Leu	Gly	Ile	His	Ile	Arg	Glu	Ser	Ser	Asn	Ile	Ile	Ile	Gln	Asn	Val
		130					135					140				
	Thr	Val	Arg	Asn	Val	Lys	Lys	Ser	Gly	Ser	Pro	Leu	Ser	Asn	Gly	Gly
	145					150					155					160
	Asp	Ala	Ile	Gly	Met	Glu	Ser	Asp	Val	Arg	Asn	Val	Trp	Val	Asp	His
					165					170					175	
20	Ser	Thr	Leu	Glu	Ala	Ser	Gly	Gly	Glu	Ser	Glu	Gly	Tyr	Asp	Gly	Leu
				180					185					190		
	Phe	Asp	Met	Lys	Asp	Asn	Thr	Arg	Tyr	Val	Thr	Leu	Ser	Tyr	Ser	Ile
			195					200					205			
	Leu	Arg	Lys	Ser	Gly	Arg	Gly	Gly	Leu	Val	Gly	Ser	Ser	Glu	Thr	Glu
		210					215					220				
25	Leu	Ser	Asn	Ser	Phe	Ile	Thr	Tyr	His	His	Asn	Leu	Tyr	Glu	Asn	Ile
						230					235					240
	Asp	Ser	Arg	Ala	Pro	Leu	Leu	Arg	Gly	Gly	Thr	Ala	His	Met	Tyr	Asn
					245					250					255	
	Asn	His	Tyr	Leu	Arg	Ile	Asn	Glu	Ser	Gly	Ile	Asn	Ser	Arg	Ala	Gly

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260 265 270
 Ala His Ala Lys Val Asp Asn Asn Tyr Phe Glu Asp Ser Lys Asp Val
 275 280 285
 Leu Gly Thr Phe Tyr Thr Asp Ala Ala Gly Tyr Trp Gln Val Ser Gly
 290 295 300
 Asn Val Tyr Asp Asn Val Thr Trp Ser Ala Arg Gly Thr Asp Asn Asn
 305 310 315 320
 Pro Ala Gly Pro Asp Pro Gln Ser Asn Thr Thr Val Ser Ile Pro Tyr
 325 330 335
 Ala Phe Ser Leu Asp Pro Ala Thr Cys Val Pro Asp Val Val Ser Arg
 340 345 350
 Thr Ala Gly Ala Gly Lys Gly Leu Gln Val Ser Asn Gly Ser Cys Ser
 355 360 365
 Pro Gln Thr Pro Thr Pro Thr Pro Thr Gly Thr Pro Thr Thr Pro Ala
 370 375 380
 Pro Thr Thr Pro Thr Pro Ser Pro Thr Pro Ser Thr Pro Gly Pro Thr
 385 390 395 400
 Gln Pro Gly Gly Thr Asn Leu Ser Ile Gly Ala Gly Ser Asp Gly Ser
 405 410 415
 Ser Lys Ala Asp Gly Thr Ser Tyr Gly Asn Val Arg Asp Gly Asp Leu
 420 425 430
 Gly Thr His Trp Ser Pro Ala Gly Ser Thr Gly Ser Val Ser Ile Lys
 435 440 445
 Trp Gly Ser Ala Thr Thr Val Ser Arg Ile Val Ile Arg Glu Ala Ala
 450 455 460
 Gly Ala Thr Gly Val Ile Gly Ser Trp Leu Val Leu Asn Gly Asp Thr
 465 470 475 480
 Gly Ala Val Leu Thr Ser Gly Ser Gly Ala Gly Thr Ile Ser Val Pro
 485 490 495
 Arg Thr Ala Leu Lys Lys Ile Thr Phe Glu Ile Thr Gly Ala Ser Gly
 500 505 510
 Thr Pro Arg Ile Ala Glu Phe Glu Thr Tyr Ala Gly
 515 520

<210> 17

<211> 1047

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 17

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gtcgtgaga	acgtgctctt	gtatcagcgc	gcggcaggag	ggaggccgaa	gaacatcaac	180
atggcggcgc	cgatgaccgc	cgctgaccgt	gcgaaagtca	cggacgagcg	cgcgcagaac	240
gacgccacga	tcgacaacac	gtcaacgacg	acgcagatcc	gttttcttgc	gtcgttctt	300
cgcggcacccg	ccgacgcacg	attcaaggac	gcggcgctga	agggcatcga	cttcctgctg	360
gctgcgcaat	acgcgaatgg	aggctggcct	cagtattttc	ccctgcgcga	cgactactcg	420
cggcgcatca	cgttcaatga	cgacgcgatg	gtgaatgtga	tgacgctgct	gcgcgagact	480
tcgcagggcc	agacgccgtt	cgagttcgtc	gacgcctcgc	ggcgcgggccg	ggcgggcgag	540
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accggctggt	gccagcagca	cgacgagcgg	aactttcagc	cggtgaaggc	gcgcgcgtac	660
gaacatccgt	cgattgccag	caaggaaacc	gcgagcatcg	caagattcct	gatggggatt	720
gaacggccgt	cgccggagat	cgtgtccgcg	gtggatggcg	cagtcgcgtg	gttgcgagcg	780
gcgcagattt	caggtgtgcg	gacggagcgc	cggcccgcgc	gacggaatcc	ggcgggcgac	840
gtcgtggcgg	tgcaggactc	cgccgcgcgc	ccaatctggg	cccgtttcta	cgagattggc	900
accaaccggc	cgatgttttc	gggtcgcgac	ggcgatcatca	agtacagcct	cagcgagatc	960
gagatcgagc	ggcgcgctgg	atacagctgg	tacggcgact	acgccgccag	actgctcaga	1020
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<210> 18
<211> 348
<212> PRT
<213> Unknown

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<220>
<223> Obtained from an environmental sample

10

<221> DOMAIN
<222> (1)...(348)
<223> Catalytic domain

<400> 18

15

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 1      5      10      15
Ser  Val  Ala  Val  Ser  Trp  Asp  Gln  Ile  Leu  Arg  Gln  Pro  Ala  Ala  Trp
      20      25      30
Tyr  Gly  Gly  Ala  Glu  Ala  Leu  Arg  Val  Ala  Glu  Asn  Val  Leu  Leu  Tyr
      35      40      45
Gln  Arg  Ala  Ala  Gly  Gly  Trp  Pro  Lys  Asn  Ile  Asn  Met  Ala  Ala  Pro
      50      55      60
Met  Thr  Ala  Ala  Asp  Arg  Ala  Lys  Val  Thr  Asp  Glu  Arg  Ala  Gln  Asn
65      70      75      80
Asp  Ala  Thr  Ile  Asp  Asn  Thr  Ser  Thr  Thr  Thr  Gln  Ile  Arg  Phe  Leu
      85      90      95
Ala  Leu  Val  Leu  Arg  Gly  Thr  Ala  Asp  Ala  Arg  Phe  Lys  Asp  Ala  Ala
      100      105      110
Leu  Lys  Gly  Ile  Asp  Phe  Leu  Leu  Ala  Ala  Gln  Tyr  Ala  Asn  Gly  Gly
      115      120      125
Trp  Pro  Gln  Tyr  Phe  Pro  Leu  Arg  Asp  Asp  Tyr  Ser  Arg  Arg  Ile  Thr
      130      135      140
Phe  Asn  Asp  Asp  Ala  Met  Val  Asn  Val  Met  Thr  Leu  Leu  Arg  Glu  Thr
145      150      155      160
Ser  Gln  Gly  Gln  Thr  Pro  Phe  Glu  Phe  Val  Asp  Ala  Ser  Arg  Arg  Gly
      165      170      175
Arg  Ala  Ala  Gln  Ser  Val  Ser  Arg  Gly  Val  Asp  Val  Met  Leu  Arg  Thr
      180      185      190
Gln  Ile  Arg  Val  Asn  Gly  Val  Leu  Thr  Gly  Trp  Cys  Gln  Gln  His  Asp
      195      200      205
Glu  Arg  Asn  Phe  Gln  Pro  Val  Lys  Ala  Arg  Ala  Tyr  Glu  His  Pro  Ser
      210      215      220
Ile  Ala  Ser  Lys  Glu  Thr  Ala  Ser  Ile  Ala  Arg  Phe  Leu  Met  Gly  Ile
225      230      235      240
Glu  Arg  Pro  Ser  Pro  Glu  Ile  Val  Ser  Ala  Val  Asp  Gly  Ala  Val  Ala
      245      250      255
Trp  Leu  Arg  Ala  Ala  Gln  Ile  Ser  Gly  Val  Arg  Thr  Glu  Arg  Arg  Pro
      260      265      270
Asp  Gly  Ser  Asn  Pro  Gly  Gly  Asp  Val  Val  Ala  Val  Gln  Asp  Ser  Ala
      275      280      285
Ala  Pro  Pro  Ile  Trp  Ala  Arg  Phe  Tyr  Glu  Ile  Gly  Thr  Asn  Arg  Pro
      290      295      300
Met  Phe  Ser  Gly  Arg  Asp  Gly  Val  Ile  Lys  Tyr  Ser  Leu  Ser  Glu  Ile
305      310      315      320
Glu  Ile  Glu  Arg  Arg  Ala  Gly  Tyr  Ser  Trp  Tyr  Gly  Asp  Tyr  Ala  Ala
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Arg  Leu  Leu  Arg  Asp  Asp  Tyr  Pro  Lys  Trp  Lys  Lys
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<210> 19
<211> 1122
<212> DNA
<213> Unknown

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<220>

<223> Obtained from an environmental sample

<400> 19

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	ggctcggccg	aggcgcgtgc	gacgcgccac	agcgttcttc	aataccagtc	gaccgctggc	180
	ggctggccca	agaacaccga	cttgacggtc	tcgccaccgt	ccgccgaatt	ccttgccgat	240
10	gcggatggtc	tcacgaacac	gacgcgacaac	gacgccacca	cgttgccgat	gcgatttctc	300
	gctctgggtg	cgcacgcgac	cggcggcatc	aagtaccgcg	ccgcgttcga	acgcggtctg	360
	gactacctgc	tcgccgctca	gtatcccaat	ggcggctggc	ctcagtattt	tcccctgcgt	420

15	gacggctatt	actcgcacat	cacctacaac	gacaatgcga	tgggtcaacgt	cctcaccggt	480
	ctgcgcgatg	cggccgcggg	ccggccccct	tactcgttcg	tcgacagggc	ccggcgcgcc	540
	agagcagaaa	cggccatcgc	tcgcggcatc	gacatcatcg	tgcgcaactca	ggtgagacgg	600
	gcccggcgtgc	tgaccgcgatg	gtgcgcccag	cacgacgaaa	agacgctcga	gccggcgtgg	660
	gcgcgcaact	acgaaccgcc	gacactctcc	gggcacgaaa	gcgtcggcat	cgtgcgcttt	720
	ctcatgggaa	tcgaaaagcc	cacgcccagg	atcgtcgcgg	cgggtgcaagg	cgccgctgac	780
20	tggttgagag	ccgtcgcgat	cagcgggttg	cgtctcgagg	aattcaccga	cgccgatggc	840
	aggcgcgaca	ggcgcgtcgt	cgccgatccg	gcagcgccgc	tcctgtgggc	gcgcttctac	900
	gagcttggca	cggaccgtcc	cgtcttcacc	ggccgcgaca	aggtgatccg	gtactcgtc	960
	agcgaaatcg	agcacgagcg	ccggaacggg	tatgcctact	atggcacatg	gccggccacg	1020
	ctcctcagcg	aggagtaccc	ccgttggcgc	gcgaaacacc	tggctcgacg	gagcgtcagg	1080
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<210> 20

<211> 373

<212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

<221> DOMAIN

<222> (1)...(373)

<223> Catalytic domain

<400> 20

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	Leu	Arg	Cys	Met	Val	Pro	Arg	Pro	Gln	Ile	His	Trp	Gly	Gly	Gly	Val
				20					25					30		
5	Ile	Arg	Gln	Glu	Pro	Glu	Trp	Tyr	Gly	Ser	Ala	Glu	Ala	Arg	Ala	Ile
			35					40					45			
	Ala	Asp	Ser	Val	Leu	Gln	Tyr	Gln	Ser	Thr	Ala	Gly	Gly	Trp	Pro	Lys
		50					55					60				
	Asn	Thr	Asp	Leu	Thr	Val	Ser	Pro	Pro	Ser	Ala	Glu	Phe	Leu	Ala	Asp
	65					70					75					80
10	Ala	Asp	Gly	Leu	Thr	Asn	Thr	Ile	Asp	Asn	Asp	Ala	Thr	Thr	Leu	Pro
				85						90					95	
	Met	Arg	Phe	Leu	Ala	Leu	Val	Ala	His	Ala	Thr	Gly	Gly	Ile	Lys	Tyr
				100					105					110		
	Arg	Ala	Ala	Phe	Glu	Arg	Gly	Leu	Asp	Tyr	Leu	Leu	Ala	Ala	Gln	Tyr
			115					120					125			
15	Pro	Asn	Gly	Gly	Trp	Pro	Gln	Tyr	Phe	Pro	Leu	Arg	Asp	Gly	Tyr	Tyr
		130					135					140				
	Ser	His	Ile	Thr	Tyr	Asn	Asp	Asn	Ala	Met	Val	Asn	Val	Leu	Thr	Val
	145					150					155					160
	Leu	Arg	Asp	Ala	Ala	Ala	Gly	Arg	Pro	Pro	Tyr	Ser	Phe	Val	Asp	Arg
				165						170					175	
20	Ala	Arg	Arg	Ala	Arg	Ala	Glu	Thr	Ala	Ile	Ala	Arg	Gly	Ile	Asp	Ile
				180					185					190		
	Ile	Val	Arg	Thr	Gln	Val	Arg	Arg	Ala	Gly	Val	Leu	Thr	Ala	Trp	Cys
			195					200					205			
	Ala	Gln	His	Asp	Glu	Lys	Thr	Leu	Glu	Pro	Ala	Trp	Ala	Arg	Asn	Tyr
		210					215					220				
25	Glu	Pro	Pro	Thr	Leu	Ser	Gly	His	Glu	Ser	Val	Gly	Ile	Val	Arg	Phe
						230					235					240
	Leu	Met	Gly	Ile	Glu	Lys	Pro	Thr	Pro	Arg	Ile	Val	Ala	Ala	Val	Gln
				245						250					255	
	Gly	Ala	Ala	Asp	Trp	Leu	Arg	Ala	Val	Ala	Ile	Ser	Gly	Leu	Arg	Leu
				260					265					270		
30	Glu	Glu	Phe	Thr	Asp	Ala	Asp	Gly	Arg	Arg	Asp	Arg	Arg	Val	Val	Ala
			275					280					285			
	Asp	Pro	Ala	Ala	Pro	Leu	Leu	Trp	Ala	Arg	Phe	Tyr	Glu	Leu	Gly	Thr
		290					295					300				
	Asp	Arg	Pro	Val	Phe	Thr	Gly	Arg	Asp	Lys	Val	Ile	Arg	Tyr	Ser	Leu
						310					315					320
35	Ser	Glu	Ile	Glu	His	Glu	Arg	Arg	Asn	Gly	Tyr	Ala	Tyr	Tyr	Gly	Thr
					325					330					335	

40	Trp	Pro	Ala	Thr	Leu	Leu	Ser	Glu	Glu	Tyr	Pro	Arg	Trp	Arg	Ala	Lys
				340					345					350		
	His	Leu	Ala	Arg	Arg	Ser	Val	Arg	Gln	Val	Glu	Glu	Gly	Ile	Ala	Ile
			355					360					365			
	Arg	Val	Pro	Asn	Pro											
				370												
45																
	<210>	21														
	<211>	1269														
	<212>	DNA														
	<213>	Unknown														
50																
	<220>															
	<223>	Obtained from an environmental sample														
55																
	<400>	21														

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	ctggcgga	ag	ccccatcg	ac	agatcacc	ct	gcgcgtgc	cg	ctcagccc	gg	cgcgctg	120		
	accgaaac	cg	cctgagca	gc	ggggcgca	tg	gcaggcct	at	ctcgccac	ca	ccgaggga	180		
5	cagcttaag	cg	acaaaggc	gg	cgctggct	gc	gcagcgcg	cg	gggtctgg	cg	aaatcccc	240		
	gccaagccg	ag	accggcag	cg	ccaacacc	at	gcgcgtcg	ac	aagccgct	gg	aatgggtac	300		
	gcgtcgtcc	agg	cgctct	gg	tcgccgat	aa	tatcgtca	gt	tatcagac	tc	ccggcaggc	360		
	ggctggggc	aaa	atcaggc	cc	gaacgaa	ccc	acgcgg	tg	aaaggta	gg	cctacact	420		
	atcgatgac	cc	gatcccac	cg	gttcgggc	aa	atggaa	tc	gtcggcac	ca	tcgacaac	480		
	gacgccacca	tc	gtgaaat	tc	gttttctc	gc	ccgcgtag	cg	ggcgccgc	ca	cggggccc	540		
10	gaaggcgac	tc	tatcgcg	ct	ccgccacg	cg	cgccatca	ct	tacttgct	gg	cgccgcag	600		
	taccccaat	gc	ggctggcc	gc	aggtcttg	cc	gttcagg	gc	ggctatca	cg	acgccatc	660		
	accctcaat	ac	ggcgcgat	ga	tccatgtg	ct	cgaactgt	tt	gacgacat	cg	ccagcgga	720		
	cagggcgact	tc	gccttcct	gc	ctgagccg	ct	gcgcgaca	ag	gtcgaggc	cg	cacaggca	780		
	aagggtcaga	ag	gtgcttct	cg	atcttcag	ct	taagcgca	ac	ggcgaaac	ca	ccctgtgg	840		
	gcgcagcagt	ac	gatccgat	ta	ccctcttg	ccc	agcgcg	cg	cgtaacta	cg	agccgtcg	900		
15	tcgatcagca	cc	ggtgaaag	cg	ccggtgtg	ct	gatctacc	tc	atgtccct	gc	ccaacccc	960		
	tcgcctgaag	tg	cgcgacgc	ca	tcgaaaaa	gg	cggtggccc	tg	ctgatcaa	ac	ttcagatc	1020		
	aacggcatgg	ca	tgggaaaa	gg	acggcatg	cg	caaacgtc	tg	gtcgccaa	gg	ctgacgcc	1080		
	tcgccgctgt	gg	tcgcgcta	tc	acgactcg	ga	aacgctgc	tg	cccatctt	cg	gtgaccgc	1140		
	gacatgcgca	tc	ttcgacga	cg	tcaacgac	at	cagcgacg	aa	cgcagccg	cg	gctatgcc	1200		
	tggtatggca	ca	agcccggc	ac	gggccatc	gc	gaatacgc	aaa	aatggaa	ac	agggcaac	1260		
20	ggcaaatga											1269		

<210> 22

<211> 422

<212> PRT

25 <213> Unknown

<220>

<223> Obtained from an environmental sample

30 <221> SIGNAL

<222> (1)...(23)

<221> DOMAIN

<222> (24)...(422)

35 <223> Catalytic domain

<400> 22

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				20					25					30		
	Val	Pro	Leu	Ser	Pro	Ala	Arg	Leu	Thr	Glu	Thr	Pro	Pro	Glu	Gln	Arg
			35					40					45			
45	Ala	Gln	Trp	Gln	Ala	Tyr	Leu	Ala	Thr	Thr	Glu	Ala	Gln	Leu	Lys	Ala
		50					55					60				
	Asp	Lys	Ala	Ala	Leu	Ala	Ala	Glu	Arg	Ala	Gly	Leu	Ala	Glu	Ile	Pro
	65					70					75				80	
	Ala	Lys	Pro	Lys	Thr	Gly	Ser	Ala	Asn	Thr	Met	Pro	Leu	Asp	Lys	Pro
				85						90				95		
50	Leu	Glu	Trp	Tyr	Ala	Ser	Ser	Glu	Ala	Arg	Leu	Val	Ala	Asp	Asn	Ile

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			100					105					110				
	Val	Ser	Tyr	Gln	Thr	Pro	Ala	Gly	Gly	Trp	Gly	Lys	Asn	Gln	Ala	Arg	
			115					120					125				
5	Asn	Glu	Pro	Thr	Arg	Leu	Lys	Gly	Gln	Ala	Tyr	Thr	Ile	Asp	Asp	Ala	
			130				135					140					
	Asp	Pro	Thr	Gly	Ser	Gly	Lys	Trp	Asn	Phe	Val	Gly	Thr	Ile	Asp	Asn	
			145			150					155					160	
	Asp	Ala	Thr	Ile	Val	Glu	Ile	Arg	Phe	Leu	Ala	Arg	Val	Ala	Ala	Ala	
				165					170						175		
10	Ala	Thr	Gly	Pro	Glu	Gly	Asp	Val	Tyr	Arg	Ala	Ser	Ala	Thr	Arg	Gly	
			180						185					190			
	Ile	Thr	Tyr	Leu	Leu	Ala	Ala	Gln	Tyr	Pro	Asn	Gly	Gly	Trp	Pro	Gln	
			195					200					205				
	Val	Trp	Pro	Leu	Gln	Gly	Gly	Tyr	His	Asp	Ala	Ile	Thr	Leu	Asn	Asp	
			210			215						220					
15	Gly	Ala	Met	Ile	His	Val	Leu	Glu	Leu	Phe	Asp	Asp	Ile	Ala	Ser	Gly	
			225			230					235					240	
	Gln	Gly	Asp	Phe	Ala	Phe	Leu	Pro	Glu	Pro	Leu	Arg	Asp	Lys	Val	Glu	
				245						250					255		
	Ala	Ala	Gln	Ala	Lys	Gly	Gln	Lys	Val	Leu	Leu	Asp	Leu	Gln	Leu	Lys	
			260						265					270			
20	Arg	Asn	Gly	Glu	Arg	Thr	Leu	Trp	Ala	Gln	Gln	Tyr	Asp	Pro	Ile	Thr	
			275					280					285				
	Leu	Leu	Pro	Ser	Ala	Ala	Arg	Asn	Tyr	Glu	Pro	Ser	Ser	Ile	Ser	Thr	
			290				295					300					
	Gly	Glu	Ser	Ala	Gly	Val	Leu	Ile	Tyr	Leu	Met	Ser	Leu	Pro	Asn	Pro	
			305			310					315					320	
25	Ser	Pro	Glu	Val	Arg	Asp	Ala	Ile	Glu	Lys	Gly	Val	Ala	Leu	Leu	Ile	
				325						330					335		
	Lys	Leu	Gln	Ile	Asn	Gly	Met	Ala	Trp	Glu	Lys	Asp	Gly	Met	Arg	Lys	
			340						345					350			
	Arg	Leu	Val	Ala	Lys	Ala	Asp	Ala	Ser	Pro	Leu	Trp	Ser	Arg	Tyr	His	
			355					360					365				
30	Asp	Ser	Glu	Thr	Leu	Leu	Pro	Ile	Phe	Gly	Asp	Arg	Asp	Met	Arg	Ile	
			370				375					380					
	Phe	Asp	Asp	Val	Asn	Asp	Ile	Ser	Asp	Glu	Arg	Ser	Arg	Gly	Tyr	Ala	
			385			390					395					400	
	Trp	Tyr	Gly	Thr	Ser	Pro	Ala	Arg	Ala	Ile	Ala	Glu	Tyr	Glu	Lys	Trp	
				405						410					415		
35	Lys	Gln	Gly	Asn	Gly	Lys											
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<210> 23

<211> 1182

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 23

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	tcggagcagg	gcgcctggcg	gggctacctc	gcccgtctcc	gcgcagccat	ggacgccgac	180
	aaggccgccc	tgggcgccga	gcgcgcgcgc	ctcgccaccg	taccgcccgc	gccgcccgc	240
5	ggcgggtggtg	atggcgggat	ggcgcgcaac	cgtccgacgg	cttgggtatgg	gacgccggaa	300
	gcgcggcaca	tcgcggacaa	tatcgtcagc	ttccagacgc	cgtccggcg	ctggggcaag	360
	aacgtggacc	gcacgggacc	tgtgcgccag	cgcgacagc	attacgtttc	cttcgatggc	420
	aaggagtcct	ggaacttcat	cggcacgatc	gacaacaacg	ccacaacgag	cgagctgaaa	480
	ttcctggcgc	gcgtgcaggc	gcaaattgcc	ggcgcgcg	gcgacgaata	ccggaaggcc	540
	gccctgcgcg	gcatcagcta	cctgttgaac	tcacaatatc	ccaacggcgg	cttcccgcag	600
10	gtctatccgc	tgcaaggcgg	ctaccacgac	gccatcacct	tcaacgacga	tgccttcgcc	660
	aacgtgctgc	aagtgcgtgc	ggaagtggcg	aaccgcaggg	gcgactatgc	cttcgtcccc	720
	gaaaccgtgg	caaccgatgc	ccgcgcggcc	gcggacaagg	cgctccaagt	cctgctggcg	780
	agccagatca	tcgtcggcgg	cgtacgcacc	gcctggtgcc	agcagcacga	tgcgatcacg	840
	ctggcgcccc	tcggcgcccc	caatttcgaa	ccggccgcgc	tgaccagcac	ggaaagcgcg	900
	cgcctgctga	tgctgttgat	gctgctgccc	gatccgagcc	cggagctgag	agcgtcaatc	960
15	catgcgggga	tggcctggct	gcagaaagcg	gcgctgccgg	gggatgtctg	gtcgcgctac	1020

	tatgacctga	acacgatgag	gccgatcttt	ggggatcgtg	aċcgagtat	ccacgatgat	1080
	gtgaaggaat	tgagcgagga	gaggcaaaaa	ggctatggct	ggttcagtaa	cggaccagcc	1140
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<210> 24
 <211> 393
 <212> PRT
 25 <213> Unknown

<220>
 <223> Obtained from an environmental sample

30 <221> SIGNAL
 <222> (1)...(18)

<221> DOMAIN
 <222> (19)...(393)
 35 <223> Catalytic domain

<400> 24

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	His	Gly	Ala	Val	Leu	Gly	Tyr	Met	Thr	Pro	Ala	Gln	Pro	Leu	Thr	Glu
				20				25					30			
5	Ala	Arg	Ile	Ala	Ala	Leu	Pro	Ala	Ser	Glu	Gln	Gly	Ala	Trp	Arg	Gly
			35					40					45			
	Tyr	Leu	Ala	Arg	Ser	Arg	Ala	Met	Asp	Ala	Asp	Lys	Ala	Ala	Leu	
		50					55				60					
	Ala	Ala	Glu	Arg	Ala	Ala	Leu	Ala	Thr	Val	Pro	Pro	Ala	Pro	Pro	His
	65					70				75					80	
10	Gly	Gly	Gly	Asp	Gly	Gly	Met	Ala	Arg	Asn	Arg	Pro	Thr	Ala	Trp	Tyr
				85						90					95	
	Gly	Thr	Pro	Glu	Ala	Arg	His	Ile	Ala	Asp	Asn	Ile	Val	Ser	Phe	Gln
				100					105					110		
	Thr	Pro	Ser	Gly	Gly	Trp	Gly	Lys	Asn	Val	Asp	Arg	Thr	Gly	Pro	Val
			115					120					125			
15	Arg	Gln	Arg	Gly	Gln	His	Tyr	Val	Ser	Phe	Asp	Gly	Lys	Glu	Ser	Trp
		130					135					140				
	Asn	Phe	Ile	Gly	Thr	Ile	Asp	Asn	Asn	Ala	Thr	Ser	Glu	Leu	Lys	
	145					150					155				160	
	Phe	Leu	Ala	Arg	Val	Gln	Ala	Gln	Met	Pro	Gly	Ala	Ala	Gly	Asp	Glu
				165						170				175		
20	Tyr	Arg	Lys	Ala	Ala	Leu	Arg	Gly	Ile	Ser	Tyr	Leu	Leu	Asn	Ser	Gln
				180					185					190		
	Tyr	Pro	Asn	Gly	Gly	Phe	Pro	Gln	Val	Tyr	Pro	Leu	Gln	Gly	Gly	Tyr
			195					200					205			
	His	Asp	Ala	Ile	Thr	Phe	Asn	Asp	Asp	Ala	Phe	Ala	Asn	Val	Leu	Gln
		210					215					220				
25	Val	Leu	Leu	Glu	Val	Ala	Asn	Arg	Arg	Gly	Asp	Tyr	Ala	Phe	Val	Pro
						230					235					240
	Glu	Thr	Val	Ala	Thr	Asp	Ala	Arg	Ala	Ala	Ala	Asp	Lys	Ala	Leu	Gln
				245						250					255	
	Val	Leu	Leu	Ala	Ser	Gln	Ile	Ile	Val	Gly	Gly	Val	Arg	Thr	Ala	Trp
				260					265					270		
30	Cys	Gln	Gln	His	Asp	Ala	Ile	Thr	Leu	Ala	Pro	Val	Gly	Ala	Arg	Asn
			275					280					285			
	Phe	Glu	Pro	Ala	Ala	Leu	Thr	Ser	Thr	Glu	Ser	Ala	Arg	Leu	Leu	Met
		290					295					300				
	Leu	Leu	Met	Leu	Leu	Pro	Asp	Pro	Ser	Pro	Glu	Leu	Arg	Ala	Ser	Ile
						310					315				320	
35	His	Ala	Gly	Met	Ala	Trp	Leu	Gln	Lys	Ala	Ala	Leu	Pro	Gly	Asp	Val
					325					330					335	
	Trp	Ser	Arg	Tyr	Tyr	Asp	Leu	Asn	Thr	Met	Arg	Pro	Ile	Phe	Gly	Asp
				340					345					350		
	Arg	Asp	Arg	Ser	Ile	His	Asp	Asp	Val	Lys	Glu	Leu	Ser	Glu	Glu	Arg
			355					360					365			
40	Gln	Lys	Gly	Tyr	Gly	Trp	Phe	Ser	Asn	Gly	Pro	Ala	Arg	Ala	Lys	Gln
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<210> 25

<211> 1194

50 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

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<400> 25

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	tcggttacgc	aatcagcagc	cacaaatcaa	ttgcaaaatg	aaaaaagcag	ttgggacagc	120
	tattacgccg	catccaaaaa	aatacatcag	gcagaccagg	atcttctcgc	cgctgaatta	180
5	aaaaaactcg	gtcagaaaaa	accaacattg	cccgcacaca	ccaaagattt	tgggttttgat	240
	gttaagcagg	taaatgcaga	ttggtttaaa	agtgcagaa	gcaaacgtgt	gatggagatt	300
	attctctcct	tccaaacccc	gtccggcggt	tgggtcaaagc	gtaccgacat	ggccaaggcg	360
	gtgcgacaac	ctggggcaagc	ctttggcggt	gaaaaagggt	atatcccaac	atttgataat	420
	ggcgctacca	gcactcaatt	gatgttgctc	gcgcaagcac	accaagccac	cggcgatcac	480
	cgcttttagcg	acgcattttg	gcgcggcctt	caattaattt	tgactgcgca	ataccggaat	540
10	ggtggctggc	cacaaaactt	tccactaacc	ggtagctacc	acgattacat	cacctacaac	600
	gacaatctta	cgcgcgacct	gatggtagtg	ctgcacaaaa	cagcgaggc	aaaaaatgat	660
	tttgatttcg	tgaccaaaagc	gcagcaaatc	gcagcgctcag	ctagcctcgc	gcgtgcactt	720
	gattgcgtat	tgaaatcaca	agttgtcgtc	aatggcacac	gcacactctg	gggcgcacag	780
	cacgatgtta	aaacactgca	accaacaaaa	gcgcgcgcac	ttgaaatggg	gtcactcact	840
	accactgaaa	gcgcagccat	gctcagtttt	ctgatggata	tcaaaaaatcc	cagcgcggat	900
15	attattcaat	ccatacatgc	agccatagcc	tggtagtagc	aaaataaaaat	cgtcggaaaaa	960
	acctggacac	gtgggtgatgc	ggaattaaaa	gataataaaa	attcgcagcc	actctgggcg	1020
	cgtttttatg	agataggcac	taataagcct	atatttgggg	atcgcgatga	cactgtgtat	1080
	tacgatttgg	caaaagtgtc	taaagagcgt	cgcggaagggt	atgcgtggta	ctccactgac	1140
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 <211> 397
 <212> PRT
 <213> Unknown

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<221> SIGNAL
 <222> (1)...(15)

30 <221> DOMAIN
 <222> (16)...(397)
 <223> Catalytic domain

35 <400> 26

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				20					25					30		
	Asn	Glu	Lys	Ser	Ser	Trp	Asp	Ser	Tyr	Tyr	Ala	Ala	Ser	Lys	Lys	Ile
			35					40					45			
	His	Gln	Ala	Asp	Gln	Asp	Phe	Leu	Ala	Ala	Glu	Leu	Lys	Lys	Leu	Gly
	50						55					60				
45	Gln	Lys	Lys	Pro	Thr	Leu	Pro	Ala	His	Thr	Lys	Asp	Phe	Gly	Phe	Asp
	65					70					75				80	
	Val	Lys	Gln	Val	Asn	Ala	Asp	Trp	Phe	Lys	Ser	Asp	Glu	Gly	Lys	Arg
					85					90					95	
	Val	Met	Glu	Ile	Ile	Leu	Ser	Phe	Gln	Thr	Pro	Ser	Gly	Gly	Trp	Ser
				100					105					110		
50	Lys	Arg	Thr	Asp	Met	Ala	Lys	Ala	Val	Arg	Gln	Pro	Gly	Gln	Ala	Phe
			115					120					125			
	Gly	Val	Glu	Lys	Gly	Tyr	Ile	Pro	Thr	Phe	Asp	Asn	Gly	Ala	Thr	Ser
		130					135					140				
	Thr	Gln	Leu	Met	Leu	Leu	Ala	Gln	Ala	His	Gln	Ala	Thr	Gly	Asp	His

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	145				150					155					160
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					165					170					175
5	Gln	Tyr	Pro	Asn	Gly	Gly	Trp	Pro	Gln	Asn	Phe	Pro	Leu	Thr	Gly
				180					185					190	
	Tyr	His	Asp	Tyr	Ile	Thr	Tyr	Asn	Asp	Asn	Leu	Thr	Arg	Asp	Leu
			195					200					205		Met
	Val	Val	Leu	His	Lys	Thr	Ala	Gln	Ala	Lys	Asn	Asp	Phe	Ala	Phe
		210					215					220			Val
10	Thr	Lys	Ala	Gln	Gln	Ile	Ala	Ala	Ser	Ala	Ser	Leu	Ala	Arg	Ala
	225					230					235				240
	Asp	Cys	Val	Leu	Lys	Ser	Gln	Val	Val	Val	Asn	Gly	Thr	Arg	Thr
					245					250					255
	Trp	Gly	Ala	Gln	His	Asp	Val	Lys	Thr	Leu	Gln	Pro	Thr	Lys	Ala
				260					265					270	Arg
15	Ala	Phe	Glu	Met	Val	Ser	Leu	Thr	Thr	Thr	Glu	Ser	Ala	Ala	Met
			275					280					285		Leu
	Ser	Phe	Leu	Met	Asp	Ile	Lys	Asn	Pro	Ser	Ala	Asp	Ile	Ile	Gln
		290					295					300			Ser
	Ile	His	Ala	Ala	Ile	Ala	Trp	Tyr	Glu	Gln	Asn	Lys	Ile	Val	Gly
	305					310					315				Lys
20	Thr	Trp	Thr	Arg	Gly	Asp	Ala	Glu	Leu	Lys	Asp	Asn	Lys	Asn	Ser
					325					330					Gln
	Pro	Leu	Trp	Ala	Arg	Phe	Tyr	Glu	Ile	Gly	Thr	Asn	Lys	Pro	Ile
				340					345					350	Phe
	Gly	Asp	Arg	Asp	Asp	Thr	Val	Tyr	Tyr	Asp	Leu	Ala	Lys	Val	Ser
			355					360					365		Lys
25	Glu	Arg	Arg	Glu	Gly	Tyr	Ala	Trp	Tyr	Ser	Thr	Asp	Pro	Asn	Lys
		370					375					380			Thr
	Leu	Lys	Lys	Tyr	Ala	Glu	Trp	Ser	Lys	Lys	Tyr	Pro	Lys		
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 <211> 1917
 <212> DNA
 <213> Unknown

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 <223> Obtained from an environmental sample

<400> 27

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	acgattcaac	aagcgggtgga	ccagggtccc	aaagacaata	cacacccggg	cttgattcag	180
5	atcaagccgg	gtgtgtatca	ggaacaagtg	cgtgtcgccg	ccggcaaacg	ctttatcact	240
	cttcgcggcg	acgacgcgag	caagaccgtc	atcacctatc	gattgagcgc	actacaagcg	300
	ggaaaataccc	ggttggcatt	caccacctta	attaatgcag	acgactttcg	cgccgagaac	360
	ctgacgtttg	aaaacitcctt	cggcaccggg	tcacaagcgg	ttgctttgtt	tgctgatgcg	420
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	ggcagccgcc	acttcttcaa	agactgctac	gtcgaaggcc	atgtcgattt	cattttcggc	540
	acggcctccg	cagtgtttga	gaactgcacc	attcacagca	aaggcgagg	ttatgtgacc	600
10	gcgcactatc	gcaccagcga	tgagatggat	accggttttg	tcittcatcg	ttgtcgtttg	660
	accggacgag	acacggggcg	cggagtttat	ctcgggaagg	cgtggcgacc	ttacgcgcgc	720
	gtcgtcttta	tcgattgctg	gctggacgca	cacatcagac	ctgaaggctg	ggataattgg	780
	agagatcctg	aacgagagaa	gaccgcgtgg	tttgccgagt	acaagtcaaa	agggcccggg	840
	gctaataccc	tagctcgtgt	cgcggtgtcc	aggcagttga	cgacagaaca	agccgccgag	900
	ttttcgcggg	aacgcttttt	cagccgcgct	gttcgcgggc	tctctgggca	ggccaaccag	960
15	gcagtcggaa	cgatcgcgtg	ggacgatgcy	cagaaaaaac	cgaacgagtg	gtatgcgagc	1020
	gccgaggcgt	tgccgatcgc	cgacaacgtt	gttctttatc	aacgtgactc	cgccgggctgg	1080
	cccaagaaca	tcgacatggg	gaagccgctc	gacgacaagg	gtcgagccgg	tcttctgcgc	1140
	gtgctgaaga	agaacgattc	caccatcgat	aacggcgcca	cttacacgca	actctcgttt	1200
	ctagcgcggg	tttacacggc	gcaaaaagcag	gagcggcatc	gcgagtcggt	tctgaaggga	1260
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	acgagtgcgg	cgaaggcggg	cgaaaaagga	atcgagtgcg	tactgaagac	gcaggtgggt	1500
	gtgaatggcc	ggcgcaccgt	gtggtgtgcy	caacatgacg	aagtcacgct	cgcgccgtgc	1560
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25							
	tttttgatgt	cgatcaagaa	cccgtcgccg	gcggttgctg	aggcgatcga	gtcggcggtt	1680
	gcgtggttcg	agcaatcgca	agtgaagat	cccggccgca	aacctgcgtg	ggcgcgattt	1740
	tatgagatcg	gcactaatcg	tccgatcttc	gccgggctg	acggcgctcg	taagtatgat	1800
30	gtgaaacaga	tcgatgagga	acgacgaaag	aattacgcat	ggtacgttga	cgacgcagcg	1860
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 <212> PRT
 35 <213> Unknown

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 <222> (1)...(21)

<221> DOMAIN
 <222> (28)...(308)
 45 <223> Pectin methyl esterase domain

<221> DOMAIN
 <222> (309)...(638)
 50 <223> Catalytic domain

<400> 28

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	Leu	Ser	Val	Gly	Ala	Glu	Thr	Arg	Ala	Ser	Lys	Arg	Ile	Val	Val	Ala
				20					25					30		
5	Ala	Asp	Gly	Ser	Gly	Asp	Val	Arg	Thr	Ile	Gln	Gln	Ala	Val	Asp	Gln
			35					40					45			
	Val	Pro	Lys	Asp	Asn	Thr	His	Pro	Val	Leu	Ile	Gln	Ile	Lys	Pro	Gly
		50					55					60				
	Val	Tyr	Gln	Glu	Gln	Val	Arg	Val	Ala	Ala	Gly	Lys	Arg	Phe	Ile	Thr
	65				70						75				80	
10	Leu	Arg	Gly	Asp	Asp	Ala	Ser	Lys	Thr	Val	Ile	Thr	Tyr	Arg	Leu	Ser
				85						90					95	
	Ala	Leu	Gln	Ala	Gly	Asn	Thr	Arg	Leu	Ala	Phe	Thr	Thr	Leu	Ile	Asn
				100					105					110		
	Ala	Asp	Asp	Phe	Arg	Ala	Glu	Asn	Leu	Thr	Phe	Glu	Asn	Ser	Phe	Gly
			115					120					125			
15	Thr	Gly	Ser	Gln	Ala	Val	Ala	Leu	Phe	Val	Asp	Ala	Asn	Arg	Ala	Thr
		130					135					140				
	Phe	Glu	Asn	Cys	Arg	Phe	Leu	Gly	Trp	Gln	Asp	Thr	Leu	Phe	Val	Asn
	145					150					155				160	
	Gly	Ser	Arg	His	Phe	Phe	Lys	Asp	Cys	Tyr	Val	Glu	Gly	His	Val	Asp
				165						170					175	
20	Phe	Ile	Phe	Gly	Thr	Ala	Ser	Ala	Val	Phe	Glu	Asn	Cys	Thr	Ile	His
				180					185					190		
	Ser	Lys	Gly	Glu	Gly	Tyr	Val	Thr	Ala	His	Tyr	Arg	Thr	Ser	Asp	Glu
			195					200					205			
	Met	Asp	Thr	Gly	Phe	Val	Phe	His	Arg	Cys	Arg	Leu	Thr	Gly	Arg	Asp
		210					215					220				
25	Thr	Gly	Arg	Gly	Val	Tyr	Leu	Gly	Arg	Pro	Trp	Arg	Pro	Tyr	Ala	Arg
	225					230					235				240	
	Val	Val	Phe	Ile	Asp	Cys	Trp	Leu	Asp	Ala	His	Ile	Arg	Pro	Glu	Gly
				245						250					255	
	Trp	Asp	Asn	Trp	Arg	Asp	Pro	Glu	Arg	Glu	Lys	Thr	Ala	Trp	Phe	Ala
				260					265					270		
30	Glu	Tyr	Lys	Ser	Lys	Gly	Pro	Gly	Ala	Asn	Pro	Val	Ala	Arg	Val	Ala
			275					280					285			
	Trp	Ser	Arg	Gln	Leu	Thr	Thr	Glu	Gln	Ala	Ala	Glu	Phe	Ser	Arg	Glu
		290					295					300				
	Arg	Phe	Phe	Ser	Arg	Ala	Val	Arg	Gly	Leu	Ser	Gly	Gln	Ala	Asn	Gln
	305					310					315				320	
35	Ala	Val	Gly	Thr	Ile	Ala	Trp	Asp	Asp	Ala	Gln	Lys	Lys	Pro	Asn	Glu
					325					330					335	

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				340					345					350		
	Tyr	Gln	Arg	Asp	Ser	Gly	Gly	Trp	Pro	Lys	Asn	Ile	Asp	Met	Gly	Lys
			355					360					365			
5	Pro	Leu	Asp	Asp	Lys	Gly	Arg	Ala	Gly	Leu	Leu	Arg	Val	Arg	Lys	Lys
		370					375					380				
	Asn	Asp	Ser	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Tyr	Thr	Gln	Leu	Ser	Phe
	385					390					395					400
	Leu	Ala	Arg	Val	Tyr	Thr	Ala	Gln	Lys	Gln	Glu	Arg	His	Arg	Glu	Ser
				405						410					415	
10	Phe	Leu	Lys	Gly	Leu	Asp	Tyr	Leu	Leu	Lys	Ala	Gln	Tyr	Pro	Asn	Gly
				420				425						430		
	Gly	Trp	Pro	Gln	Phe	Tyr	Pro	Asn	Leu	Asn	Gly	Tyr	Tyr	Lys	His	Ile
			435					440					445			
	Thr	Phe	Asn	Asp	Asn	Ala	Met	Ile	Gly	Val	Met	Lys	Leu	Leu	Arg	Asp
		450					455					460				
15	Val	Ala	Ala	Ala	Lys	Pro	Ala	Tyr	Ala	Phe	Val	Asp	Glu	Ala	Arg	Arg
	465					470					475					480
	Thr	Ser	Ala	Ala	Lys	Ala	Val	Glu	Lys	Gly	Ile	Glu	Cys	Ile	Leu	Lys
				485						490					495	
	Thr	Gln	Val	Val	Val	Asn	Gly	Arg	Arg	Thr	Val	Trp	Cys	Ala	Gln	His
				500					505					510		
20	Asp	Glu	Val	Thr	Leu	Ala	Pro	Ala	Pro	Ala	Arg	Thr	Phe	Glu	Leu	Val
			515					520					525			
	Ser	Leu	Ser	Gly	Gly	Glu	Ser	Val	Glu	Ile	Val	Arg	Phe	Leu	Met	Ser
		530					535					540				
	Ile	Lys	Asn	Pro	Ser	Pro	Ala	Val	Val	Glu	Ala	Ile	Glu	Ser	Ala	Val
	545					550					555					560
25	Ala	Trp	Phe	Glu	Gln	Ser	Gln	Val	Lys	Asp	Pro	Ala	Gly	Lys	Pro	Ala
				565						570					575	
	Trp	Ala	Arg	Phe	Tyr	Glu	Ile	Gly	Thr	Asn	Arg	Pro	Ile	Phe	Ala	Gly
				580					585					590		
	Arg	Asp	Gly	Val	Val	Lys	Tyr	Asp	Val	Lys	Gln	Ile	Asp	Glu	Glu	Arg
			595					600					605			
30	Arg	Lys	Asn	Tyr	Ala	Trp	Tyr	Val	Asp	Asp	Ala	Ala	Lys	Leu	Leu	Lys
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 <212> DNA
 <213> Unknown

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<400> 29

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	gcgcggatca	aaccgccgaa	atttccgaag	cgcgatttcg	tcatacagaa	gttcggcgcg	180
5	aaggcgggaa	ccgatatgcac	gcaagcgatc	gctaaagccc	tcgacgcttg	cgcgaaagcc	240
	ggcggcgggac	gcgtcgctcgt	acccgccggc	gaattttctca	ccggtgcgat	ccatctcaag	300
	tcgaacacca	atctctacgt	ctcaaaaggc	gcgactctga	agttttcgac	cgacccccgaa	360
	aaatatctgc	cgatcgttca	cacgcggtgg	gaaggggatgg	agttgatgca	tctctcgccg	420
	ttcatctacg	cgtacgagca	gacgaacatc	gcgatcaccg	gcgagggcac	gctcgacggc	480
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10	gtgatcagtc	agcaaaaagc	gcgggcgcg	ctttacgaga	tgatggacaa	gaacgtaccc	600
	gtcgcggagc	gcgtgttcgg	tatcggggcac	tatctccggc	cgcagttcat	ccagccgtac	660
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	catccggtgc	tttgcgagaa	tgtcaccgtc	cgcaatcttc	acatctcgtc	gcacgggtccg	780
	aacaacgacg	gctgcgatcc	cgagtcgtgc	aaagacgtcc	tgatcgacaa	ctgcttcttc	840
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	atcacggtcg	gaagcgagat	ctcgggcggc	gtgcgaaact	tgttcgaca	cgattgcaag	1020
	atggacagtg	cggatctgtg	gaccgcgctc	cgggtaaaga	acaacgcac	gcggggcggc	1080
	atgctggaga	atttctatct	ccgcaacatc	accgtcgggc	aagtcgcgcg	tgctgtggtc	1140
	gagatcgatt	tcaactatga	agaaggcgcg	aagggatcgt	acacaccggt	catgcgcaac	1200

20	tacgtgggtcg	aggatctgac	gtgcaccagc	gggaaccggc	ccgtcgatct	gcaaggatta	1260
	gacaacgcgc	caattttacga	tgtgtcgctg	cgtaacacga	ccttcggcgc	gatgaagaac	1320
	aagagcgctc	tgaagaatgt	ccgaggactg	aagatcgaaa	acgttaccgt	cagcggcacg	1380
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 <211> 465
 <212> PRT
 <213> Unknown

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<220>
 <223> Obtained from an environmental sample

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<221> SIGNAL
 <222> (1)...(27)

<221> DOMAIN
 <222> (77)...(459)
 <223> Catalytic domain

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<400> 30

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[illegible]

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<211> 1401
<212> DNA

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<213> Unknown

<220>

<223> Obtained from an environmental sample

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10	gtggtctaca	tcccggccgg	acggttcctc	accggtcccc	tccgcctcaa	aagccacgtc	180
	cggctccatc	tcgaggccgg	agcgcacttg	ctcttttagtc	aggacccggc	cgattatcct	240
	gttctggaga	cgaggtggga	ggggaaggag	gtcttgacct	atgcacacca	gatctacggc	300
	gaggacctcg	aaggggtcgc	gattaccggg	cgggggacca	tcgacggccg	gggagagact	360
	tggtggcgac	tcttccgcgc	caaagccttc	acccatcccc	gaccccgcc	catcgctttt	420
15	acccgctgca	aggacatcct	gatagaagga	gtaaccctcg	tcaattcacc	ggcctggacc	480
	atcaatcctg	tgatgtgcga	gcgggtgacc	atcgataagg	tgactatcat	caacccgccc	540
	gactcgccca	acaccgacgg	gatcgacccc	gattcctccc	ggaacgtcta	tactactaac	600
	tgctacattg	acgtaggcga	tgactgcatc	gccatcaaag	cgggcccaga	ggactccctt	660
	tatcggacgc	cttgtgaaaa	cattgtcatc	gccaactgcc	tcatgcgcca	cggtcacggc	720
	ggggtggtca	tcggcagcga	gaccagcggg	ggtattcgca	aggtagtcat	taccaactgc	780
20	atcttcgagg	acaccgaccg	gggcattaga	cttaagtccc	ggcgcggacg	cggcgggttc	840
	gtcaggagac	tccgggagac	gaatattatc	atggaaaagg	tgctctgtcc	cttcgtcctc	900
	aacatgtact	atgataccgg	gggaggcggtg	atcgacgagc	gcgcgcatga	cttagaacc	960
	catccggtaa	gcgaggctac	accctccttc	cgccgcctct	ccttcagtca	cattactgcc	1020
	cgggaagtgc	aggccgcccgc	ggccttcctc	tacggcctgc	ccgaacagcc	tctggaggac	1080
	gtcttatttg	acgatattctg	gatagagctg	gccgcgacg	cttctcctgc	ccgtccggcc	1140
25	atgatgcggg	ccgtcccgc	catgagccaa	ggtggtgtgc	tctgctacgg	tgcgcgggcg	1200
	atctccttcc	ggcacatgca	cctccgcggg	caccgcggtc	cggccttcca	gatcgaacgc	1260
	gcggaggcgg	tgcagttgat	gggctgctcg	accgacggca	gtgaagaccc	ccagcttgtc	1320
	ttgggtcaag	cggaggaggt	caccatccgt	gactgcacct	ttaccgccc	gcaggacccc	1380
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30 <210> 32

<211> 466

<212> PRT

<213> Unknown

35 <220>

<223> Obtained from an environmental sample

<221> DOMAIN

<222> (1)...(348)

40 <223> Catalytic domain

<400> 32

45	Met	Ile	Asn	Leu	Tyr	Gly	Val	Phe	Asp	Ile	Arg	Thr	Phe	Gly	Ala	Gln
	1				5					10				15		
	Pro	Asp	Gly	Glu	Thr	Pro	Ser	Thr	Ala	Ala	Ile	Thr	Ala	Ala	Ile	Glu
			20					25					30			
	Thr	Cys	Ala	Ala	Ala	Gly	Gly	Gly	Val	Val	Tyr	Ile	Pro	Ala	Gly	Arg
			35				40					45				
50	Phe	Leu	Thr	Gly	Pro	Leu	Arg	Leu	Lys	Ser	His	Val	Arg	Leu	His	Leu

55

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[illegible]

<210> 33

<211> 1041

50 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

55

<400> 33

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	cggattacgg	ccgacaagat	caacaacaag	cccgaactcct	ggcttaccag	cgacgaaggc	120
	atcaagctga	tcgacaacat	catcacctgg	cagaacccccg	aggggtggctg	ggccaagtac	180
5	tacgacgcga	ccaatccgca	caaacaaggc	gaagtctacg	gcgactggga	cggcgtcggc	240
	accatcgaca	acggctacac	ctacaccgag	ctgaatctcc	tggcgcacgt	ctacaccctc	300

	accaagcgcc	cggagatcct	cgattcgttc	aacaagggcc	tggagtttct	gctcaaagcc	360
10	caatacccca	gcggcggtg	gccgcaacgg	tttccggtgc	ccaacaacta	cggcaagtgc	420
	atcacgctca	acgacaacgc	gatgggtgaac	gtgatgcagt	tcctgcagaa	cgtcgcaaag	480
	ggcaaggaag	acttcgcttt	cgtcgacgag	cagcgtcgcg	ccaaagcgaa	ggaggcgttt	540
	gaccgcggga	tcgactgcct	tctgaagctc	cagattaccg	tgaacggcaa	gcttaccgcc	600
	tggggcccagc	agtatgaccc	gaagacactc	gccgcggcgc	ccgcccgggc	gtacgagctc	660
	ccgggcctca	gcggctgcga	aagcgcgccc	gtcatgcgct	tgttcatgtc	tttgagaaac	720
15	cccagtcccg	aagttcagcg	cgccgtccac	gcggcggtcg	cttggtacga	ggcgtcgaag	780
	atcacccgga	agaagctggt	gcgcgagaac	aacgacgtga	caactggccga	cgaccccaac	840
	ggcgagccgc	tttgggcgcg	cttctacgac	atcgaaacca	accgcccgtt	ctattgcggt	900
	cgcgacggcg	tgaagaagtg	gtcgctggac	gagatcgagc	ccgaacgccg	caagggctac	960
	gcttggtgcc	gcccctgggc	gacgagcgta	ctggagcagt	atcgcaagtg	ggcggcgaag	1020
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20
 <210> 34
 <211> 346
 <212> PRT
 <213> Unknown

25
 <220>
 <223> Obtained from an environmental sample

30
 <221> SIGNAL
 <222> (1)...(18)

<221> DOMAIN
 <222> (19)...(346)
 <223> Catalytic domain

35
 <400> 34

40

45

50

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	Met	Lys	Leu	Arg	Cys	Leu	Met	Leu	Thr	Leu	Leu	Leu	Cys	Gly	Ser	Ala
	1				5					10					15	
	Phe	Ala	Ala	Asp	Arg	Ile	Thr	Ala	Asp	Lys	Ile	Asn	Asn	Lys	Pro	Asp
5				20					25					30		
	Ser	Trp	Leu	Thr	Ser	Asp	Glu	Gly	Ile	Lys	Leu	Ile	Asp	Asn	Ile	Ile
			35					40					45			
	Thr	Trp	Gln	Asn	Pro	Glu	Gly	Gly	Trp	Ala	Lys	Tyr	Tyr	Asp	Ala	Thr
		50					55					60				
	Asn	Pro	His	Lys	Gln	Gly	Glu	Val	Tyr	Gly	Asp	Trp	Asp	Gly	Val	Gly
10	65				70					75					80	
	Thr	Ile	Asp	Asn	Gly	Tyr	Thr	Tyr	Thr	Glu	Leu	Asn	Leu	Leu	Ala	His
				85						90					95	
	Val	Tyr	Thr	Leu	Thr	Lys	Arg	Pro	Glu	Ile	Leu	Asp	Ser	Phe	Asn	Lys
				100					105					110		
	Gly	Leu	Glu	Phe	Leu	Leu	Lys	Ala	Gln	Tyr	Pro	Ser	Gly	Gly	Trp	Pro
15			115					120					125			
	Gln	Arg	Phe	Pro	Val	Pro	Asn	Asn	Tyr	Gly	Lys	Cys	Ile	Thr	Leu	Asn
		130					135					140				
	Asp	Asn	Ala	Met	Val	Asn	Val	Met	Gln	Phe	Leu	Gln	Asn	Val	Ala	Lys
	145					150					155					160
	Gly	Lys	Glu	Asp	Phe	Ala	Phe	Val	Asp	Glu	Gln	Arg	Arg	Ala	Lys	Ala
20				165					170					175		
	Lys	Glu	Ala	Phe	Asp	Arg	Gly	Ile	Asp	Cys	Leu	Leu	Lys	Leu	Gln	Ile
				180					185					190		
	Thr	Val	Asn	Gly	Lys	Leu	Thr	Ala	Trp	Ala	Gln	Gln	Tyr	Asp	Pro	Lys
			195				200						205			
	Thr	Leu	Ala	Ala	Ala	Pro	Ala	Arg	Ala	Tyr	Glu	Leu	Pro	Gly	Leu	Ser
25		210					215					220				
	Gly	Cys	Glu	Ser	Ala	Pro	Val	Met	Arg	Leu	Phe	Met	Ser	Leu	Glu	Asn
	225					230					235					240
	Pro	Ser	Pro	Glu	Val	Gln	Arg	Ala	Val	His	Ala	Ala	Ala	Ala	Trp	Tyr
				245						250					255	
	Glu	Ala	Ser	Lys	Ile	Thr	Gly	Lys	Lys	Leu	Val	Arg	Glu	Asn	Asn	Asp
30				260					265					270		
	Val	Thr	Leu	Ala	Asp	Asp	Pro	Asn	Gly	Glu	Pro	Leu	Trp	Ala	Arg	Phe
			275					280					285			
	Tyr	Asp	Ile	Glu	Thr	Asn	Arg	Pro	Phe	Tyr	Cys	Gly	Arg	Asp	Gly	Val
		290					295					300				
35																
	Lys	Lys	Trp	Ser	Leu	Asp	Glu	Ile	Glu	Pro	Glu	Arg	Arg	Lys	Gly	Tyr
	305					310					315					320
	Ala	Trp	Val	Arg	Pro	Trp	Ala	Thr	Ser	Val	Leu	Glu	Gln	Tyr	Arg	Lys
				325						330					335	
40		Trp	Ala	Ala	Lys	His	Pro	Pro	Val	Asn	Ser					
				340						345						

<210> 35

<211> 1071

<212> DNA

45 <213> Unknown

<220>

<223> Obtained from an environmental sample

50 <400> 35

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acatggagcg acgtattcaa acagaagcct ctctggtacc aaaccgacga ggcggctcga      120
gtcgcggacc aactcctcat ctatcaaaaa gagaacggcg ggtttgagaa gaatgtcgac      180
atggcggttg tgctgacgca gaaggaaaaa gaagagctca ccgcaaagcg gtcagacgctc      240
tccgaaacga cgatcgacaa ccggaccacg tatcctcagg tcgcgtaict cggtcgagta      300
atcacccgcaa gccttcttaa accttcgccg ccggcggaatc ttccgaaata caaagacgcc      360
ttcaacaaag gtcttgatta cctgcttgcc tcccagtatg agaacggagg atttccgcaa      420
ttctatccgt tgaaaaaaagg ctattacaca cacatcacct tcaacgacga cgcgatgac      480
ggcgtcctga aggtgcttcg cgacatcgca aataagaaag aggtattacgt gttcgtggat      540
gaagcgcgaa gacttcgcgc cgagcaagcg gtcgccaaag cgctgcctct tattctgaag      600
cttcagggttg tcgtcgacgg aaagaaaacc gtctgggctg cgcagtatga cgagactacg      660
ttggcgccctg cagcggctcg caagtttgag cccgtgtcgt tgaccgctgg tgagagcgtc      720
ggcatcgtcc gatacctgat gcaggaaaaa ccgacgccgg agatcaccga tgcgatcgag      780
tctgcgatcg attggtatcg aaagaacaag atcgacggaa tacgttgga ggcgatcaaa      840
ggcgagaaca cggttgtgaa agacaaatcg gctcccccta tatgggcacg gttctatcag      900
atcgaacga tgcgtccgat cttcatcgga cgtgattcgg ttatcaagta tgacgtgacg      960
caggtcgaag ccgagcgtcg gaatgggtac gcctggtacg tcaccgcacc gaatgaattg     1020
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<210> 36

<211> 356

<212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

<221> DOMAIN

<222> (1)...(356)

<223> Catalytic domain

<400> 36

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Trp  Ser  Leu  Val  Thr  Trp  Ser  Asp  Val  Phe  Lys  Gln  Lys  Pro  Leu  Trp
20     25     30
Tyr  Gln  Thr  Asp  Glu  Ala  Ala  Arg  Val  Ala  Asp  Gln  Leu  Leu  Ile  Tyr
35     40     45
Gln  Lys  Glu  Asn  Gly  Gly  Phe  Glu  Lys  Asn  Val  Asp  Met  Ala  Leu  Met
50     55     60
Leu  Thr  Gln  Lys  Glu  Lys  Glu  Glu  Leu  Thr  Ala  Lys  Arg  Ser  Asp  Val
65     70     75     80
Ser  Glu  Thr  Thr  Ile  Asp  Asn  Arg  Thr  Thr  Tyr  Pro  Gln  Val  Ala  Tyr
85     90     95
Leu  Gly  Arg  Val  Ile  Thr  Ala  Ser  Leu  Lys  Pro  Ser  Pro  Pro  Ala
100    105    110
Asn  Leu  Pro  Lys  Tyr  Lys  Asp  Ala  Phe  Asn  Lys  Gly  Leu  Asp  Tyr  Leu
115    120    125
Leu  Ala  Ser  Gln  Tyr  Glu  Asn  Gly  Gly  Phe  Pro  Gln  Phe  Tyr  Pro  Leu
130    135    140
Lys  Lys  Gly  Tyr  Tyr  Thr  His  Ile  Thr  Phe  Asn  Asp  Asp  Ala  Met  Ile
145    150    155    160

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	Gly	Val	Leu	Lys	Val	Leu	Arg	Asp	Ile	Ala	Asn	Lys	Lys	Glu	Asp	Tyr
					165					170					175	
	Val	Phe	Val	Asp	Glu	Ala	Arg	Arg	Leu	Arg	Ala	Glu	Gln	Ala	Val	Ala
				180					185					190		
5	Lys	Ala	Leu	Pro	Leu	Ile	Leu	Lys	Leu	Gln	Val	Val	Val	Asp	Gly	Lys
			195					200					205			
	Lys	Thr	Val	Trp	Ala	Ala	Gln	Tyr	Asp	Glu	Thr	Thr	Leu	Ala	Pro	Ala
		210					215					220				
	Ala	Ala	Arg	Lys	Phe	Glu	Pro	Val	Ser	Leu	Thr	Ala	Gly	Glu	Ser	Val
	225					230					235					240
10	Gly	Ile	Val	Arg	Tyr	Leu	Met	Gln	Glu	Lys	Pro	Thr	Pro	Glu	Ile	Thr
				245						250					255	
	Asp	Ala	Ile	Glu	Ser	Ala	Ile	Asp	Trp	Tyr	Arg	Lys	Asn	Lys	Ile	Asp
				260					265					270		
	Gly	Ile	Arg	Trp	Glu	Arg	Ile	Lys	Gly	Glu	Asn	Thr	Val	Val	Lys	Asp
			275					280					285			
15	Lys	Ser	Ala	Pro	Pro	Ile	Trp	Ala	Arg	Phe	Tyr	Gln	Ile	Glu	Thr	Met
		290					295					300				
	Arg	Pro	Ile	Phe	Ile	Gly	Arg	Asp	Ser	Val	Ile	Lys	Tyr	Asp	Val	Thr
	305					310					315					320
	Gln	Val	Glu	Ala	Glu	Arg	Arg	Asn	Gly	Tyr	Ala	Trp	Tyr	Val	Thr	Ala
				325						330					335	
20	Pro	Asn	Glu	Leu	Val	Asn	Glu	Asp	Tyr	Leu	Lys	Trp	Lys	Gly	Lys	Ser
				340					345					350		
	Ala	Gly	Ala	Lys												
			355													

25 <210> 37
 <211> 1860
 <212> DNA
 <213> Unknown

30 <220>
 <223> Obtained from an environmental sample

<400> 37

35

40

45

50

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	atagcagccg	ctgttgcggt	gatggcaggc	acttcagcat	ttgcagctgc	gacgggtggc	120
	ttctctacca	ctgatgggtg	caacgtatcg	ggcgcccgtt	cgttttactgc	atcgacttac	180
	cagcaaatca	acaccattat	tgccaacgca	aaactggatg	atgcaggtaa	aaaagtcact	240
5	gggggtgctt	acccgcttat	cattacctac	accggtaatg	aagactcgct	gattaaccag	300
	atgatcaaa	accacacggt	gaattcatcg	ggcaactgcc	ctaaccgcg	ttggagcgaa	360
	gcctatcgct	acgtggaaat	taaagagttt	accaagggtg	ttaccattca	aggcgcgaa	420
	ggttcttcag	caaacttcgg	cattgtgatt	aataaatctg	acaatgtgat	tgtgctgaat	480
	atgaaaatcg	gtgcgcttgc	tgggtgcgag	aacgatgcgg	atatgattcg	tatcgacacc	540
	ggcggttaac	tgtggattga	tcacaacgaa	ttgtttgcgg	ttaaataatga	atgtaaaggt	600
10	tcacccgatg	gtgacctgac	atttgaaagt	gcgattgata	ttaaaaaagc	atcgcaaaat	660
	attacgggtg	cctacaacat	tatccgcgat	agtaaaaaag	tagggctcga	tggttcgagt	720
	agcagtgata	ttgcagggtg	ccgtaagatt	acgttccatc	acaatattta	tcgcaatggt	780
	gggtgcacgt	taccgttgca	acgcggtggg	tggacacaca	tgtataacaa	tctttacgac	840
	ggagttacca	gctcgggtat	taacgttcgt	caagggtggc	acgcgcta	cgagaacaac	900
	tgtttccaaa	atgctgtcaa	cccgtttacc	tgccgttttg	acagtagtaa	ctgcggttac	960
15	tgggatctgc	gcaacaacaa	cgtgcgcaac	cctggtgatt	tctccaccta	caacattacc	1020
	tggaccagcg	gtggcaccat	cgacgccacc	aactggacta	ccactcaacc	tttcccga	1080
	agcattccct	acagctactc	gcctgttagc	ccgcagtggt	tcaaagacaa	gttggcaaat	1140
	tatgctggtg	tcggtaaaaa	caatgcgcaa	ttaacggcgt	ctgcgtgcag	cggaaatact	1200
	tcacggtag	caccttcac	agtgccagca	tcacggcg	caccttcaag	ccgttcaccc	1260
	agcagtgac	cgccatccag	cacaccaact	acatcaagct	cgagttcagt	tgccgcaacc	1320
20	ggttcaattt	cgctcgggtc	aacggcaacc	aacaacagca	ttgtgttgag	ttggtcacc	1380
	aacaatgtga	cgctcgggtc	gcaagaagt	tatcgcgata	ccgacgctga	tccatcgggg	1440
	cgtgtgcgta	tcgcatccct	ggctgcttca	gcgcgtatgt	ataccgatag	cacagcggca	1500
	tcggggccaaa	cctattacta	ctggattaaa	aataccactt	ctggtgttgt	caccaattcc	1560
	aatgctgcat	cagcgcgtat	tggtagcacg	gcgtccagtt	ctgttgcatc	aagcagctca	1620
	agttcaagcg	gcggcgcgcc	cgatttaggt	ggtagctggg	attatccaag	cggcttctcc	1680
25	aagtgcgctg	atttggggcg	gacttgttca	gtgtcatcgg	gcgatggctg	ggttgcgttt	1740
	ggtcgcaaa	gcaagtgggt	taccaagaaa	gtatcggtag	gtagttcaat	cgcctgtacc	1800
	gttgcgcat	ttggttcgga	tccacagggc	aaccctaaca	agtgttctta	caaacgttaa	1860

30 <210> 38
 <211> 619
 <212> PRT
 <213> Unknown

35 <220>
 <223> Obtained from an environmental sample

40 <221> SIGNAL
 <222> (1)...(35)
 <221> DOMAIN
 <222> (36)...(387)
 <223> Catalytic domain

45 <400> 38

50

55

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	Ser	Leu	Thr	Ala	Ile	Ala	Ala	Ala	Val	Ala	Leu	Met	Ala	Gly	Thr	Ser
				20					25					30		
5	Ala	Phe	Ala	Ala	Ala	Thr	Gly	Gly	Phe	Ser	Thr	Thr	Asp	Gly	Gly	Asn
			35					40					45			
	Val	Ser	Gly	Ala	Arg	Ser	Phe	Thr	Ala	Ser	Thr	Tyr	Gln	Gln	Ile	Asn
		50					55					60				
	Thr	Ile	Ile	Ala	Asn	Ala	Lys	Leu	Asp	Asp	Ala	Gly	Lys	Lys	Val	Thr
	65				70					75						80
10	Gly	Gly	Ala	Tyr	Pro	Leu	Ile	Ile	Thr	Tyr	Thr	Gly	Asn	Glu	Asp	Ser
				85						90					95	
	Leu	Ile	Asn	Gln	Met	Ile	Lys	Asp	His	Thr	Val	Asn	Ser	Ser	Gly	Asn
			100						105					110		
	Cys	Pro	Asn	Pro	Arg	Trp	Ser	Glu	Ala	Tyr	Arg	Tyr	Val	Glu	Ile	Lys
			115					120					125			
15	Glu	Phe	Thr	Lys	Gly	Ile	Thr	Ile	Gln	Gly	Ala	Asn	Gly	Ser	Ser	Ala
		130					135					140				
	Asn	Phe	Gly	Ile	Val	Ile	Asn	Lys	Ser	Asp	Asn	Val	Ile	Val	Arg	Asn
	145				150						155					160
	Met	Lys	Ile	Gly	Ala	Leu	Ala	Gly	Ala	Ser	Asn	Asp	Ala	Asp	Met	Ile
				165					170						175	
20	Arg	Ile	Asp	Thr	Gly	Val	Asn	Val	Trp	Ile	Asp	His	Asn	Glu	Leu	Phe
			180						185					190		
	Ala	Val	Asn	Asn	Glu	Cys	Lys	Gly	Ser	Pro	Asp	Gly	Asp	Leu	Thr	Phe
			195					200					205			
	Glu	Ser	Ala	Ile	Asp	Ile	Lys	Lys	Ala	Ser	Gln	Asn	Ile	Thr	Val	Ser
		210					215					220				
25	Tyr	Asn	Ile	Ile	Arg	Asp	Ser	Lys	Lys	Val	Gly	Leu	Asp	Gly	Ser	Ser
	225				230						235					240
	Ser	Ser	Asp	Ile	Ala	Gly	Gly	Arg	Lys	Ile	Thr	Phe	His	His	Asn	Ile
				245						250					255	
	Tyr	Arg	Asn	Val	Gly	Ala	Arg	Leu	Pro	Leu	Gln	Arg	Gly	Gly	Trp	Thr
			260						265					270		
30	His	Met	Tyr	Asn	Asn	Leu	Tyr	Asp	Gly	Val	Thr	Ser	Ser	Gly	Ile	Asn
			275					280					285			
	Val	Arg	Gln	Gly	Gly	Tyr	Ala	Leu	Ile	Glu	Asn	Asn	Trp	Phe	Gln	Asn
		290					295					300				
	Ala	Val	Asn	Pro	Val	Thr	Cys	Arg	Phe	Asp	Ser	Ser	Asn	Cys	Gly	Tyr
	305					310					315					320
35	Trp	Asp	Leu	Arg	Asn	Asn	Val	Arg	Asn	Pro	Gly	Asp	Phe	Ser	Thr	
				325					330					335		
	Tyr	Asn	Ile	Thr	Trp	Thr	Ser	Gly	Gly	Thr	Ile	Asp	Ala	Thr	Asn	Trp
			340						345					350		
	Thr	Thr	Thr	Gln	Pro	Phe	Pro	Ile	Ser	Ile	Pro	Tyr	Ser	Tyr	Ser	Pro
			355					360					365			
40	Val	Ser	Pro	Gln	Cys	Val	Lys	Asp	Lys	Leu	Ala	Asn	Tyr	Ala	Gly	Val
		370					375					380				
	Gly	Lys	Asn	Asn	Ala	Gln	Leu	Thr	Ala	Ser	Ala	Cys	Ser	Gly	Asn	Thr
	385				390						395					400
	Ser	Ser	Val	Ala	Pro	Ser	Ser	Val	Pro	Ala	Ser	Ser	Ala	Ala	Pro	Ser
					405					410					415	

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Ser Arg Ser Ser Ser Ser Ala Ala Pro Ser Ser Thr Pro Thr Thr Ser
 Ser Ser Ser Ser Val Ala Ala Thr Gly Ser Ile Ser Leu Gly Ala Thr
 Ala Thr Asn Asn Ser Ile Val Leu Ser Trp Ser Pro Asn Asn Val Thr
 Leu Gly Ser Gln Glu Val Tyr Arg Asp Thr Asp Ala Asp Pro Ser Gly
 Arg Val Arg Ile Ala Ser Leu Ala Ala Ser Ala Arg Met Tyr Thr Asp
 Ser Thr Ala Ala Ser Gly Gln Thr Tyr Tyr Trp Ile Lys Asn Thr
 Thr Ser Gly Val Val Thr Asn Ser Asn Ala Ala Ser Ala Arg Ile Gly
 Ser Thr Ala Ser Ser Ser Val Ala Ser Ser Ser Ser Ser Ser Ser Gly
 Gly Ala Pro Val Leu Gly Thr Gly Asp Tyr Pro Ser Gly Phe Ser
 Lys Cys Ala Asp Leu Gly Gly Thr Cys Ser Val Ser Ser Gly Asp Gly
 Trp Val Ala Phe Gly Arg Lys Gly Lys Trp Val Thr Lys Lys Val Ser
 Val Gly Ser Ile Ala Cys Thr Val Ala Ala Phe Gly Ser Asp Pro
 Gln Gly Asn Pro Asn Lys Cys Ser Tyr Lys Arg

<210> 39
 <211> 1077
 <212> DNA
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<400> 39

35	atggcgccga	tcctccgacc	caacctcctt	tgcacttacg	cgctctgcat	gggcttgctc	60
	gccgtggtga	gctgcgcggc	ggggccggtg	tcagcgcagc	agccggcgcc	atggagcacg	120
	gccatcgtgg	agcaggagga	gagcgcgttc	gcctccccgt	cgatgcgcag	cgtcgccgac	180
	aacgtcgtgc	gccatcagtc	ggccgaaggc	ggctggccta	agaacaccaa	tctggcgggc	240
	ccgccatcgg	ggccggcgcc	ggagggcgtc	gccaatacga	tcgacaatga	tgcgacgacg	300
40	ctgcccgatgg	agtttctggc	gcgtgtgata	cacgccggcg	gcgtccgata	caagccggcc	360
	ttcgagcgcg	ggctggatta	tctgcttgcg	gctcagtagc	cgaacggcgg	ctggccgcag	420
	ttctatccgc	tgccgcgggg	ctattacgat	cacgtgacgt	tcaacgacga	cgccatgatc	480
	cgggtgatga	ttctgctcgg	cgagtgggcg	cgccggcggg	cgccctatga	atttgctgac	540
	gccggggcggc	gcgcgcgcgc	tgacgcccgc	gtcgagcggg	gcctggcgct	catcctgcgc	600
	acgcagatcc	ggcagggcgg	ggcgctgacg	gtctggtgcg	cgagtgatga	cagcgccacc	660
45	ttgcagcccg	cctggggcgc	cgccatagag	ccgccgtccc	tgtccggcgc	ggaaagtgtg	720
	gggatcgtgc	gctatctcat	gtcgatcgac	catccctcgc	ccgaagtcgt	cgccgccgtc	780
	gacggcgctg	tggcatggct	gcgcgcggcc	gccattggcg	gcgtgcgcgt	ggagaatttc	840
	acggacgccg	acggccgccc	tgaccgcccg	gccgtggccg	acgcggggcg	gccgccgatc	900
	tggggcgcggt	tctacgagtt	cggcgccaac	cgccgatctt	tcctggggcg	tgattccggt	960
	tttactaca	cgttcggaga	aatcgagcgc	gagcggcgcg	caggctacaa	ttattacgga	1020
50	tactgggcgc	gctccgtgct	ggaagactat	ccggcctggc	gcgcgcgcgt	gcatga	1077

<210> 40
 <211> 358
 <212> PRT
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

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<221> SIGNAL

<222> (1)...(32)

<221> DOMAIN

<222> (33)...(358)

5

<223> Catalytic domain

<400> 40

10	Met	Ala	Pro	Ile	Leu	Arg	Pro	Asn	Leu	Leu	Cys	Thr	Tyr	Ala	Leu	Cys
	1				5					10					15	
	Met	Gly	Leu	Leu	Ala	Val	Val	Ser	Cys	Ala	Ala	Gly	Pro	Val	Ser	Ala
				20					25					30		
	Gln	Gln	Pro	Ala	Pro	Trp	Ser	Thr	Ala	Ile	Val	Glu	Gln	Glu	Glu	Ser
			35					40					45			
15	Ala	Phe	Ala	Ser	Pro	Ser	Met	Arg	Ser	Val	Ala	Asp	Asn	Val	Val	Arg
	50						55					60				
	His	Gln	Ser	Ala	Glu	Gly	Gly	Trp	Pro	Lys	Asn	Thr	Asn	Leu	Ala	Ala
	65				70					75					80	
	Pro	Pro	Ser	Gly	Pro	Ala	Pro	Glu	Gly	Val	Ala	Asn	Thr	Ile	Asp	Asn
				85					90					95		
20	Asp	Ala	Thr	Thr	Leu	Pro	Met	Glu	Phe	Leu	Ala	Arg	Val	Ile	His	Ala
			100						105					110		
	Gly	Gly	Val	Arg	Tyr	Lys	Pro	Ala	Phe	Glu	Arg	Gly	Leu	Asp	Tyr	Leu
			115					120					125			
	Leu	Ala	Ala	Gln	Tyr	Ala	Asn	Gly	Gly	Trp	Pro	Gln	Phe	Tyr	Pro	Leu
	130						135					140				
25	Arg	Gly	Gly	Tyr	Tyr	Asp	His	Val	Thr	Phe	Asn	Asp	Asp	Ala	Met	Ile
	145					150					155					160
	Arg	Val	Met	Ile	Leu	Leu	Gly	Ala	Val	Ala	Arg	Gly	Gly	Ala	Pro	Tyr
				165						170					175	
	Glu	Phe	Val	Asp	Ala	Gly	Arg	Arg	Ala	Arg	Ala	Ala	Ala	Ala	Val	Glu
				180					185					190		
30	Arg	Gly	Leu	Ala	Leu	Ile	Leu	Arg	Thr	Gln	Ile	Arg	Gln	Gly	Gly	Ala
			195					200					205			
	Leu	Thr	Val	Trp	Cys	Ala	Gln	Tyr	Asp	Ser	Ala	Thr	Leu	Gln	Pro	Ala
	210						215					220				
	Trp	Ala	Arg	Ala	Tyr	Glu	Pro	Pro	Ser	Leu	Ser	Gly	Ala	Glu	Ser	Val
	225					230					235					240
35	Gly	Ile	Val	Arg	Tyr	Leu	Met	Ser	Ile	Asp	His	Pro	Ser	Pro	Glu	Val
				245						250					255	
	Val	Ala	Ala	Val	Asp	Gly	Ala	Val	Ala	Trp	Leu	Arg	Ala	Ala	Ala	Ile
				260					265					270		
	Ala	Gly	Val	Arg	Val	Glu	Asn	Phe	Thr	Asp	Ala	Asp	Gly	Arg	Pro	Asp
			275					280					285			
40	Arg	Arg	Ala	Val	Ala	Asp	Ala	Gly	Ala	Pro	Pro	Ile	Trp	Ala	Arg	Phe
			290				295					300				
	Tyr	Glu	Phe	Gly	Ala	Asn	Arg	Pro	Ile	Phe	Leu	Gly	Arg	Asp	Ser	Val
	305					310					315					320
	Phe	His	Tyr	Thr	Phe	Gly	Glu	Ile	Glu	Arg	Glu	Arg	Arg	Ala	Gly	Tyr
				325						330					335	
45	Asn	Tyr	Tyr	Gly	Tyr	Trp	Ala	Arg	Ser	Val	Leu	Glu	Asp	Tyr	Pro	Ala
				340					345					350		
	Trp	Arg	Ala	Arg	Val	Arg										
			355													

50

<210> 41

<211> 1080

<212> DNA

<213> Unknown

55

<220>

<223> Obtained from an environmental sample

<400> 41

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	acgcaagaaa	aaaaagtaac	ttggaaaagc	atcacagaaa	ataacgatga	aaattgggtt	120
	gtaagcgaag	aagccaaaaa	aatagccgaa	aatgttttgt	tatatcaacg	cgatattggg	180
5	ggttggccaa	aaaacactga	aattcaaaat	gaactttcag	aaaaagaaaa	actaacatta	240
	aaagaattaa	aatcggatcc	aaaaggatgt	accatcgaca	atgggtgcaac	gtgtcaggaa	300
	ttacttttct	tatccaaaat	atataaatcc	aatccagatg	agcgatataa	aatggctttc	360
	ttaaaagggtg	tgattttacct	gattacagct	caatacaaaa	atgggtgggtg	gccacaatat	420
	tacccttttga	gagaaggata	ttacactcat	attactttaca	acgataatgc	aatgggtgaat	480
	gttttaaaagt	tgttgaaaga	agtttaaagat	aaatctgatt	actactcaat	tcaagcacc	540
10							
	gatgaaat	ccaaaatggc	tgaagtatca	tttaataaag	gagtcgattg	catattaaaa	600
	acacagtaca	aacaaaatgg	aatattaacc	gcttgggtgtg	cacaacatga	cagggaaaca	660
	ttgaaacctg	ctaaagcaag	agcttatgaa	ttgccttcgt	taagcggaaa	agaatcagcc	720
15	aaaatttgtgt	tgttattaat	gtcaatcgaa	aatccatcta	agaagtaaat	tactgccgta	780
	aattcagcag	ttaattgggt	tgaaaaaaca	aaaatcaacg	gaattaaaaat	tgaaaccatt	840
	tccaccggga	aaaaggatga	aaaagataga	attgtttgtg	aaagtcctga	tgctccgccg	900
	ctttgggcaa	gattttatgga	attaagtgc	aacaaaccat	ttttttgtga	tcgtgacgga	960
	aagaaaaaat	acagcatgtc	agaaattagt	caagagcgta	gaaccggcta	tgcatgggtac	1020
	accaacgaac	caaaagaagt	tttaaaaaaa	tacgatgatt	ggaagtcac	attaaactaa	1080
20							
	<210> 42						
	<211> 359						
	<212> PRT						
	<213> Unknown						
25							
	<220>						
	<223> Obtained from an environmental sample						
	<221> SIGNAL						
30	<222> (1)...(21)						
	<221> DOMAIN						
	<222> (22)...(359)						
	<223> Catalytic domain						
35							
	<400> 42						
40							
45							
50							
55							

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	Met	Lys	Asn	Leu	Lys	Tyr	Ser	Leu	Val	Ser	Phe	Val	Leu	Leu	Ile	Thr
	1				5					10					15	
	Met	Asn	Val	Phe	Thr	Gln	Glu	Lys	Lys	Val	Thr	Trp	Lys	Ser	Ile	Thr
			20						25					30		
5	Glu	Asn	Asn	Asp	Glu	Asn	Trp	Phe	Val	Ser	Glu	Glu	Ala	Lys	Lys	Ile
			35					40					45			
	Ala	Glu	Asn	Val	Leu	Leu	Tyr	Gln	Arg	Asp	Ile	Gly	Gly	Trp	Pro	Lys
	50						55					60				
	Asn	Thr	Glu	Ile	Gln	Asn	Glu	Leu	Ser	Glu	Lys	Glu	Lys	Leu	Thr	Leu
	65					70					75				80	
10	Lys	Glu	Leu	Lys	Ser	Asp	Pro	Lys	Gly	Cys	Thr	Ile	Asp	Asn	Gly	Ala
				85						90				95		
	Thr	Cys	Gln	Glu	Leu	Leu	Phe	Leu	Ser	Lys	Ile	Tyr	Lys	Ser	Asn	Pro
				100					105					110		
	Asp	Glu	Arg	Tyr	Lys	Met	Ala	Phe	Leu	Lys	Gly	Val	Ile	Tyr	Leu	Ile
			115					120					125			
15	Thr	Ala	Gln	Tyr	Lys	Asn	Gly	Gly	Trp	Pro	Gln	Tyr	Tyr	Pro	Leu	Arg
	130						135					140				
	Glu	Gly	Tyr	Tyr	Thr	His	Ile	Thr	Tyr	Asn	Asp	Asn	Ala	Met	Val	Asn
	145					150					155					160
	Val	Leu	Lys	Leu	Leu	Lys	Glu	Val	Lys	Asp	Lys	Ser	Asp	Tyr	Tyr	Ser
				165						170					175	
20	Ile	Gln	Ala	Pro	Asp	Glu	Ile	Ser	Lys	Met	Ala	Glu	Val	Ser	Phe	Asn
				180					185					190		
	Lys	Gly	Val	Asp	Cys	Ile	Leu	Lys	Thr	Gln	Tyr	Lys	Gln	Asn	Gly	Ile
			195					200					205			
	Leu	Thr	Ala	Trp	Cys	Ala	Gln	His	Asp	Arg	Glu	Thr	Leu	Lys	Pro	Ala
	210						215					220				
25	Lys	Ala	Arg	Ala	Tyr	Glu	Leu	Pro	Ser	Leu	Ser	Gly	Lys	Glu	Ser	Ala
	225					230					235					240
	Lys	Ile	Val	Leu	Leu	Leu	Met	Ser	Ile	Glu	Asn	Pro	Ser	Lys	Glu	Val
				245						250					255	
	Ile	Thr	Ala	Val	Asn	Ser	Ala	Val	Asn	Trp	Phe	Glu	Lys	Thr	Lys	Ile
				260					265					270		
30	Asn	Gly	Ile	Lys	Ile	Glu	Thr	Ile	Ser	Thr	Gly	Lys	Lys	Asp	Glu	Lys
			275					280					285			
	Asp	Arg	Ile	Val	Val	Glu	Ser	Pro	Asp	Ala	Pro	Pro	Leu	Trp	Ala	Arg
	290					295					300					
35	Phe	Met	Glu	Leu	Ser	Asp	Asn	Lys	Pro	Phe	Phe	Cys	Asp	Arg	Asp	Gly
	305					310				315						320
	Lys	Lys	Lys	Tyr	Ser	Met	Ser	Glu	Ile	Ser	Gln	Glu	Arg	Arg	Thr	Gly
				325						330					335	
40	Tyr	Ala	Trp	Tyr	Thr	Asn	Glu	Pro	Lys	Glu	Val	Leu	Lys	Lys	Tyr	Asp
				340					345					350		
	Asp	Trp	Lys	Ser	Ser	Leu	Asn									
			355													

<210> 43

45 <211> 1902

<212> DNA

<213> Unknown

<220>

50 <223> Obtained from an environmental sample

<400> 43

55

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	gtggatccaa	agaattgggg	cagcggat	accggcgaaa	tcaaagtaac	taacaacaca	60
	agccaaacag	tcaatagctg	gtctgtgtca	tggcaagagg	caggagccag	tgtaactaat	120
	tcctggaatg	caaccttggg	agggacgaat	ccttataccg	caaccgggtt	aggatggaac	180
	tcaaccctgg	cgcccggagc	ctctgccagt	tttgggttttc	aagcaaacgg	cactgcggggg	240
5	gcaccaaagg	taaatggcag	tttgtgtggt	gcgactgcat	catctgcagc	gaccagcaaa	300
	tccagtgcga	gtgttgcgag	ttcaaagatt	gcaagttcaa	ttcaatcaag	tgcaactagc	360
	agttcaaaat	cgtccagttc	tgctgcacct	tcaagcacgc	caaaatccag	tagctctgct	420
	ccaacggctg	catcattcac	tattcaagaa	gagcaagccg	gtttttgccg	tgtagacggt	480
	attgcaacgg	aaagtaccaa	caccggattc	accggcaacg	gctacaccaa	ttccaataat	540
	gtacaagggt	ctgccattgt	gtgggcggtg	aatgcaacta	ccagtgcacg	ccatacaatt	600
10	actttccgct	tcgctaattg	tggcactgcg	aatcgcaatg	gctcgctagt	cattaacggc	660
	ggcagcaatg	gtaattacac	ggtgcaatta	ccacgcaccg	cgagctgggc	tgactggcaa	720
	acagtaagtc	tggaaattga	tttgggtacaa	ggcaataaca	atttgcaact	caccgcattg	780
	actgcagatg	gcctcgcaaa	tatcgacttc	atcaaaattg	aaggagcatc	aaccaaagcg	840
	ggaacctgtg	caggtgcggt	cagcagtagc	agtgttgcc	cttcggtaaa	atccagtgtc	900
	agcgcggcaa	gcagttctgt	accaacgaac	accggcgcca	tgctaacttt	ggatggcaac	960
15	cctgccgcaa	gctggcctaa	caaatcgcg	acaaagtgga	gcgcatcgcg	cgctgacatt	1020
	gttgccctct	atcaacagtc	caacggcggc	tggccaaaaa	atctggatta	caattcagtg	1080
	agcgtggtg	atggcgggcag	tgcaagcggc	accatcgata	atggtgcaac	tattactgaa	1140
	atggtttatc	tcgctgaggt	ttacaaaacc	ggaacaataa	ccaagtaccg	cgatgcagtt	1200
	cgccgtgcag	caaactttat	cgtgagttcg	caatatagca	ctggcgcggt	gccgcaattt	1260
	tatccgctca	aagggtggcta	tgcagaccac	gccaccttta	atgataacgg	catggccttac	1320
20	gcattaactg	tattggattt	cgctgcaaac	aagcgcgcgc	cttttgatac	ggatgtcttt	1380
	aatgacacag	accgcgcaaa	atttaaaaca	gcggtaacca	aagggtgttg	ttacatttta	1440
	aaagcgcaat	ggaaacaaaa	tggaaaatta	acagcctggt	gcgcacaaca	tggcgcgact	1500
	gactatcaac	ctaaaaaagc	acgcgcttat	gaattggaat	cactgagtgg	tagcgagtct	1560
	gttggtgtaa	ttgcattttt	aatgacgcag	ccgcagacag	cacaaatcca	aacggccggt	1620
	aaagcaggcc	tcaactgggt	caatagcccg	agcacctatt	tgggaagggt	cacctacgat	1680
25	tcattccaaag	cgtccactaa	tcccatagtg	cagaaagcgg	gaagtagaat	gtggtatcgc	1740
	ttttacgatt	taaataccaa	ccgtgggttt	ttcagcgacc	gggacggcag	caaattctat	1800
	gacattacca	aaatgtctga	agaacgtcgc	acgggttata	gttgggggtg	cgcttatggt	1860
	gagagcatca	tcgccttttg	caaaaaagtg	ggctatctat	aa		1902

30 <210> 44
 <211> 633
 <212> PRT
 <213> Unknown

35 <220>
 <223> Obtained from an environmental sample

<221> BINDING
 <222> (4)...(89)
 40 <223> Carbohydrate binding module

<221> BINDING
 <222> (152)...(275)
 <223> Carbohydrate binding module

45 <221> DOMAIN
 <222> (277)...(633)
 <223> Catalytic domain

50 <400> 44

55

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	1				5					10					15	
	Thr	Asn	Asn	Thr	Ser	Gln	Thr	Val	Asn	Ser	Trp	Ser	Val	Ser	Trp	Gln
5				20					25					30		
	Glu	Ala	Gly	Ala	Ser	Val	Thr	Asn	Ser	Trp	Asn	Ala	Thr	Leu	Gly	Gly
			35					40					45			
	Thr	Asn	Pro	Tyr	Thr	Ala	Thr	Gly	Leu	Gly	Trp	Asn	Ser	Thr	Leu	Ala
		50					55					60				
	Pro	Gly	Ala	Ser	Ala	Ser	Phe	Gly	Phe	Gln	Ala	Asn	Gly	Thr	Ala	Gly
10	65					70					75					80
	Ala	Pro	Lys	Val	Asn	Gly	Ser	Leu	Cys	Gly	Ala	Thr	Ala	Ser	Ser	Ala
				85					90						95	
	Ala	Thr	Ser	Lys	Ser	Ser	Ala	Ser	Val	Ala	Ser	Ser	Lys	Ile	Ala	Ser
				100					105					110		
	Ser	Ile	Gln	Ser	Ser	Ala	Thr	Ser	Ser	Ser	Lys	Ser	Ser	Ser	Ser	Ala
			115					120					125			
15	Ala	Pro	Ser	Ser	Thr	Pro	Lys	Ser	Ser	Ser	Ser	Ala	Pro	Thr	Ala	Ala
		130					135					140				
	Ser	Phe	Thr	Ile	Gln	Glu	Gln	Ala	Gly	Phe	Cys	Arg	Val	Asp	Gly	
	145					150					155				160	
	Ile	Ala	Thr	Glu	Ser	Thr	Asn	Thr	Gly	Phe	Thr	Gly	Asn	Gly	Tyr	Thr
				165						170					175	
20	Asn	Ser	Asn	Asn	Val	Gln	Gly	Ala	Ala	Ile	Val	Trp	Ala	Val	Asn	Ala
				180					185					190		
	Thr	Thr	Ser	Ala	Arg	His	Thr	Ile	Thr	Phe	Arg	Phe	Ala	Asn	Gly	Gly
			195					200					205			
	Thr	Ala	Asn	Arg	Asn	Gly	Ser	Leu	Val	Ile	Asn	Gly	Gly	Ser	Asn	Gly
		210					215					220				
25	Asn	Tyr	Thr	Val	Gln	Leu	Pro	Arg	Thr	Ala	Ser	Trp	Ala	Asp	Trp	Gln
	225					230					235				240	
	Thr	Val	Ser	Leu	Glu	Ile	Asp	Leu	Val	Gln	Gly	Asn	Asn	Asn	Leu	Gln
				245						250					255	
	Leu	Thr	Ala	Leu	Thr	Ala	Asp	Gly	Leu	Ala	Asn	Ile	Asp	Phe	Ile	Lys
				260					265					270		
30	Ile	Glu	Gly	Ala	Ser	Thr	Lys	Ala	Gly	Thr	Cys	Ala	Gly	Ala	Val	Ser
			275					280					285			
	Ser	Ser	Ser	Val	Ala	Ser	Ser	Val	Lys	Ser	Ser	Ala	Ser	Ala	Ala	Ser
		290					295					300				
	Ser	Ser	Val	Pro	Thr	Asn	Thr	Gly	Ala	Met	Leu	Thr	Leu	Asp	Gly	Asn
	305					310					315				320	
35	Pro	Ala	Ala	Ser	Trp	Leu	Asn	Lys	Ser	Arg	Thr	Lys	Trp	Ser	Ala	Ser
				325						330					335	
	Arg	Ala	Asp	Ile	Val	Ala	Ser	Tyr	Gln	Gln	Ser	Asn	Gly	Gly	Trp	Pro
				340					345					350		
	Lys	Asn	Leu	Asp	Tyr	Asn	Ser	Val	Ser	Ala	Gly	Asn	Gly	Gly	Ser	Ala
			355					360					365			
40	Ser	Gly	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Ile	Thr	Glu	Met	Val	Tyr	Leu
		370					375					380				
	Ala	Glu	Val	Tyr	Lys	Thr	Gly	Asn	Asn	Thr	Lys	Tyr	Arg	Asp	Ala	Val
	385					390					395				400	
	Arg	Arg	Ala	Ala	Asn	Phe	Ile	Val	Ser	Ser	Gln	Tyr	Ser	Thr	Gly	Ala
				405						410					415	
45	Leu	Pro	Gln	Phe	Tyr	Pro	Leu	Lys	Gly	Gly	Tyr	Ala	Asp	His	Ala	Thr
				420					425					430		
	Phe	Asn	Asp	Asn	Gly	Met	Ala	Tyr	Ala	Leu	Thr	Val	Leu	Asp	Phe	Ala
			435					440					445			
	Ala	Asn	Lys	Arg	Ala	Pro	Phe	Asp	Thr	Asp	Val	Phe	Asn	Asp	Thr	Asp
		450					455					460				
50	Arg	Ala	Lys	Phe	Lys	Thr	Ala	Val	Thr	Lys	Gly	Val	Asp	Tyr	Ile	Leu
	465					470					475				480	
	Lys	Ala	Gln	Trp	Lys	Gln	Asn	Gly	Lys	Leu	Thr	Ala	Trp	Cys	Ala	Gln
				485						490					495	
	His	Gly	Ala	Thr	Asp	Tyr	Gln	Pro	Lys	Lys	Ala	Arg	Ala	Tyr	Glu	Leu
				500					505					510		
55	Glu	Ser	Leu	Ser	Gly	Ser	Glu	Ser	Val	Gly	Val	Ile	Ala	Phe	Leu	Met
			515					520					525			
	Thr	Gln	Pro	Gln	Thr	Ala	Gln	Ile	Gln	Thr	Ala	Val	Lys	Ala	Gly	Leu
				530			535					540				

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Asn Trp Phe Asn Ser Pro Ser Thr Tyr Leu Glu Gly Tyr Thr Tyr Asp
 545 550 555 560
 Ser Ser Lys Ala Ser Thr Asn Pro Ile Val Gln Lys Ala Gly Ser Arg
 565 570 575
 Met Trp Tyr Arg Phe Tyr Asp Leu Asn Thr Asn Arg Gly Phe Phe Ser
 580 585 590
 Asp Arg Asp Gly Ser Lys Phe Tyr Asp Ile Thr Lys Met Ser Glu Glu
 595 600 605
 Arg Arg Thr Gly Tyr Ser Trp Gly Gly Ala Tyr Gly Glu Ser Ile Ile
 610 615 620
 Ala Phe Gly Lys Lys Val Gly Tyr Leu
 625 630

<210> 45
 <211> 987
 <212> DNA
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<400> 45

25	atgactagac	gcgcccttcac	cgcggtttatc	tgttttcttcg	cggccgtctg	cgcgcacgcg	60
	cagtccaccg	tgcgctggaa	ggacgtgctc	gagcagtcg	agggctggta	ttccacgacc	120
	gccgcgcacg	tcgtcgccga	cacggtgctg	ctgtatcaac	gtccatccgg	tggatggccg	180
	aaggacatcg	acatgacggc	gccgcggcg	gaccgcactc	ctcccgcg	tccagacg	240
	accatcgaca	acggcgccac	gaccacgcag	atccgcctgc	tcgctcgtgc	ggcctcgggc	300
	gcaccggcgg	ctgccgccc	cacctacacg	gcggcgggcg	ttcgcgggat	cgattacctg	360
30	ctcgaggcgc	agtatcccaa	cggcggtctg	ccgcagttct	tccccctgcg	caaggactat	420
	tcgcgctacg	tcacgttcaa	cgacgacgcg	atgatgaacg	tgatgttcct	gctggacgag	480
	gtctcgggcg	gagatgcgcc	gttcacgttc	gtggacgaac	aacgccgcga	ccgcgcgcgc	540
	gctgccgtcg	ccaagggggg	ctccgtcatc	ctgaagtcgc	aggtccggat	cgacgggacg	600
	ctgaccgcct	ggtgcgcgca	acacgacgag	atcaccctgg	caccgcgtcc	ggcgcgaccc	660
	ttcgagcacg	cgtcgctcag	cggcaacgag	tctgtcgcga	tcgtgcgctt	cctgatgacc	720
35	ggtccgcccga	cgccagcgat	cgtcgcgcgc	gtcgatgcgg	cggtcgcctg	gctcagacgc	780
	gtccgcctcc	ctgacggacg	gtgggcccga	ttctacgagt	tcgggtaccaa	tcgtccgatc	840
	ttctcggggc	gagacagtgt	cgtcgcgtac	aaactcgagg	agatcgaaca	ggaacgtcag	900
	gagggctacg	cgtaggtacg	cacgtggccg	aggacgcttg	ttgagaagat	gtaccctgca	960
	tggaagtcgc	ggcttccggg	caagtag				987

<210> 46
 <211> 328
 <212> PRT
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(20)

<221> DOMAIN
 <222> (21)...(328)

<223> Catalytic domain

<400> 46

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Met Thr Arg Arg Ala Phe Ile Ala Val Ile Cys Phe Phe Ala Ala Val
 1 5 10 15
 Cys Ala His Ala Gln Ser Thr Val Arg Trp Lys Asp Val Leu Glu Gln
 20 25 30
 Ser Glu Gly Trp Tyr Ser Thr Thr Ala Ala His Val Val Ala Asp Thr
 35 40 45
 Val Leu Leu Tyr Gln Arg Pro Ser Gly Gly Trp Pro Lys Asp Ile Asp
 50 55 60
 Met Thr Ala Pro Pro Ala Asp Arg Thr Pro Pro Ala Arg Pro Asp Ala
 65 70 75 80
 Thr Ile Asp Asn Gly Ala Thr Thr Thr Gln Ile Arg Leu Leu Ala Arg
 85 90 95

Ala Ala Ser Gly Ala Pro Ala Ala Ala Ala His Thr Tyr Thr Ala Ala
 100 105 110
 Ala Leu Arg Gly Ile Asp Tyr Leu Leu Glu Ala Gln Tyr Pro Asn Gly
 115 120 125
 Gly Trp Pro Gln Phe Phe Pro Leu Arg Lys Asp Tyr Ser Arg Tyr Val
 130 135 140
 Thr Phe Asn Asp Asp Ala Met Met Asn Val Met Phe Leu Leu Asp Glu
 145 150 155 160
 Val Ser Ala Gly Asp Ala Pro Phe Thr Phe Val Asp Glu Gln Arg Arg
 165 170 175
 Asp Arg Ala Arg Ala Val Ala Lys Gly Val Ser Val Ile Leu Lys
 180 185 190
 Ser Gln Val Arg Ile Asp Gly Thr Leu Thr Ala Trp Cys Ala Gln His
 195 200 205
 Asp Glu Ile Thr Leu Ala Pro Arg Pro Ala Arg Thr Phe Glu His Ala
 210 215 220
 Ser Leu Ser Gly Asn Glu Ser Val Ala Ile Val Arg Phe Leu Met Thr
 225 230 235 240
 Arg Pro Pro Thr Pro Ala Ile Val Ala Ala Val Asp Ala Ala Val Ala
 245 250 255
 Trp Leu Arg Arg Val Arg Leu Pro Asp Gly Arg Trp Ala Arg Phe Tyr
 260 265 270
 Glu Phe Gly Thr Asn Arg Pro Ile Phe Ser Gly Arg Asp Ser Val Val
 275 280 285
 Arg Tyr Lys Leu Glu Glu Ile Glu Gln Glu Arg Gln Glu Gly Tyr Ala
 290 295 300
 Trp Tyr Gly Thr Trp Pro Arg Thr Leu Val Glu Lys Met Tyr Pro Ala
 305 310 315 320
 Trp Lys Ser Arg Leu Pro Gly Lys
 325

<210> 47

<211> 1077

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 47

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	gggcaggtaa	acaaaaaatc	ctggcgggct	attacacagt	ctaacgacga	tgcatgggtt	120
	gcatctgatg	gagctgcaca	gattgcagat	aatgtattac	tctatcagcg	caatgttggc	180
5	ggatggccta	aaaatattga	aatgcaggaa	ccgcttagtg	aggccgacaa	aaaaaagctg	240
	atagatctta	agtctacggc	caaagaaaagt	actacagata	atggggctac	gtgtcaggaa	300
	atgggtattcc	tctctaagat	atataaacia	aagcccgaag	agaagtataa	agaggctttt	360
	ttaaaaggac	ttaattattt	gcttgaagca	cagtataaaa	atgggtggatg	gccacagttc	420
	taccctttta	aaaaaggtta	ttatacccac	attacctata	atgacgattc	tatggtaaac	480
	attctttatga	tcttaaagaa	tattaaggaa	gatgccaaact	attacagtat	tacgccaaagc	540
	gataaagttt	taaagcagggt	atcgacagct	tttgacagag	gcattgactg	catttctaaaa	600
10	acacagtaca	agcaaaaagg	tgtgctttaca	agctgggtgtg	cccagcacga	tgagggttaca	660
	ttagaacctg	caaatgcaag	ggctttttgag	ttggcatcac	taagtggtaa	agaatctgct	720
	aaaataacgt	tgttgctaata	gtctgttaaaa	aatccgtcta	aagagggttgt	tgctgctgta	780
	gatgctgctg	tggcgtgggt	tgaaaaaaca	aaaattgaag	gcattaaagt	agaagaagta	840
	accggagctg	atggcaaaaa	ggatagggtta	gtagtacaaa	gggctgatgc	cgaaccattg	900
	tgggcgcggt	ttatggaact	ggataccaac	aggccatttt	tttgcgacag	ggacggtata	960
15	aaaaaatatt	cgcttgctga	gatagggtcat	gaacgccgta	acggatatgg	ctggtacacc	1020
	aacgaaccaa	aagaagtttt	aaagaaatac	accaaattgga	aaaacagttc	taaatag	1077

<210> 48

<211> 358

20 <212> PRT

<213> Unknown

<220>

25 <223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(21)

<221> DOMAIN

30 <222> (22)...(358)

<223> Catalytic domain

<400> 48

35

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Met Lys Asn Phe Lys Asn Ile Val Gly Ala Leu Leu Ile Ser Val Thr
1 5 10 15
Phe Cys Val His Gly Gln Val Asn Lys Lys Ser Trp Arg Ala Ile Thr
20 25 30
5 Gln Ser Asn Asp Asp Ala Trp Phe Ala Ser Asp Gly Ala Ala Gln Ile
35 40 45
Ala Asp Asn Val Leu Leu Tyr Gln Arg Asn Val Gly Gly Trp Pro Lys
50 55 60
Asn Ile Glu Met Gln Glu Pro Leu Ser Glu Ala Asp Lys Lys Lys Leu
65 70 75 80
10 Ile Asp Leu Lys Ser Thr Ala Lys Glu Ser Thr Thr Asp Asn Gly Ala
85 90 95
Thr Cys Gln Glu Met Val Phe Leu Ser Lys Ile Tyr Lys Gln Lys Pro
100 105 110
Glu Glu Lys Tyr Lys Glu Ala Phe Leu Lys Gly Leu Asn Tyr Leu Leu
115 120 125
15 Glu Ala Gln Tyr Lys Asn Gly Gly Trp Pro Gln Phe Tyr Pro Leu Lys
130 135 140
Lys Gly Tyr Tyr Thr His Ile Thr Tyr Asn Asp Asp Ser Met Val Asn
145 150 155 160
Ile Leu Met Ile Leu Lys Asn Ile Lys Glu Asp Ala Asn Tyr Tyr Ser
165 170 175
20 Ile Thr Pro Ser Asp Lys Val Leu Lys Gln Val Ser Thr Ala Phe Asp
180 185 190
Arg Gly Ile Asp Cys Ile Leu Lys Thr Gln Tyr Lys Gln Lys Gly Val
195 200 205
Leu Thr Ser Trp Cys Ala Gln His Asp Glu Val Thr Leu Glu Pro Ala
210 215 220
25 Asn Ala Arg Ala Phe Glu Leu Ala Ser Leu Ser Gly Lys Glu Ser Ala
225 230 235 240
Lys Ile Thr Leu Leu Leu Met Ser Val Lys Asn Pro Ser Lys Glu Val
245 250 255
30 Val Ala Ala Val Asp Ala Ala Val Ala Trp Phe Glu Lys Thr Lys Ile
260 265 270
Glu Gly Ile Lys Val Glu Glu Val Thr Gly Ala Asp Gly Lys Lys Asp
275 280 285
Arg Val Val Val Gln Arg Ala Asp Ala Glu Pro Leu Trp Ala Arg Phe
290 295 300
35 Met Glu Leu Asp Thr Asn Arg Pro Phe Phe Cys Asp Arg Asp Gly Ile
305 310 315 320
Lys Lys Tyr Ser Leu Ala Glu Ile Gly His Glu Arg Arg Asn Gly Tyr
325 330 335
Gly Trp Tyr Thr Asn Glu Pro Lys Glu Val Leu Lys Lys Tyr Thr Lys
340 345 350
40 Trp Lys Asn Ser Leu Lys
355

<210> 49

<211> 1023

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 49

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acaaaaatt cagtggccga aaagatgctt cagtaccagt tgtcaaatgg cgcctggccc 120
aaacagttgg tagacaaaag tgtcgttgat tacagtcttc cattaacgaa agagcgccta 180
cagcagatca agaaaacaga tattgatcat gctacgctcg acaacagtgc gacaacccgg 240
gaaataactg aattgatcaa ggcttttaag gacactaaaa ataaggcata ttgtactgct 300

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	gtagaaaagg	ggattgcata	tatatttatcg	gctcaatatg	ag̃aatggcgg	atttccacaa	360
	tactacccaa	ataaattata	ctatagagct	gagataacat	acaacgatga	tgcgatgatc	420
	aatgcattac	tagtgcttta	caaagtagcc	aataagcgag	agggggttga	ggctatcaat	480
	cccataattt	tgtaaaaagc	gcaaaaagca	gttgaaaagg	gtataacctg	tatcctaaaa	540
5	acacaggtca	tacaagacgg	aaaaaggagt	atttgggctg	cgcaatacga	tcagaacact	600
	ttacaacctg	ctcaggcaag	aaagtttgaa	ccagcttcat	tgagcacaag	tgaatctggt	660
	tccatcgttc	gcttttctcat	gctacagcct	gcaaccactg	aaattaagca	agcgatcgaa	720
	catgcaatac	aatgggttcga	acagcatgat	attgaagggt	accgtttcga	ccgcatacaa	780
	gatagggtga	ctggaaaata	tcaacggcaa	cttgctgcag	atcggacttc	cacgatttgg	840
	gcgcgatttt	ataatctcga	agacaaccgc	ccattgtttg	gagatcgggg	caataacaatc	900
10	aaatacaact	ttgaggaggt	ttcagaggag	cgtagaaatg	gctatgcttg	gttcgggaac	960
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	taa						1023

<210> 50
 <211> 340
 <212> PRT
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<221> SIGNAL
 <222> (1)...(16)
 <221> DOMAIN
 <222> (17)...(340)
 <223> Catalytic domain

<400> 50

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[illegible]

<210> 51

<211> 1131

<212> DNA

<213> Unknown

$\langle 220 \rangle$

<223> Obtained from an environmental sample

<400> 51

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	cggatcgcga	atctggtcct	gctgtaccag	cgcgcgacgg	ggggctggcc	caagaacatc	120
	gacatggcgc	ggtcgttgct	tccggacgat	cgcacgacgc	tcgcggcgga	acggggccctc	180
	accgactcga	cgatcgacaa	tggatcgacg	acgacgcagt	tgcggtttct	cgcgatgggtg	240
5	cagcacgccc	agcaggcacc	cgtgcgcgac	gccatcacgc	acggcctgga	ctatctgctg	300
	aacgcgcaat	actcgaacgg	cggatggccg	cagtactttc	cgctccgaga	cgactactcg	360
	cgtcacatca	cggtcaacga	cgacgcgatg	atcaatgtaa	tgacgggtgct	acgcgatgtc	420
	gcagaagctc	gcatgccctt	cgaagggatc	gacgcggtcc	gtcgggaccg	ggcgcgtgtc	480
	gccatcacgc	gtggcatcga	cgtgattctc	gggacgcaaa	tccgcgtcgg	ggaccgtctg	540
	acgggctggt	gccagcagca	tgacgagcgc	tccttcgccc	ccaccaaggc	tcgcgcctac	600
10	gagcaccat	cgatcgccag	caaggaaacg	gtaaccatca	cgcgcttcct	catgaccctc	660
	gatcgcccga	gtcagcagat	catcgcgggc	atcgaggcgg	ctgtcgagtg	gttgcgcgtg	720
	gcgaccctgt	cgggtgtgcg	agttgagcgt	cggccggacc	cggcgagtcc	gaccggatat	780
	gacgtcgctc	ccgcgcccga	tgccgcccga	cctccgacct	gggcacgggt	ctacgagatc	840
	ggcacgaacc	gccaatgtt	ttccggccgc	gacggcgtga	tcagattccg	gctcgcggac	900
	atcgagattg	agcgcgcac	cggctacagc	tggtatggcg	actatgccgc	gaggttgctg	960
15	aacgaggagt	atccggcgtg	ggcgaggcta	cgccgggcga	gctttcagaa	cgccgagctg	1020
	cacaaggagt	ccggtgaagt	cgtaacacac	gcgatcgtgc	acgatcttgc	cttccttgat	1080
	gtcgaagaca	aagaccagcc	gcagccgaaa	gtgcttttcg	ctgggcggta	g	1131

<210> 52
 <211> 376
 <212> PRT
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<221> DOMAIN
 <222> (1)...(376)
 <223> Catalytic domain

<400> 52

	Met	Thr	Trp	Asp	Gln	Ile	Leu	Arg	Gln	Pro	Ala	Ala	Trp	Tyr	Gly	Gly
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35	Pro	Glu	Ala	Arg	Arg	Ile	Ala	Asn	Leu	Val	Leu	Leu	Tyr	Gln	Arg	Ala
				20					25					30		
	Thr	Gly	Gly	Trp	Pro	Lys	Asn	Ile	Asp	Met	Ala	Arg	Ser	Leu	Ser	Pro
			35				40						45			
	Asp	Asp	Arg	Thr	Thr	Leu	Ala	Glu	Arg	Ala	Leu	Thr	Asp	Ser	Thr	
		50				55				60						
40	Ile	Asp	Asn	Gly	Ser	Thr	Thr	Thr	Gln	Leu	Arg	Phe	Leu	Ala	Met	Val
	65					70			75						80	
	Gln	His	Ala	Gln	Gln	Ala	Pro	Val	Arg	Asp	Ala	Ile	Thr	His	Gly	Leu
				85					90					95		
	Asp	Tyr	Leu	Leu	Asn	Ala	Gln	Tyr	Ser	Asn	Gly	Gly	Trp	Pro	Gln	Tyr
			100						105					110		
45	Phe	Pro	Leu	Arg	Asp	Asp	Tyr	Ser	Arg	His	Ile	Thr	Phe	Asn	Asp	Asp
			115					120					125			
	Ala	Met	Ile	Asn	Val	Met	Thr	Val	Leu	Arg	Asp	Val	Ala	Glu	Ala	Arg
		130				135					140					
	Met	Pro	Phe	Glu	Gly	Ile	Asp	Ala	Val	Arg	Arg	Asp	Arg	Ala	Arg	Val

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	145				150				155				160
	Ala	Ile	Thr	Arg	Gly	Ile	Asp	Val	Ile	Leu	Gly	Thr	Gln
					165					170			175
5	Gly	Asp	Arg	Leu	Thr	Gly	Trp	Cys	Gln	Gln	His	Asp	Glu
				180					185				190
	Ala	Pro	Thr	Lys	Ala	Arg	Ala	Tyr	Glu	His	Pro	Ser	Ile
			195					200				205	
	Glu	Thr	Val	Thr	Ile	Thr	Arg	Phe	Leu	Met	Thr	Leu	Asp
		210				215						220	
10	Gln	Gln	Ile	Ile	Ala	Ala	Ile	Glu	Ala	Ala	Val	Glu	Trp
	225				230						235		240
	Ala	Thr	Leu	Ser	Gly	Val	Arg	Val	Glu	Arg	Arg	Pro	Asp
				245					250				255
	Pro	Thr	Gly	Tyr	Asp	Val	Val	Ala	Ala	Pro	Asp	Ala	Ala
			260					265				270	
15	Thr	Trp	Ala	Arg	Phe	Tyr	Glu	Ile	Gly	Thr	Asn	Arg	Pro
		275					280					285	
	Gly	Arg	Asp	Gly	Val	Ile	Arg	Phe	Arg	Leu	Ala	Asp	Ile
		290				295						300	
	Arg	Arg	Thr	Gly	Tyr	Ser	Trp	Met	Gly	Asp	Tyr	Ala	Ala
	305				310						315		320
20	Asn	Glu	Glu	Tyr	Pro	Ala	Trp	Ala	Arg	Leu	Arg	Arg	Ala
				325						330			335
	Asn	Ala	Glu	Leu	His	Lys	Glu	Ser	Gly	Glu	Val	Val	His
			340						345				350
	Val	His	Asp	Leu	Ala	Phe	Leu	Asp	Val	Glu	Asp	Lys	Asp
		355					360					365	
25	Pro	Lys	Val	Leu	Phe	Ala	Gly	Arg					
		370					375						

<210> 53

<211> 1977

30 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

35

<400> 53

40

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50

55

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30	aattacactt aaaatgtggg ggcagcaagt ggtggtgact	acgattcttc atcgctttta tctacgacat acggcagctc	caaagcttcg tgacctgaat cacccaaattg gattatcagc	accaatccaa accaaccgcg tcagaagagc ttcgcacaaa	tcgtgccaaa gtttcttcag gtcgcactgg aagtgggata	atccggaagc tgatcgtagc ttacagttgg tctctaa	1800 1860 1920 1977
35	<210> 54 <211> 658 <212> PRT <213> Unknown						
40	<220> <223> Obtained from an environmental sample						
45	<221> SIGNAL <222> (1)...(31)						
50	<221> BINDING <222> (32)...(124) <223> Carbohydrate binding module						
55	<221> BINDING <222> (180)...(303) <223> Carbohydrate binding module						
	<221> DOMAIN <222> (304)...(658) <223> Catalytic domain						
	<400> 54						

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	Met	Asn	Asn	Ser	Thr	Lys	Lys	Met	Ile	Arg	Pro	Leu	Lys	Ala	Ser	Phe
	1				5					10					15	
	Ala	Leu	Gly	Ala	Leu	Ala	Leu	Ala	Ile	Ala	Ser	Pro	Ser	Trp	Ala	Ala
5				20					25					30		
	Cys	Ser	Tyr	Ser	Val	Thr	Asn	Asn	Trp	Gly	Ser	Gly	Phe	Thr	Gly	Glu
			35					40					45			
	Ile	Lys	Val	Thr	Asn	Asp	Thr	Thr	Ser	Thr	Val	Asn	Asn	Trp	Ser	Val
		50				55						60				
	Ser	Trp	Gln	Glu	Ser	Gly	Val	Thr	Val	Thr	Asn	Ala	Trp	Asn	Ala	Thr
10	65					70				75					80	
	Leu	Ser	Gly	Ser	Asn	Pro	Tyr	Thr	Ala	Thr	Ser	Leu	Gly	Trp	Asn	Gly
					85				90						95	
	Thr	Leu	Ala	Pro	Lys	Ala	Ser	Ala	Ser	Phe	Gly	Phe	Gln	Ala	Asn	Gly
				100				105						110		
	Thr	Ala	Gly	Ala	Pro	Lys	Val	Asn	Gly	Thr	Leu	Cys	Gly	Thr	Ser	Thr
15			115					120					125			
	Ser	Ser	Thr	Gly	Thr	Ser	Ser	Val	Ala	Pro	Ser	Ser	Val	Ala	Ser	Ser
		130					135					140				
	Val	Ala	Val	Ser	Ser	Ser	Lys	Ser	Ser	Ser	Ser	Val	Ala	Thr	Ile	Ser
	145					150				155					160	
	Ser	Ser	Lys	Ser	Ser	Ser	Ser	Val	Pro	Thr	Val	Ser	Ser	Phe	Thr	Ile
20					165				170						175	
	Gln	Glu	Glu	Gln	Ala	Gly	Phe	Cys	Arg	Val	Asp	Gly	Ile	Ala	Thr	Glu
				180					185					190		
	Ser	Thr	Asn	Thr	Gly	Tyr	Thr	Gly	Asn	Gly	Tyr	Thr	Asn	Thr	Thr	Asn
			195					200					205			
	Ala	Gln	Gly	Ala	Ala	Ile	Glu	Trp	Ala	Ile	Asn	Ala	Pro	Asn	Ser	Ser
25		210					215					220				
	Arg	Tyr	Thr	Leu	Thr	Phe	Arg	Tyr	Ala	Asn	Ala	Gly	Thr	Ala	Asn	Arg
	225					230				235						240
	Asn	Gly	Ser	Leu	Leu	Ile	Asn	Asp	Gly	Ser	Asn	Gly	Asn	Tyr	Thr	Val
				245					250					255		
	Gln	Leu	Pro	Ser	Thr	Gly	Ala	Trp	Ala	Thr	Trp	Gln	Thr	Val	Ser	Val
30				260					265					270		
	Glu	Val	Asp	Leu	Val	Gln	Gly	Asn	Asn	Ile	Leu	Lys	Leu	Ala	Ser	Leu
			275					280					285			
	Thr	Ala	Asp	Gly	Leu	Ala	Asn	Ile	Asp	Ser	Leu	Lys	Ile	Glu	Gly	Ala
		290					295					300				
	Gln	Ala	Lys	Ala	Gly	Val	Cys	Ser	Thr	Thr	Val	Ser	Ser	Ser	Ser	Ser

35

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	305				310						315					320
	Ser	Ile	Lys	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Thr	Ala	Ala
					325					330					335	
5	Val	Lys	Thr	Leu	Thr	Leu	Asp	Gly	Asn	Pro	Ala	Ala	Asn	Trp	Phe	Asn
				340					345					350		
	Lys	Ser	Arg	Thr	Lys	Trp	Asn	Val	Ser	Arg	Ala	Asp	Ile	Val	Leu	Ser
			355					360					365			
	Tyr	Gln	Gln	Ser	Asn	Gly	Gly	Trp	Pro	Lys	Asn	Leu	Asp	Tyr	Asn	Ser
		370				375						380				
10	Val	Gly	Ser	Gly	Asn	Gly	Gly	Ser	Asp	Ser	Gly	Thr	Ile	Asp	Asn	Gly
	385				390						395					400
	Ala	Thr	Ile	Thr	Glu	Met	Val	Tyr	Leu	Ala	Glu	Val	Tyr	Lys	Asn	Gly
					405					410					415	
	Gly	Asn	Thr	Lys	Tyr	Arg	Asp	Ala	Val	Arg	Arg	Ala	Ala	Asn	Phe	Ile
				420					425					430		
15	Val	Ser	Ser	Gln	Tyr	Ser	Thr	Gly	Ala	Leu	Pro	Gln	Phe	Tyr	Pro	Leu
			435					440					445			
	Lys	Gly	Gly	Tyr	Ala	Asp	His	Ala	Thr	Phe	Asn	Asp	Asn	Gly	Met	Ala
		450					455					460				
	Tyr	Ala	Leu	Thr	Val	Leu	Asp	Phe	Ala	Val	Asn	Lys	Arg	Ala	Pro	Phe
	465				470						475					480
20	Asp	Asn	Asp	Ile	Phe	Ser	Asp	Ser	Asp	Arg	Ser	Lys	Phe	Lys	Thr	Ala
				485						490					495	
	Val	Thr	Lys	Gly	Val	Asp	Tyr	Ile	Leu	Lys	Ala	Gln	Trp	Lys	Gln	Asn
				500					505					510		
	Gly	Lys	Leu	Thr	Val	Trp	Cys	Ala	Gln	His	Gly	Ala	Asn	Asp	Tyr	Gln
			515					520					525			
25	Pro	Lys	Lys	Ala	Arg	Ala	Tyr	Glu	Leu	Glu	Ser	Leu	Ser	Gly	Ser	Glu
		530					535					540				
	Ser	Val	Gly	Val	Leu	Ala	Phe	Leu	Met	Thr	Gln	Pro	Gln	Thr	Thr	Gln
	545				550						555					560
	Ile	Glu	Ala	Ala	Val	Arg	Ala	Gly	Val	Ala	Trp	Phe	Asn	Ser	Pro	Ser
				565						570					575	
30	Thr	Tyr	Leu	Asn	Asn	Tyr	Thr	Tyr	Asp	Ser	Ser	Lys	Ala	Ser	Thr	Asn
				580					585					590		
	Pro	Ile	Val	Pro	Lys	Ser	Gly	Ser	Lys	Met	Trp	Tyr	Arg	Phe	Tyr	Asp
			595					600					605			
	Leu	Asn	Thr	Asn	Arg	Gly	Phe	Phe	Ser	Asp	Arg	Asp	Gly	Ser	Lys	Phe
		610				615					620					
35	Tyr	Asp	Ile	Thr	Gln	Met	Ser	Glu	Glu	Arg	Arg	Thr	Gly	Tyr	Ser	Trp
	625				630						635					640
	Gly	Gly	Asp	Tyr	Gly	Ser	Ser	Ile	Ile	Ser	Phe	Ala	Gln	Lys	Val	Gly
					645					650					655	

<210> 55

<211> 1125

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 55

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	ggacccgggtg	gacctcgacc	gccgggtgaaa	ccggccgtcc	aggcgcccgt	taggggttacc	120
	tggagcgcac	gcctagtcca	gcggcccga	tggtagcggga	gtgacgaagc	gatccgcac	180
	gcggacaacg	tcctcctcta	ccagcgcaac	accggcgggt	ggccgaagga	catagatatg	240
5	gccgagccca	ccccggaaca	caggaagtcc	tttttcctca	ccgagaagga	gcggaccgat	300
	gactcgacca	tcgacaacgg	tgccaccgtg	accagctca	agtatctcgc	ccgcgtctac	360
	aaggcgacca	ggctggaacg	gttcaaggag	ggcttcctca	aaggctctga	ctacctcttg	420
	gccgcccagt	acccgaacgg	cggctggccc	cagtattatc	ctaacttgag	gggtactac	480
	gccaacatca	cttataacga	caatgccatg	gtgaacgtgc	tcacctctct	ccagagcatc	540
	gccaaaaagg	ccccggagta	cgacttcgtc	gaccggcg	gccgggagaa	ggccgcccgg	600
10	gccgtggcga	aagggatcga	ctgcacctc	aagaccaga	tccgtgtcaa	tgaaaaactt	660
	accgcctggt	gcgcccagca	tgacccaag	acgctggcgc	ccgcgcccgc	ccgttcgtat	720
	gagcttgagt	ccatcagcgg	tttcgagagc	gtcgggatcg	tccggttctt	aatgagcctc	780

15	gagaatccga	ccccgaagg	catcgaggcg	gtagaggccg	c̃gtgaaatg	gttcgaggag	840
	gtcaagctta	ccgggatcaa	ggtggctcga	aaacccgacc	cgtcccttcc	ggcggttac	900
	gaccgcgtgg	tggtcgaaga	ccccaacgcg	ccgccatct	gggcccggtt	ctacgagatc	960
	ggcaccaacc	gtcccttctt	ctgcggccgc	gatggtatca	aaaaatacag	cctggcggag	1020
	atcgaacacg	aacgccgggt	cggttactcc	tggtacacca	atgccccggc	ctacctcatc	1080
	gagaaggagt	atccgctctg	gcggggccaaa	caccctacca	agtaa		1125

20

<210> 56

<211> 374

<212> PRT

<213> Unknown

25

<220>

<223> Obtained from an environmental sample

30

<221> DOMAIN

<222> (1)...(374)

<223> Catalytic domain

35

<400> 56

40

45

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	Met	Val	Leu	Gly	Asn	Asn	Gly	Gly	Ser	Leu	Ser	Cys	Val	Gln	Tyr	Ile
	1				5				10					15		
	Val	Ile	Val	Lys	Gly	Pro	Gly	Gly	Pro	Arg	Pro	Pro	Val	Lys	Pro	Ala
				20					25					30		
5	Val	Gln	Ala	Pro	Val	Arg	Val	Thr	Trp	Ser	Ala	Cys	Leu	Val	Gln	Arg
			35				40						45			
	Pro	Glu	Trp	Tyr	Gly	Ser	Asp	Glu	Ala	Ile	Arg	Ile	Ala	Asp	Asn	Val
		50				55					60					
	Leu	Leu	Tyr	Gln	Arg	Asn	Thr	Gly	Gly	Trp	Pro	Lys	Asp	Ile	Asp	Met
	65				70					75					80	
10	Ala	Glu	Pro	Ile	Pro	Glu	His	Arg	Lys	Ser	Phe	Phe	Leu	Thr	Glu	Lys
				85					90					95		
	Glu	Arg	Thr	Asp	Asp	Ser	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Val	Thr	Gln
			100					105					110			
	Leu	Lys	Tyr	Leu	Ala	Arg	Val	Tyr	Lys	Ala	Thr	Arg	Leu	Glu	Arg	Phe
			115				120						125			
15	Lys	Glu	Gly	Phe	Leu	Lys	Gly	Leu	Asp	Tyr	Leu	Leu	Ala	Ala	Gln	Tyr
		130					135				140					
	Pro	Asn	Gly	Gly	Trp	Pro	Gln	Tyr	Tyr	Pro	Asn	Leu	Arg	Gly	Tyr	Tyr
	145				150					155					160	
	Ala	Asn	Ile	Thr	Tyr	Asn	Asp	Asn	Ala	Met	Val	Asn	Val	Leu	Thr	Leu
				165					170					175		
20	Leu	Gln	Ser	Ile	Ala	Lys	Lys	Ala	Pro	Glu	Tyr	Asp	Phe	Val	Asp	Pro
			180					185					190			
	Ala	Arg	Arg	Glu	Lys	Ala	Ala	Arg	Ala	Val	Ala	Lys	Gly	Ile	Asp	Cys
			195				200						205			
	Ile	Leu	Lys	Thr	Gln	Ile	Arg	Val	Asn	Gly	Lys	Leu	Thr	Ala	Trp	Cys
		210				215						220				
25	Ala	Gln	His	Asp	Pro	Lys	Thr	Leu	Ala	Pro	Ala	Pro	Ala	Arg	Ser	Tyr
	225				230				235						240	
	Glu	Leu	Glu	Ser	Ile	Ser	Gly	Phe	Glu	Ser	Val	Gly	Ile	Val	Arg	Phe
				245					250					255		
	Leu	Met	Ser	Leu	Glu	Asn	Pro	Ser	Pro	Lys	Val	Ile	Glu	Ala	Val	Glu
			260					265					270			
30	Ala	Ala	Val	Lys	Trp	Phe	Glu	Glu	Val	Lys	Leu	Thr	Gly	Ile	Lys	Val
			275				280						285			
	Val	Glu	Lys	Pro	Asp	Pro	Ser	Leu	Pro	Gly	Gly	Tyr	Asp	Arg	Val	Val
		290				295						300				
	Val	Glu	Asp	Pro	Asn	Ala	Pro	Pro	Ile	Trp	Ala	Arg	Phe	Tyr	Glu	Ile
		305			310					315					320	
35	Gly	Thr	Asn	Arg	Pro	Phe	Phe	Cys	Gly	Arg	Asp	Gly	Ile	Lys	Lys	Tyr
				325					330					335		
	Ser	Leu	Ala	Glu	Ile	Glu	His	Glu	Arg	Arg	Val	Gly	Tyr	Ser	Trp	Tyr
			340					345					350			
	Thr	Asn	Ala	Pro	Ala	Tyr	Leu	Ile	Glu	Lys	Glu	Tyr	Pro	Leu	Trp	Arg
			355				360						365			
40	Ala	Lys	His	Pro	Thr	Lys										
		370														

<210> 57

<211> 1170

45 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

50

<400> 57

55

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atggacaaac gcgtcaaatt gattcatcag ctttcaaaag aagaagcaaa gcagttcgag      60
cccgaaaatt tcctcaaagg caaagacggc tggaaatccga aaaaggcgga tgaccgctgg      120
ctcgaaaaaa caaaacctga ctggcagctc gttacgtgga acgacgcgtt acgccaggcg      180
ccgctctggg atcaaaccga tgaagcggcg cgcattgccg accagggtgat tttgtaccag      240
5 aaagacaacg gcggctggga aaaaaatctc gatatgacgg cgatgctcac gcaagccgaa      300
cgcgaaaagc tcgccaaaga aaaatcgaac acgtcggaaa cgacgatcga caaccgcacg      360
acctacacgc aagtcgcttt tctcgccaaa gtcattacgg gcagcttgca gaaaacgact      420
ccgccgacca atttcccgaa acataaggaa gcttttttca agggcttgga ttacctgctc      480
gcgtcgcagt acgaatcggg cggcttttccg cagttttatc cgctcaaaaa aggttattac      540
acgcacatca cgttcaacga cgatgcgatg attggcggtt tgaagggttt gcgcgaaatc      600
10 gccaaaaaga aggaagacta tctttttgtt gacgaagaac gccgcctgaa agcggaaaaa      660
tcggtcgaaa aagcgtgccc gctgattctg aaattgcagg ttgaagtcgg cggcaaaaaa      720
acggttttgg cggcgcagta tgacgaaaaa acttttaaac ccgcagcggc gcgaaagttt      780
gaaccggttt ctttaacggc gggcgaatcg gtcggcatcg tccgggtttt aatgtacgat      840
tcaaagcccc accaggcgac gattgacgcg attgaatctg ccattcagtg gtatcgcgcg      900
15 aacaaaatcg aaggcattcg atgggtgccc gaaaacggcg aaaaccgcgt cgtcaaggac      960
aaaaacgcgc cgccgatttg ggcgcggttt tacgaaatcg aaacgatgaa gccgattttc      1020
atcggggcgc acgccatcat tcgttacgac gtgtctgaaa tcgaagccga gcgccgcaac      1080
ggctacgcgt ggtacgtctc ggagccgaac gagctgcttg aaaaagatta cccgaaatgg      1140
ctggaaaaaa ttaaaaaatc agtaaagtaa

```

20 <210> 58
 <211> 389
 <212> PRT
 <213> Unknown

25 <220>
 <223> Obtained from an environmental sample

<221> DOMAIN
 <222> (1)...(389)
 30 <223> Catalytic domain

<400> 58

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Lys Gln Phe Glu Pro Glu Asn Phe Leu Lys Gly Lys Asp Gly Trp Asn
20 25 30
Pro Lys Lys Ala Asp Asp Arg Trp Leu Glu Lys Thr Lys Pro Asp Trp
35 40 45
Gln Leu Val Thr Trp Asn Asp Ala Leu Arg Gln Ala Pro Leu Trp Tyr
40 50 55 60
Gln Thr Asp Glu Ala Ala Arg Ile Ala Asp Gln Val Ile Leu Tyr Gln
65 70 75 80
Lys Asp Asn Gly Gly Trp Glu Lys Asn Leu Asp Met Thr Ala Met Leu
85 90 95
Thr Gln Ala Glu Arg Glu Lys Leu Ala Lys Glu Lys Ser Asn Thr Ser
45 100 105 110
Glu Thr Thr Ile Asp Asn Arg Thr Thr Tyr Thr Gln Val Ala Phe Leu
115 120 125
Ala Lys Val Ile Thr Gly Ser Leu Gln Lys Thr Thr Pro Pro Thr Asn
130 135 140
Phe Pro Lys His Lys Glu Ala Phe Phe Lys Gly Leu Asp Tyr Leu Leu
50 145 150 155 160
Ala Ser Gln Tyr Glu Ser Gly Gly Phe Pro Gln Phe Tyr Pro Leu Lys
165 170 175
Lys Gly Tyr Tyr Thr His Ile Thr Phe Asn Asp Asp Ala Met Ile Gly
180 185 190

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55

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Val Leu Lys Val Leu Arg Glu Ile Ala Lys Lys Lys Glu Asp Tyr Leu
 195 200 205
 Phe Val Asp Glu Glu Arg Arg Leu Lys Ala Glu Lys Ser Val Glu Lys
 210 215 220
 5 Ala Leu Pro Leu Ile Leu Lys Leu Gln Val Glu Val Gly Gly Lys Lys
 225 230 235 240
 Thr Val Trp Ala Ala Gln Tyr Asp Glu Asn Thr Phe Lys Pro Ala Ala
 245 250 255
 Ala Arg Lys Phe Glu Pro Val Ser Leu Thr Ala Gly Glu Ser Val Gly
 260 265 270
 10 Ile Val Arg Phe Leu Met Tyr Asp Ser Lys Pro Asp Gln Ala Thr Ile
 275 280 285
 Asp Ala Ile Glu Ser Ala Ile Gln Trp Tyr Arg Ala Asn Lys Ile Glu
 290 295 300
 Gly Ile Arg Trp Val Arg Glu Asn Gly Glu Asn Arg Val Val Lys Asp
 305 310 315 320
 15 Lys Asn Ala Pro Pro Ile Trp Ala Arg Phe Tyr Glu Ile Glu Thr Met
 325 330 335
 Lys Pro Ile Phe Ile Gly Arg Asp Ala Ile Ile Arg Tyr Asp Val Ser
 340 345 350
 Glu Ile Glu Ala Glu Arg Arg Asn Gly Tyr Ala Trp Tyr Val Ser Glu
 355 360 365
 20 Pro Asn Glu Leu Leu Glu Lys Asp Tyr Pro Lys Trp Leu Glu Lys Ile
 370 375 380
 Lys Lys Ser Val Lys
 385

25
 <210> 59
 <211> 1080
 <212> DNA
 <213> Unknown

30
 <220>
 <223> Obtained from an environmental sample

35
 <400> 59

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 tatggcaccg atgaagcccg tcgaattgcc gaccacgtgc tcgagcatca acgagcggaa 180
 ggcggatggc caaagaacac ggacatgacc gcagcgcccg atccggcggt gctcacagcc 240
 40 gcgcgagtga agccagactc gacgatcgat aacggcgcgga ccgtcactga aatgcgcgtc 300
 ctcgcgcgcg tctaccgttc atcaccgat ccccggtatc gcgatgcgct gctcaagggt 360
 ctcgactatc tgttggcagc gcagtatgcc aacggcggtt ggccgcagtt ctaccgcctc 420
 cggcaggact attcgcgcta tatcacgttc aacgacaacg cgatgatcaa tgtcgtgacg 480
 ctgctctcag acgtcgctgc cggaaatggc gactgggctt ttgctgatgc cagccggcgc 540
 gagaaaagcc ggacggctgt agagaaggcc gtagaagtca tcctgcgcgc gcaggtgaga 600
 45 gttgacggcc ggctgaccgc gtggtgcgcc caacacgacg aggtgacact cgagccgcgc 660
 aaggcccgcg cctacgaaca tccgtcgctg agcggacag agacgggtgg gatcatccgg 720
 tttctcatga cccgcgataa accggatcag agagtcgtcg atgcaatcga ggcgtcagtg 780
 gcatggctga aggcggtgca gctcaaagga cttcgcgtcg accagcgccg cgatccctcg 840
 ctgccggagg ggcgtgacgt ggtgaccgtc gctgaccctg cggcgccgcc gctctgggcg 900
 cgcttctacg aaatcgggac caatcgcccg atcttctctg gacgcgacgg cgtgatccga 960
 50 tactcgctgg cagagatcga gcacgaacgc cggatagggt acgcctggct cggaacctgg 1020
 cccgcgaagc tgctcgatac cgaataccca tcctggcgac ggactcaaca aaggccgtga 1080

55
 <210> 60
 <211> 359
 <212> PRT
 <213> Unknown

<220>

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<223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(24)

<221> DOMAIN

<222> (25)...(359)

<223> Catalytic domain

<400> 60

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      Ala Leu Arg Val Pro Ala Gln Ala Gln Val Thr Val Arg Trp Ala Asp
      Val Leu Asn Gln Pro Ala Ala Trp Tyr Gly Thr Asp Glu Ala Arg Arg
      Ile Ala Asp His Val Leu Glu His Gln Arg Ala Glu Gly Gly Trp Pro
      Lys Asn Thr Asp Met Thr Ala Ala Pro Asp Pro Ala Val Leu Thr Ala
      Ala Arg Val Lys Pro Asp Ser Thr Ile Asp Asn Gly Ala Thr Val Thr
      Glu Met Arg Val Leu Ala Arg Val Tyr Arg Ser Ser Pro Asp Pro Arg
      Tyr Arg Asp Ala Leu Leu Lys Gly Leu Asp Tyr Leu Leu Ala Ala Gln
      Tyr Ala Asn Gly Gly Trp Pro Gln Phe Tyr Pro Leu Arg Gln Asp Tyr
      Ser Arg Tyr Ile Thr Phe Asn Asp Asn Ala Met Ile Asn Val Val Thr
      Leu Leu Ser Asp Val Ala Ala Gly Asn Gly Asp Trp Ala Phe Ala Asp
      Ala Ser Arg Arg Glu Lys Ser Arg Thr Ala Val Glu Lys Ala Val Glu
      Val Ile Leu Arg Ala Gln Val Arg Val Asp Gly Arg Leu Thr Ala Trp
      Cys Ala Gln His Asp Glu Val Thr Leu Glu Pro Arg Lys Ala Arg Ala
      Tyr Glu His Pro Ser Leu Ser Gly Gln Glu Thr Val Gly Ile Ile Arg
      Phe Leu Met Thr Arg Asp Lys Pro Asp Gln Arg Val Val Asp Ala Ile
      Glu Ala Ser Val Ala Trp Leu Lys Ala Val Gln Leu Lys Gly Leu Arg
      Val Asp Gln Arg Arg Asp Pro Ser Leu Pro Glu Gly Arg Asp Val Val
      Thr Val Ala Asp Pro Ser Ala Pro Pro Leu Trp Ala Arg Phe Tyr Glu
      Ile Gly Thr Asn Arg Pro Ile Phe Ser Gly Arg Asp Gly Val Ile Arg
      Tyr Ser Leu Ala Glu Ile Glu His Glu Arg Arg Ile Gly Tyr Ala Trp
      Leu Gly Thr Trp Pro Ala Lys Leu Leu Asp Thr Glu Tyr Pro Ser Trp
      Arg Arg Thr Gln Gln Arg Pro
      355

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<210> 61

<211> 1224

<212> DNA

<213> Unknown

<220>

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<223> Obtained from an environmental sample

<400> 61

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	ttgggtgcaag	gtaacaacct	gttaaaactt	tctgcatca	cggtgatgg	tttgcaaat	120
	atcgattcgt	tgaaaattga	cggcgcacaa	accaaagccg	gcgtgtgcag	caactgtggca	180
	agcagcagct	cttcattcgt	tgcttcattc	attaaatcaa	gctccagttc	atcctcttcc	240
	agttcaacga	cgacggtaaa	aacattaaca	ctggatggca	accccgagc	aaactggttt	300
10	aacaaatcca	gaaccaaaatg	gaataaccagc	agagccgatg	ttgtactttc	ctatcaacaa	360
	tccaacggcg	gctggccaaa	aaatctcgat	tacaattcag	taagcgcagg	taatggcggc	420
	agcgatagcg	gcaccatcga	taacgggtgca	accattactg	aaatggttta	tctcgcggaa	480
	gtttacaaaa	atggcaacaa	caccaagtat	cgcgatgcgg	tgcgagagc	cgcaaatatt	540
15	attgtcagct	cgcaatacag	caactgggtga	ttaccacaat	tttatccatt	gaaaggcggc	600
	tatgcagacc	acgccacctt	taacgataac	ggcatggcat	atgcattaac	ggatttggat	660
	tttgagtcga	acaaacgcgc	cccatttgat	actgatgttt	tctccgattc	tgatcgcgcg	720
	aaattcaaaa	ccgctgttgc	caaagggtgtg	gattacattt	tgaaagcgca	gtggaaacaa	780
	aacggaaaat	taaccgtgtg	gtgtgcacaa	catgggtgcta	ccgattatca	accgaaaaaa	840
20	gcgcgcgcct	atgaattgga	atcactgagt	ggcagcgaat	ctgttggtgt	actcgctttc	900
	ttgatgacc	aaccgcaaac	cgcacaaatt	gaagccgctg	taaaagccgg	tgtagcctgg	960
	ttcaatagcc	ccaacacgta	tttgaacaat	tacacttacg	actcttcaaa	agcgtcaact	1020
	aatccaatag	ttgccaagtc	tggaagcaaa	atgtggtatc	gcttttacga	tttaaatacc	1080
	aatcgtggct	tcttcagtga	tcgcgatggc	agcaaattct	atgacatcac	ccagatgtca	1140
	gaagagcgct	gcactggata	tagctggggg	ggtgattacg	gcacgtcgat	tatttccttc	1200
25	gcgcaaaaag	tgggatattct	gtaa				1224

<210> 62

<211> 407

<212> PRT

30 <213> Unknown

<220>

<223> Obtained from an environmental sample

35 <221> DOMAIN

<222> (0)...(407)

<223> Catalytic domain

<400> 62

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50

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	Val	Glu	Ile	Asp 20	Leu	Val	Gln	Gly	Asn 25	Asn	Leu	Leu	Lys	Leu	Ser	Ala
	Ile	Thr	Ala 35	Asp	Gly	Leu	Ala	Asn 40	Ile	Asp	Ser	Leu	Lys 45	Ile	Asp	Gly
	Ala	Gln 50	Thr	Lys	Ala	Gly	Val 55	Cys	Ser	Thr	Val	Ala 60	Ser	Ser	Ser	Ser
10	Ser 65	Ser	Val	Ala	Ser	Ser 70	Ile	Lys	Ser	Ser	Ser 75	Ser	Ser	Ser	Ser	Ser
	Ser	Ser	Thr	Thr	Thr 85	Val	Lys	Thr	Leu	Thr 90	Leu	Asp	Gly	Asn	Pro 95	Ala
	Ala	Asn	Trp	Phe 100	Asn	Lys	Ser	Arg	Thr 105	Lys	Trp	Asn	Thr	Ser 110	Arg	Ala
	Asp	Val	Val 115	Leu	Ser	Tyr	Gln	Gln 120	Ser	Asn	Gly	Gly	Trp 125	Pro	Lys	Asn
15	Leu	Asp 130	Tyr	Asn	Ser	Val	Ser 135	Ala	Gly	Asn	Gly	Gly 140	Ser	Asp	Ser	Gly
	Thr 145	Ile	Asp	Asn	Gly	Ala 150	Thr	Ile	Thr	Glu	Met 155	Val	Tyr	Leu	Ala	Glu 160
	Val	Tyr	Lys	Asn	Gly 165	Asn	Asn	Thr	Lys	Tyr 170	Arg	Asp	Ala	Val	Arg 175	Arg
	Ala	Ala	Asn	Phe 180	Ile	Val	Ser	Ser	Gln 185	Tyr	Ser	Thr	Gly	Ala 190	Leu	Pro
20	Gln	Phe 195	Tyr	Pro	Leu	Lys	Gly 200	Gly	Tyr	Ala	Asp	His	Ala 205	Thr	Phe	Asn
	Asp	Asn 210	Gly	Met	Ala	Tyr	Ala 215	Leu	Thr	Val	Leu	Asp 220	Phe	Ala	Val	Asn
	Lys 225	Arg	Ala	Pro	Phe	Asp 230	Thr	Asp	Val	Phe	Ser 235	Asp	Ser	Asp	Arg	Ala 240
	Lys	Phe	Lys	Thr	Ala 245	Val	Ala	Lys	Gly	Val 250	Asp	Tyr	Ile	Leu	Lys 255	Ala
30	Gln	Trp	Lys	Gln 260	Asn	Gly	Lys	Leu	Thr 265	Val	Trp	Cys	Ala	Gln 270	His	Gly
	Ala	Thr 275	Asp	Tyr	Gln	Pro	Lys	Lys 280	Ala	Arg	Ala	Tyr	Glu 285	Leu	Glu	Ser
	Leu	Ser 290	Gly	Ser	Glu	Ser	Val 295	Gly	Val	Leu	Ala	Phe 300	Leu	Met	Thr	Gln
	Pro 305	Gln	Thr	Ala	Gln	Ile 310	Glu	Ala	Ala	Val	Lys 315	Ala	Gly	Val	Ala	Trp 320
35	Phe	Asn	Ser	Pro	Asn 325	Thr	Tyr	Leu	Asn	Asn 330	Tyr	Thr	Tyr	Asp	Ser 335	Ser
	Lys	Ala	Ser	Thr 340	Asn	Pro	Ile	Val	Ala 345	Lys	Ser	Gly	Ser	Lys 350	Met	Trp
	Tyr	Arg	Phe 355	Tyr	Asp	Leu	Asn	Thr 360	Asn	Arg	Gly	Phe	Phe 365	Ser	Asp	Arg
	Asp	Gly 370	Ser	Lys	Phe	Tyr	Asp 375	Ile	Thr	Gln	Met	Ser 380	Glu	Glu	Arg	Arg
45	Thr 385	Gly	Tyr	Ser	Trp	Gly 390	Gly	Asp	Tyr	Gly	Thr 395	Ser	Ile	Ile	Ser	Phe 400
	Ala	Gln	Lys	Val	Gly 405	Tyr	Leu									

50 <210> 63
 <211> 1023
 <212> DNA
 <213> Unknown

55 <223> Obtained from an environmental sample

<400> 63

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	atgttaagtt	tcacgcggt	atcagtggtt	cataattact	gtacagggca	gactgcgtcc	60
	accaaaaatt	cagtggccga	aaagatgctt	cagtaccagt	tgtcaaattg	cgcttgccc	120
	aaacagtttg	tagacaaaag	tgtcgttgat	tacagtcttc	cattaacgaa	agagctccta	180
5	cagcagatca	agaaaacaga	tattgatcat	gctacgctcg	acaacagtg	gacaacccgg	240
	gaaataactg	aattgatcaa	ggcttttaag	gacactaaaa	ataaggcata	tttgactgct	300
	gcagaaaagg	ggattgcata	tattttatcg	gctcaatatg	agaatggcgg	atttccacaa	360
	tactacccaa	ataaattata	ctatagagct	gagataacat	acaacgatga	tgcgatgatc	420
	aatgcattac	tagtgcttta	caaagtagcc	aataagcgag	aggggtttga	ggctatcaat	480
	cccatatattg	tgtcaaaagc	gcaaaaagca	gttgaaaagg	gtataacctg	tatcctaaaa	540
10	acacaggtca	tacaagacgg	aaaaaggagt	atttgggctg	cgcaatacga	tcagaacact	600
	ttacaacctg	ctcaggcaag	aaagtttgaa	ccagcttcat	tgagcacaag	tgaatctgtt	660
	tccatcgttc	gctttctcat	gctacagcct	gcaaccactg	aaattaagca	agcgatcgaa	720
	catgcaatac	aatggttcga	acagcatgat	attgaagggt	accgtttcga	ccgcatacaa	780
	gataggggtga	ctggaaaata	tcaacggcag	cttgctcgctg	atcggacttc	cacgatttgg	840
	gcgcgatttt	ataatctcga	agacaaccgt	ccattgtttg	gagatcgggg	caatacaatc	900
15	aaatacaact	ttgaggaggt	ttcagaggag	cgtagaaatg	gctatgcttg	gttcggcaac	960
	tggccggaat	agctgatcca	aaaggactat	caaaatgga	aaaaacaata	caaaattcaa	1020
	taa						1023

<210> 64

<211> 340

20 <212> PRT

<213> Unknown

<220>

25 <223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(16)

<221> DOMAIN

30 <222> (17)...(340)

<223> Catalytic domain

<400> 64

35	Met	Leu	Ser	Phe	Ile	Ala	Val	Ser	Val	Phe	His	Asn	Tyr	Cys	Thr	Gly
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	Gln	Thr	Ala	Ser	Thr	Lys	Asn	Ser	Val	Ala	Glu	Lys	Met	Leu	Gln	Tyr
				20					25					30		
	Gln	Leu	Ser	Asn	Gly	Ala	Trp	Pro	Lys	Gln	Leu	Val	Asp	Lys	Ser	Val
40			35					40					45			
	Val	Asp	Tyr	Ser	Leu	Pro	Leu	Thr	Lys	Glu	Leu	Leu	Gln	Gln	Ile	Lys
		50					55					60				
	Lys	Thr	Asp	Ile	Asp	His	Ala	Thr	Leu	Asp	Asn	Ser	Ala	Thr	Thr	Arg
	65					70					75				80	
	Glu	Ile	Thr	Glu	Leu	Ile	Lys	Ala	Phe	Lys	Asp	Thr	Lys	Asn	Lys	Ala
45				85						90				95		
	Tyr	Leu	Thr	Ala	Ala	Glu	Lys	Gly	Ile	Ala	Tyr	Ile	Leu	Ser	Ala	Gln

50

55

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				100				105					110			
	Tyr	Glu	Asn	Gly	Gly	Phe	Pro	Gln	Tyr	Tyr	Pro	Asn	Lys	Leu	Tyr	Tyr
			115					120					125			
5	Arg	Ala	Glu	Ile	Thr	Tyr	Asn	Asp	Asp	Ala	Met	Ile	Asn	Ala	Leu	Leu
		130					135					140				
	Val	Leu	Tyr	Lys	Val	Ala	Asn	Lys	Arg	Glu	Gly	Phe	Glu	Ala	Ile	Asn
		145				150					155					160
	Pro	Ile	Phe	Val	Ser	Lys	Ala	Gln	Lys	Ala	Val	Glu	Lys	Gly	Ile	Thr
				165						170					175	
10	Cys	Ile	Leu	Lys	Thr	Gln	Val	Ile	Gln	Asp	Gly	Lys	Arg	Ser	Ile	Trp
			180						185					190		
	Ala	Ala	Gln	Tyr	Asp	Gln	Asn	Thr	Leu	Gln	Pro	Ala	Gln	Ala	Arg	Lys
			195					200					205			
	Phe	Glu	Pro	Ala	Ser	Leu	Ser	Thr	Ser	Glu	Ser	Val	Ser	Ile	Val	Arg
		210					215					220				
15	Phe	Leu	Met	Leu	Gln	Pro	Ala	Thr	Thr	Glu	Ile	Lys	Gln	Ala	Ile	Glu
		225				230					235					240
	His	Ala	Ile	Gln	Trp	Phe	Glu	Gln	His	Asp	Ile	Glu	Gly	Tyr	Arg	Phe
				245						250					255	
	Asp	Arg	Ile	Gln	Asp	Arg	Val	Thr	Gly	Lys	Tyr	Gln	Arg	Gln	Leu	Val
				260					265					270		
20	Ala	Asp	Arg	Thr	Ser	Thr	Ile	Trp	Ala	Arg	Phe	Tyr	Asn	Leu	Glu	Asp
			275					280					285			
	Asn	Arg	Pro	Leu	Phe	Gly	Asp	Arg	Asp	Asn	Thr	Ile	Lys	Tyr	Asn	Phe
		290					295					300				
	Glu	Glu	Val	Ser	Glu	Glu	Arg	Arg	Asn	Gly	Tyr	Ala	Trp	Phe	Gly	Asn
		305				310					315					320
25	Trp	Pro	Glu	Lys	Leu	Ile	Gln	Lys	Asp	Tyr	Pro	Lys	Trp	Lys	Lys	Gln
				325						330				335		
	Tyr	Lys	Ile	Gln												
				340												

<210> 65

30 <211> 1311

<212> DNA

<213> Bacteria

35 <400> 65

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	gccccgcgcg	ccggtgcccc	cgccgcgcgt	ccccacgccg	cgccacgccc	ggtcgcccgat	120
	ccggtcgcgcg	ccacgtgccc	cgccggcgac	ggctgggcgt	ccgaggggac	cggcacgacc	180
40	ggtggggccc	ccgcccgggc	ctcccgggtc	ttcacgcgtc	ccacctggga	ggagttccgg	240
	gccgcgctcg	cggtgcccgg	ctccgagccc	aggatcgtca	agggtggtggg	cacgctgaac	300
	gccaccgccc	ccggtcgcgcg	cgcccttcgag	gcgcccgggt	acgacttcgc	ccgctacctc	360
	gccgactacg	acccggccgt	gtgggggtac	gagaaggagg	tcagcggccc	gcaggaggag	420
	ctgcgggcgcg	cgccgcgcgc	cgcgccgggc	caggccatca	agggtcaagg	gccggcgaac	480
	accacgatcg	tcggggtcgcg	caggcacgcg	gggatcacgg	gcggcagcct	ccaggtgcag	540
45	ggcgtcgaca	acgtcgtggt	ccgcaacctg	acgctggaga	gcccgcctcg	ctgcttccc	600
	cagtgggacc	cgaccgacgg	cgcgaccggg	gcgtggaact	ccgagtacga	cagcctcgtc	660
	gtgtacggct	ccacccatgt	ctggatcgac	cacaacacct	tcaccgacgg	cgccccaccg	720
	gacagttcgc	tgccctcgta	ctacggcgag	gtctaccagc	agcacgacgg	cgaactggac	780
	gtcgtgcggg	gcgcggacct	cgtcacggtc	tcgtggaacg	ccttcaccga	ccacgacaag	840
	accctgatga	tcggcaacag	cgacagcgcg	ggcgccaccg	accggggcaa	gctgcgggtc	900
50	accctgcacc	acaacctggt	cgagaacgtc	gtccgagcgg	cgccccgggt	caggttcggg	960
	caggtcgacg	cgtacaacaa	ccacttcgtc	gtgccgagtt	cggcctacgc	gtacagcctg	1020
	ggcgtcgggc	aggagtccca	gctcttcgcg	gagaagaacg	cgttcaccct	cgccgggggc	1080
	gtgccggccg	ggaagatcct	caagaagtgg	aaggacgcgc	ccgtcaccac	cgtcggcaac	1140
	tacgtgaacg	gcaggccggt	cgacctgctc	gccgtccaca	acaccagtt	cccggaggag	1200
	cagttgcggg	ccgacgcggg	ctggaccccc	gtcctgcgca	ccagggtcga	ccacccgagg	1260
55	gccgtccccg	cgctcgtcga	ccaccgcgcg	ggcgccggcc	gctcctgctg	a	1311

<210> 66

<211> 436

<212> PRT
<213> Bacteria

<220>
<221> SIGNAL
<222> (1)...(28)

<221> DOMAIN
<222> (29)...(436)
<223> Catalytic domain

<400> 66

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	Leu	Thr	Val	Thr	Ala	Pro	Ala	Ala	Gly	Ala	His	Ala	Ala	Ala	Pro	His
5				20					25					30		
	Ala	Ala	Pro	Arg	Pro	Val	Ala	Asp	Pro	Ala	Arg	Ala	Thr	Leu	Pro	Ala
			35					40					45			
	Gly	Asp	Gly	Trp	Ala	Ser	Glu	Gly	Thr	Gly	Thr	Thr	Gly	Gly	Ala	Ala
		50					55					60				
	Ala	Glu	Ala	Ser	Arg	Val	Phe	Thr	Val	Ala	Thr	Trp	Glu	Glu	Phe	Arg
10	65					70					75				80	
	Ala	Ala	Leu	Ala	Val	Pro	Gly	Ser	Glu	Pro	Arg	Ile	Val	Lys	Val	Val
				85						90				95		
	Gly	Thr	Leu	Asn	Ala	Thr	Ala	Ala	Gly	Cys	Gly	Ala	Phe	Glu	Ala	Pro
				100					105					110		
	Gly	Tyr	Asp	Phe	Ala	Arg	Tyr	Leu	Ala	Asp	Tyr	Asp	Pro	Ala	Val	Trp
			115					120					125			
15	Gly	Tyr	Glu	Lys	Glu	Val	Ser	Gly	Pro	Gln	Glu	Glu	Leu	Arg	Ala	Ala
							135					140				
	Ser	Ala	Thr	Ala	Gln	Gly	Gln	Ala	Ile	Lys	Val	Lys	Val	Pro	Ala	Asn
	145					150					155				160	
	Thr	Thr	Ile	Val	Gly	Val	Gly	Arg	His	Ala	Gly	Ile	Thr	Gly	Gly	Ser
					165					170					175	
20	Leu	Gln	Val	Gln	Gly	Val	Asp	Asn	Val	Val	Arg	Asn	Leu	Thr	Leu	
				180					185				190			
	Glu	Ser	Pro	Leu	Asp	Cys	Phe	Pro	Gln	Trp	Asp	Pro	Thr	Asp	Gly	Ala
			195					200					205			
	Thr	Gly	Ala	Trp	Asn	Ser	Glu	Tyr	Asp	Ser	Leu	Val	Val	Tyr	Gly	Ser
							215					220				
25	Thr	His	Val	Trp	Ile	Asp	His	Asn	Thr	Phe	Thr	Asp	Gly	Ala	His	Pro
						230					235					240
	Asp	Ser	Ser	Leu	Pro	Ser	Tyr	Tyr	Gly	Glu	Val	Tyr	Gln	Gln	His	Asp
					245					250					255	
	Gly	Glu	Leu	Asp	Val	Val	Arg	Gly	Ala	Asp	Leu	Val	Thr	Val	Ser	Trp
30				260					265					270		
	Asn	Ala	Phe	Thr	Asp	His	Asp	Lys	Thr	Leu	Met	Ile	Gly	Asn	Ser	Asp
			275					280					285			
	Ser	Ala	Gly	Ala	Thr	Asp	Arg	Gly	Lys	Leu	Arg	Val	Thr	Leu	His	His
							295					300				
	Asn	Leu	Phe	Glu	Asn	Val	Glu	Arg	Ala	Pro	Arg	Val	Arg	Phe	Gly	
35	305					310				315					320	
	Gln	Val	Asp	Ala	Tyr	Asn	Asn	His	Phe	Val	Pro	Ser	Ser	Ala	Tyr	
					325					330				335		
	Ala	Tyr	Ser	Leu	Gly	Val	Gly	Gln	Glu	Ser	Gln	Leu	Phe	Ala	Glu	Lys
				340					345					350		
	Asn	Ala	Phe	Thr	Leu	Ala	Gly	Gly	Val	Pro	Ala	Gly	Lys	Ile	Leu	Lys
40				355				360					365			
	Lys	Trp	Lys	Asp	Ala	Pro	Val	Thr	Thr	Val	Gly	Asn	Tyr	Val	Asn	Gly
							375					380				
	Arg	Pro	Val	Asp	Leu	Leu	Ala	Val	His	Asn	Thr	Gln	Phe	Pro	Glu	Glu
						390					395				400	
	Gln	Leu	Arg	Ala	Asp	Ala	Gly	Trp	Thr	Pro	Val	Leu	Arg	Thr	Arg	Val
45					405					410					415	
	Asp	His	Pro	Arg	Ala	Val	Pro	Ala	Leu	Val	Asp	His	Arg	Ala	Gly	Ala
				420					425					430		
	Gly	Arg	Ser	Cys												
			435													

50 <210> 67
 <211> 1995
 <212> DNA
 <213> Unknown

55 <220>
 <223> Obtained from an environmental sample

<400> 67

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	aataattggg	gttctggcct	caccggtgaa	atcaaagtta	ccaacaacac	atcatcggt	180
5	gtaaatgggt	ggtctgtgtc	ttggcaggaa	tcaggcgcat	cagtcacca	ctcatggaac	240
	gcaactctga	gcggatcaaa	tccttatacg	gcagccgcct	taggttggaa	tgcaactctc	300
	gcacccggtg	cttctgccag	ttttggcctt	caagcaaagt	gcactgctag	cgcacctaaa	360
	gtgaatggca	ctttatgtgg	aacagctact	tcataaacac	ctgcgtcatc	cagcagtggt	420
	gcgagttcgg	ttaaatcaag	cgcacccggt	tcgtccagca	gtaaatcatc	cagctcaatc	480
	actgtgagta	gtagttctat	cgccagcagc	agcgcacca	gtgtttcttc	attaacaatt	540
10	caggaagagc	aagctggcct	ctgtcgtgtt	gatggcattg	caacagaaag	caccaacacc	600
	ggctttaccg	gcaacggcta	taccaatgca	aacaacgcac	aaggtgcagc	gattgaatgg	660
	gcggtaaatg	caccgagcag	tggccgctac	acactcacat	tccgttttgc	aaatggcggc	720
	actgcagcac	gcaatgggtc	actgttaatt	aacggcggtg	gcaatggtaa	ctacaccgtg	780
	gatttaccac	taaccggcgc	atgggcgact	tggcaaacag	cgactgtaga	aatcgatttg	840
	gtacaaggca	ccaacacgct	gaaactttct	gcattaaccg	cagatggctt	agctaataatc	900
15	gattcattaa	aaattgatgg	caaccaaccg	aaagcaggca	cttgagcaaa	tacatcaagc	960
	agtgttgcca	gcagttcttc	atccgttaaa	tccagttcaa	gttcttcatc	aagctcatcc	1020
	accactgcaa	aaatgctgac	tcttgatggc	aaccccgccg	caagttggtt	caacaaatcc	1080
	agaaccaaat	ggaatagcag	tcgtgcggac	attgtgttgt	cttaccagca	agctaacggc	1140
	ggctggccaa	aaaatctgga	ttacaactca	gtaagcgcag	gtaatgggtg	tagcgacagc	1200
	ggcactatcg	acaacggcgc	aaccatcacg	gaaatgggtt	atctcgcaga	agtttataaa	1260
20	aacgggggca	atacaaaata	tcgcgatgca	gtacgtaaag	cggcaaactt	tattgtgagt	1320
	tcgcaatata	gcactgggtg	gttaccacaa	ttttacccat	tgaagggtgg	ttatgcagat	1380
	cacgccacct	ttaacgataa	cggcattggt	tacgcattaa	cggtattgga	ttttgcggtg	1440
	aacaaacgtg	cgccgtttga	taacgatgta	ttttctgatg	cagaccgcgc	aaaattcaaa	1500
	actgccgtga	ccaaaggat	tgattacatt	ttgaaagccc	aatggaaaca	aaatggaaaa	1560
	ctcaccgcat	ggtgtgcgca	acacggagca	aacgactatc	aaccaaagc	ggcgcggtgt	1620
25	tatgagttag	tatctttaag	cggcagcgaa	tccgttggca	tcacgcgttt	cctgatgacc	1680
	caaccacaaa	ctgcgcaaat	cgaagcagcg	gttaaagccg	gtgtaaactg	gttcgctagc	1740
	ccgaatacat	acttggctaa	ttacacctac	gactcgtaaa	aagcctctac	caatccgatt	1800
	gtgtacaaat	ccggcagcag	aatgtggtat	cgcttctacg	atctgaacac	caatcgcgga	1860
	ttcttttagtg	atcgcgatgg	cagcaaattc	tatgacatca	ctcaaagtgc	tgaagaacgt	1920
	cgcaccggct	acagctgggg	cggttcttac	ggtgaatcga	ttatcagctt	cgcgcaaaaa	1980
30	gtgggttatc	tctaa					1995

<210> 68

<211> 664

<212> PRT

35 <213> Unknown

<220>

<223> Obtained from an environmental sample

40 <221> SIGNAL

<222> (1)...(32)

<221> BINDING

<222> (33)...(126)

45 <223> Carbohydrate binding module

<221> BINDING

<222> (184)...(307)

<223> Carbohydrate binding module

50

<221> DOMAIN

<222> (308)...(664)

<223> Catalytic domain

55 <400> 68

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Met Lys Asn Ser Lys Thr Val Phe Thr Ala Gln Lys Lys Leu Met His
 1 5 10 15
 Ser Cys Ile Ala Ala Ala Ile Gly Leu Ala Ile Ser Ser Gly Ala Trp
 - 51

5

10

15

20

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40

45

50

55

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				20					25					30		
	Ser	Ala	Cys	Thr	Tyr	Thr	Val	Thr	Asn	Asn	Trp	Gly	Ser	Gly	Phe	Thr
			35					40					45			
5	Gly	Glu	Ile	Lys	Val	Thr	Asn	Asn	Thr	Ser	Ser	Ala	Val	Asn	Gly	Trp
		50					55					60				
	Ser	Val	Ser	Trp	Gln	Glu	Ser	Gly	Ala	Ser	Val	Thr	Asn	Ser	Trp	Asn
	65				70					75					80	
	Ala	Thr	Leu	Ser	Gly	Ser	Asn	Pro	Tyr	Thr	Ala	Ala	Ala	Leu	Gly	Trp
				85					90					95		
10	Asn	Ala	Thr	Leu	Ala	Pro	Gly	Ala	Ser	Ala	Ser	Phe	Gly	Phe	Gln	Ala
			100					105					110			
	Asn	Gly	Thr	Ala	Ser	Ala	Pro	Lys	Val	Asn	Gly	Thr	Leu	Cys	Gly	Thr
			115					120					125			
	Ala	Thr	Ser	Ser	Thr	Pro	Ala	Ser	Ser	Ser	Ser	Val	Ala	Ser	Ser	Val
		130					135					140				
15	Lys	Ser	Ser	Ala	Pro	Val	Ser	Ser	Ser	Ser	Lys	Ser	Ser	Ser	Ser	Ile
	145				150						155					160
	Thr	Val	Ser	Ser	Ser	Ser	Ile	Ala	Ser	Ser	Ser	Ala	Pro	Ser	Val	Ser
				165					170					175		
	Ser	Leu	Thr	Ile	Gln	Glu	Glu	Gln	Ala	Gly	Phe	Cys	Arg	Val	Asp	Gly
			180					185						190		
20	Ile	Ala	Thr	Glu	Ser	Thr	Asn	Thr	Gly	Phe	Thr	Gly	Asn	Gly	Tyr	Thr
			195				200						205			
	Asn	Ala	Asn	Asn	Ala	Gln	Gly	Ala	Ala	Ile	Glu	Trp	Ala	Val	Asn	Ala
		210					215					220				
	Pro	Ser	Ser	Gly	Arg	Tyr	Thr	Leu	Thr	Phe	Arg	Phe	Ala	Asn	Gly	Gly
	225				230						235					240
	Thr	Ala	Ala	Arg	Asn	Gly	Ser	Leu	Leu	Ile	Asn	Gly	Gly	Ser	Asn	Gly
				245						250					255	
25	Asn	Tyr	Thr	Val	Asp	Leu	Pro	Leu	Thr	Gly	Ala	Trp	Ala	Thr	Trp	Gln
			260					265						270		
	Thr	Ala	Thr	Val	Glu	Ile	Asp	Leu	Val	Gln	Gly	Thr	Asn	Thr	Leu	Lys
			275					280					285			
	Leu	Ser	Ala	Leu	Thr	Ala	Asp	Gly	Leu	Ala	Asn	Ile	Asp	Ser	Leu	Lys
		290					295					300				
30	Ile	Asp	Gly	Asn	Gln	Pro	Lys	Ala	Gly	Thr	Cys	Ser	Asn	Thr	Ser	Ser
	305				310						315					320
	Ser	Val	Ala	Ser	Ser	Ser	Ser	Ser	Val	Lys	Ser	Ser	Ser	Ser	Ser	Ser
				325						330					335	
	Ser	Ser	Ser	Ser	Thr	Thr	Ala	Lys	Met	Leu	Thr	Leu	Asp	Gly	Asn	Pro
				340				345						350		
35	Ala	Ala	Ser	Trp	Phe	Asn	Lys	Ser	Arg	Thr	Lys	Trp	Asn	Ser	Ser	Arg
			355					360					365			
	Ala	Asp	Ile	Val	Leu	Ser	Tyr	Gln	Gln	Ala	Asn	Gly	Gly	Trp	Pro	Lys
		370					375					380				
	Asn	Leu	Asp	Tyr	Asn	Ser	Val	Ser	Ala	Gly	Asn	Gly	Gly	Ser	Asp	Ser
	385				390						395					400
40	Gly	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Ile	Thr	Glu	Met	Val	Tyr	Leu	Ala
				405						410					415	
	Glu	Val	Tyr	Lys	Asn	Gly	Gly	Asn	Thr	Lys	Tyr	Arg	Asp	Ala	Val	Arg
				420				425						430		
	Lys	Ala	Ala	Asn	Phe	Ile	Val	Ser	Ser	Gln	Tyr	Ser	Thr	Gly	Ala	Leu
			435					440					445			
45	Pro	Gln	Phe	Tyr	Pro	Leu	Lys	Gly	Gly	Tyr	Ala	Asp	His	Ala	Thr	Phe
		450					455					460				
	Asn	Asp	Asn	Gly	Met	Ala	Tyr	Ala	Leu	Thr	Val	Leu	Asp	Phe	Ala	Val
	465				470						475					480
	Asn	Lys	Arg	Ala	Pro	Phe	Asp	Asn	Asp	Val	Phe	Ser	Asp	Ala	Asp	Arg
				485						490					495	
50	Ala	Lys	Phe	Lys	Thr	Ala	Val	Thr	Lys	Gly	Ile	Asp	Tyr	Ile	Leu	Lys
				500					505					510		
	Ala	Gln	Trp	Lys	Gln	Asn	Gly	Lys	Leu	Thr	Ala	Trp	Cys	Ala	Gln	His
			515					520					525			
	Gly	Ala	Asn	Asp	Tyr	Gln	Pro	Lys	Ala	Ala	Arg	Ala	Tyr	Glu	Leu	Val
		530					535					540				
55	Ser	Leu	Ser	Gly	Ser	Glu	Ser	Val	Gly	Ile	Ile	Ala	Phe	Leu	Met	Thr
	545				550					555						560
	Gln	Pro	Gln	Thr	Ala	Gln	Ile	Glu	Ala	Ala	Val	Lys	Ala	Gly	Val	Asn

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5 Trp Phe Ala Ser 565 Pro Asn Thr Tyr Leu 570 Ala Asn Tyr Thr Tyr Asp Ser 575
 Ser Lys Ala 580 Ser Thr Asn Pro Ile Val 585 Tyr Lys Ser Gly 590 Ser Arg Met
 10 Trp Tyr Arg Phe Tyr Asp Leu 600 Thr Asn Arg Gly 605 Phe Phe Ser Asp
 Arg Asp Gly Ser Lys Phe Tyr Asp Ile Thr Gln Met Ser Glu Glu Arg
 625 630 635 640
 10 Arg Thr Gly Tyr Ser Trp Gly Gly Ser Tyr 650 Gly Glu Ser Ile Ile Ser 655
 Phe Ala Gln Lys Val Gly Tyr Leu 660

15 <210> 69
 <211> 1035
 <212> DNA
 <213> Unknown

20 <220>
 <223> Obtained from an environmental sample

<400> 69

25 atggcgcggtt tgttccggtg cgtgtgtgcc agcctgggag gatggggccgc ggttctggcc 60
 gccgcggcg gcccggattg gtcccgcctg ctccgcgaac cggacccttg gtttcgcagt 120
 ccggcggggc aacaggcggt gacgaacgtt ttgtcctggc agagcgcgac aggcgcctgg 180
 ccgaaaaacc tggacaccac ccgcgagccg cgtcggcagg attccgcccc gcccgagggc 240
 actttcgaca acggcgccac caccggcgag ttgcggtttc tggcgcgggc gtttgcggcc 300
 accggcgatc cgcgctgcga agccgcggtg ctccgggggc tggacggcat cctcgcggcc 360
 cagcttccca gcggcggtg gccgcagtgt catcctccgc gcgcgcctta tcagcgccac 420
 30 atcaccttca acgacggtgt catggtgcgc atcctggagc tgctgcgcga gatagaccgc 480
 gcgcccggagt ttcgctgggt ggacgaggcg cggcgcgcg gcggtgcgcgc ggccttact 540
 cgcgggctgg agtgccctct gcgctgccag gtggtcgtcg agggcagact caccgtgtgg 600
 tgtgcccagc atgacgcgga gaactttcaa ccgcgaccgg cacgcgccta cgaactggaa 660
 tcgctcagcg gcgcggaaag cgccggcatc ctggtgttcc tcatgagcct ggagccgcca 720
 35 accccggaga tcgcgcgcgc ggtcgaggcc ggggcggcct ggttttcggc ggtaaagctt 780
 gaaggggttcc gtctcgaacg aacggccgac gacgcgcggg tgggtggaaga gccgggcgcg 840
 ccgcccgtct gggcgcggtt ctacgagatc gggaccaatc gccccatctt tgccggtcgc 900
 gacgggtgtca agaagtacgc cctgagcgag atcgagcggg aacgccgggt cggctatgcg 960
 tgggtacggcg cctggggtga accggtcgcc cgccattatg cccagtggcg ggagcgttac 1020
 gggacgcaga aatga 1035

40 <210> 70
 <211> 344
 <212> PRT
 <213> Unknown

45 <220>
 <223> Obtained from an environmental sample

50 <221> SIGNAL
 <222> (1)...(22)
 <221> DOMAIN
 <222> (23)...(344)
 <223> Catalytic domain

55 <400> 70

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5 Met Ala Arg Leu Phe Arg Cys Val Cys Ala Ser Leu Gly Gly Trp Ala
 1 1 5 10 15
 Ala Val Leu Ala Ala Ala Ala Gly Pro Asp Trp Ser Arg Leu Ala
 20 25 30
 10 Gln Pro Asp Pro Trp Phe Arg Ser Ala Gly Gln Gln Ala Val Thr
 35 40 45
 Asn Val Leu Ser Trp Gln Ser Ala Thr Gly Ala Trp Pro Lys Asn Leu
 50 55 60
 Asp Thr Thr Arg Glu Pro Arg Arg Gln Asp Ser Ala Pro Pro Glu Gly
 65 70 75 80
 15 Thr Phe Asp Asn Gly Ala Thr Thr Gly Glu Leu Arg Phe Leu Ala Arg
 85 90 95
 Ala Phe Ala Ala Thr Gly Asp Pro Arg Cys Glu Ala Ala Val Leu Arg
 100 105 110
 20 Gly Leu Asp Gly Ile Leu Ala Ala Gln Leu Pro Ser Gly Gly Trp Pro
 115 120 125
 Gln Cys His Pro Pro Arg Ala Pro Tyr Gln Arg His Ile Thr Phe Asn
 130 135 140
 Asp Gly Val Met Val Arg Ile Leu Glu Leu Leu Arg Glu Ile Asp Arg
 145 150 155 160
 25 Ala Pro Glu Phe Arg Trp Val Asp Glu Ala Arg Arg Ala Arg Val Arg
 165 170 175
 Ala Ala Phe Thr Arg Gly Leu Glu Cys Leu Leu Arg Cys Gln Val Val
 180 185 190
 Val Glu Gly Arg Leu Thr Val Trp Cys Ala Gln His Asp Ala Glu Asn
 195 200 205
 30 Phe Gln Pro Arg Pro Ala Arg Ala Tyr Glu Leu Glu Ser Leu Ser Gly
 210 215 220
 Ala Glu Ser Ala Gly Ile Leu Val Phe Leu Met Ser Leu Glu Pro Pro
 225 230 235 240
 Thr Pro Glu Ile Ala Arg Ala Val Glu Ala Gly Ala Ala Trp Phe Ser
 245 250 255
 35 Ala Val Lys Leu Glu Gly Phe Arg Leu Glu Arg Thr Ala Asp Ala
 260 265 270
 Arg Val Val Glu Glu Pro Gly Ala Pro Pro Leu Trp Ala Arg Phe Tyr
 275 280 285
 Glu Ile Gly Thr Asn Arg Pro Ile Phe Ala Gly Arg Asp Gly Val Lys
 290 295 300
 40 Lys Tyr Ala Leu Ser Glu Ile Glu Arg Glu Arg Val Gly Tyr Ala
 305 310 315 320
 Trp Tyr Gly Ala Trp Gly Glu Pro Val Ala Arg His Tyr Ala Gln Trp
 325 330 335
 Arg Glu Arg Tyr Gly Thr Gln Lys
 340

<210> 71

<211> 1038

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 71

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	gtgactcgtg	tcgcccttgc	gatggggcctt	gttgcatggg	ttccggcgct	cgcttcagct	60
	gggcccgcgtg	catattttgca	gaagccggac	gactgggttcg	ccagtcccga	ggccagggca	120
	atcgccgcga	acgtactcgc	gcatcaggcc	gatctcggcg	ggtggccgaa	gaacatcgac	180
	acaacgaagc	cgttcaccgg	cgaccggacg	caaatcaaac	cgaccttcga	taacagcgcg	240
5	acaaccgacg	agctccggtt	tctggcgcg	atccacaacg	cgactcgca	cgagaagtac	300
	cgacccgcgt	tcgagaaggg	gctcgattac	atcttgaaag	cacaatacgc	aaacggcggt	360
	tggccgcagt	cgacccgc	cggcaccggc	taccaccggc	acatcacctt	caacgacaat	420
	gccatggtcc	gtttgatgga	gctcgtgcgc	gaagtcgcga	cctcgaatcg	gtacgacttc	480
	ctggacgccg	accgccgcaa	ggcctgccgc	gccgctttcg	atcgcggcg	cgaaatgcac	540
	ctgaagtgcc	agatcaaggt	cgacagtaag	ctgacggcat	ggtgcgcca	gcacgacgag	600
10	aaggacctcg	ctccccggcc	ggcgcgacc	tacgagctcg	tctcactcag	cggtcggag	660
	tcggtcggga	tcgtccgcct	actcatgagc	ctcgatcgac	caagcccgga	ggtcgctcgg	720
	gccatcgacg	gcgcggtcgc	gtggttccag	tcggcgaagc	tcgaaggcac	caaggtcgtt	780
	gtcgagcgcg	acccgaagta	tccgggcggc	cggaacgcg	tgggtggtgaa	ggatccaaag	840
	gcaccgccac	tctgggcgcg	cttctacgaa	atcggcacga	atcgccccat	cttctccgac	900
	cgcgacggca	tcaagaagta	cgcgctcgcc	gagatcggcc	ccgaacggcg	gaatggctat	960
15	gcctggtatg	gcacctggcc	gcgcgacctg	ctggagaagg	aatacccagg	gtggaaaaag	1020
	aagctggccc	ggccgtga					1038

<210> 72

<211> 345

20 <212> PRT

<213> Unknown

<220>

25 <223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(20)

<221> DOMAIN

30 <222> (21)...(345)

<223> Catalytic domain

<400> 72

35

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45

50

55

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Met Thr Arg Val Ala Leu Ala Met Gly Leu Val Ala Trp Val Pro Ala
1 5 10 15
Leu Ala Ser Ala Gly Pro Ala Ala Tyr Leu Gln Lys Pro Asp Asp Trp
20 25 30
5 Phe Ala Ser Pro Glu Ala Arg Ala Ile Ala Ala Asn Val Leu Ala His
35 40 45
Gln Ala Asp Leu Gly Gly Trp Pro Lys Asn Ile Asp Thr Thr Lys Pro
50 55 60
10 Phe Thr Gly Asp Arg Thr Gln Ile Lys Pro Thr Phe Asp Asn Ser Ala
65 70 75 80
Thr Thr Asp Glu Leu Arg Phe Leu Ala Arg Ile His Asn Ala Thr Arg
85 90 95
Asp Glu Lys Tyr Arg Thr Ala Phe Glu Lys Gly Leu Asp Tyr Ile Leu
100 105 110
15 Lys Ala Gln Tyr Ala Asn Gly Gly Trp Pro Gln Ser His Pro Pro Gly
115 120 125
Thr Gly Tyr His Arg His Ile Thr Phe Asn Asp Asn Ala Met Val Arg
130 135 140
Leu Met Glu Leu Val Arg Glu Val Ala Thr Ser Asn Arg Tyr Asp Phe
145 150 155 160
Leu Asp Ala Asp Arg Arg Lys Ala Cys Arg Ala Ala Phe Asp Arg Gly
165 170 175
20 Ile Glu Cys Ile Leu Lys Cys Gln Ile Lys Val Asp Ser Lys Leu Thr
180 185 190
Ala Trp Cys Ala Gln His Asp Glu Lys Asp Leu Ala Pro Arg Pro Ala
195 200 205
Arg Thr Tyr Glu Leu Val Ser Leu Ser Gly Ser Glu Ser Val Gly Ile
210 215 220
25 Val Arg Leu Leu Met Ser Leu Asp Arg Pro Ser Pro Glu Val Ala Arg
225 230 235 240
Ala Ile Asp Gly Ala Val Ala Trp Phe Gln Ser Ala Lys Leu Glu Gly
245 250 255 260
Thr Lys Val Val Val Glu Arg Asp Pro Lys Tyr Pro Gly Gly Arg Glu
265 270 275
30 Arg Val Val Val Lys Asp Pro Lys Ala Pro Pro Leu Trp Ala Arg Phe
275 280 285
Tyr Glu Ile Gly Thr Asn Arg Pro Ile Phe Ser Asp Arg Asp Gly Ile
290 295 300
35 Lys Lys Tyr Ala Leu Ala Glu Ile Gly Pro Glu Arg Arg Asn Gly Tyr
305 310 315 320
Ala Trp Tyr Gly Thr Trp Pro Arg Asp Leu Leu Glu Lys Glu Tyr Pro
325 330 335
Gly Trp Lys Lys Lys Leu Ala Arg Pro
340 345

40 <210> 73
<211> 1221
<212> DNA
<213> Unknown

45 <220>
<223> Obtained from an environmental sample

<400> 73

50 atgctcacca aaacatcact acttattgca ttgctaggca gttgttgat cgcaccatta 60
catgcggaca caccagcaag caatgcaccg acaaccaatg catcaattcc gctacagcaa 120
actgcgagcg atgctgccgc ctggaaaaat tatctcgcca aatccaacga gttgcgcaaa 180

55

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	gcagaccagg	cgcagctcaa	agccgagctg	aaaaaactcg	ggcaaaaaac	cgcgagtttg	240
	cctgagtaca	ccaaagaatt	tggttttgaa	gtgaagcagt	catctgagtg	gtttaaaagc	300
	actgaaggta	aacgagtgat	ggatattatc	ctatcgtttc	aaactccttc	tggcggctgg	360
5	tcaaaacgca	ctgacatgag	caaagcgccg	cgcaaaccgg	gccaggcatt	tgggtgttgaa	420
	aaaaattaca	tccccacctt	tgataatggc	gcgaccagca	cacaattaat	gctactggca	480
	caggcgcatc	aagccactgg	cgataaacgc	tacagcgatg	catttgcgcg	cgggcttgaa	540
	tttatcatca	ccgctcaata	tccaatggc	ggctggccac	aaaattttcc	attggttggc	600
	aagtatcacg	atcacatcac	ttacaacgat	gccctgatgc	gcgatttaat	ggtagtgcta	660
	cacaaggttg	ccatggccaa	ggatgaattt	gcctttgtat	ccaaggcgca	gcaacaggcc	720
10	gcacaagcga	gcctcgaacg	cgcgctggac	tgcgttttga	aaaccagggt	gatggccaat	780
	ggccaattaa	ctatatgggg	tgcgcagcac	gatgccaaaa	ccttaaaacc	cgccaaagcg	840
	cgcgccatg	aatgattttc	actcaccagt	tctgaaagcg	tgtggatgct	cgatttttta	900
	atggatttgc	aacagcccag	cgctgacatt	attaaatccg	tgcacgcggc	tgccgcttgg	960
	tatgagcaaa	ataaaattat	cggaataaac	tggaccgggg	gcgacacagt	tctgaaagac	1020
	gataaggatg	caccgccaat	ctgggcgcgt	ttttatgaga	taggtacgaa	caaacccttg	1080
15	tttggcgacc	gcgatgactc	tgtccattac	gatctggcaa	aggtatcgga	agagcgccgc	1140
	acgggttatg	cctgggtacac	aacctcacc	aatcaggtat	taaaaaagta	cgcgcgctgg	1200
	gctaaacaat	atccgcaata	a				1221

20 <210> 74
 <211> 406
 <212> PRT
 <213> Unknown

25 <220>
 <223> Obtained from an environmental sample

30 <221> SIGNAL
 <222> (1)...(22)
 <221> DOMAIN
 <222> (23)...(406)
 <223> Catalytic domain

35 <400> 74

40

45

50

55

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	Met	Leu	Thr	Lys	Thr	Ser	Leu	Leu	Ile	Ala	Leu	Leu	Gly	Ser	Cys	Cys
	1				5					10					15	
	Ile	Ala	Pro	Leu	His	Ala	Asp	Thr	Pro	Ala	Ser	Asn	Ala	Pro	Thr	Thr
5				20					25				30			
	Asn	Ala	Ser	Ile	Pro	Leu	Gln	Gln	Thr	Ala	Ser	Asp	Ala	Ala	Ala	Trp
			35				40					45				
	Lys	Asn	Tyr	Leu	Ala	Lys	Ser	Asn	Glu	Leu	Arg	Lys	Ala	Asp	Gln	Ala
		50					55					60				
	Gln	Leu	Lys	Ala	Glu	Leu	Lys	Lys	Leu	Gly	Gln	Lys	Thr	Ala	Ser	Leu
10		65			70					75					80	
	Pro	Glu	Tyr	Thr	Lys	Glu	Phe	Gly	Phe	Glu	Val	Lys	Gln	Ser	Ser	Glu
				85					90					95		
	Trp	Phe	Lys	Ser	Thr	Glu	Gly	Lys	Arg	Val	Met	Asp	Ile	Ile	Leu	Ser
				100					105					110		
	Phe	Gln	Thr	Pro	Ser	Gly	Gly	Trp	Ser	Lys	Arg	Thr	Asp	Met	Ser	Lys
			115					120					125			
15	Ala	Pro	Arg	Lys	Pro	Gly	Gln	Ala	Phe	Gly	Val	Glu	Lys	Asn	Tyr	Ile
		130					135					140				
	Pro	Thr	Phe	Asp	Asn	Gly	Ala	Thr	Ser	Thr	Gln	Leu	Met	Leu	Leu	Ala
		145			150						155					160
	Gln	Ala	His	Gln	Ala	Thr	Gly	Asp	Lys	Arg	Tyr	Ser	Asp	Ala	Phe	Ala
				165						170					175	
20	Arg	Gly	Leu	Glu	Phe	Ile	Ile	Thr	Ala	Gln	Tyr	Pro	Asn	Gly	Gly	Trp
			180						185					190		
	Pro	Gln	Asn	Phe	Pro	Leu	Val	Gly	Lys	Tyr	His	Asp	His	Ile	Thr	Tyr
			195					200					205			
	Asn	Asp	Ala	Leu	Met	Arg	Asp	Leu	Met	Val	Val	Leu	His	Lys	Val	Ala
		210					215					220				
25	Met	Ala	Lys	Asp	Glu	Phe	Ala	Phe	Val	Ser	Lys	Ala	Gln	Gln	Gln	Ala
		225			230						235					240
	Ala	Gln	Ala	Ser	Leu	Glu	Arg	Ala	Leu	Asp	Cys	Val	Leu	Lys	Thr	Gln
				245					250					255		
	Val	Met	Ala	Asn	Gly	Gln	Leu	Thr	Ile	Trp	Gly	Ala	Gln	His	Asp	Ala
30																
				260					265				270			
	Lys	Thr	Leu	Lys	Pro	Ala	Lys	Ala	Arg	Ala	Tyr	Glu	Met	Ile	Ser	Leu
			275					280					285			
	Thr	Ser	Ser	Glu	Ser	Val	Trp	Met	Leu	Asp	Phe	Leu	Met	Asp	Leu	Gln
35			290				295					300				
	Gln	Pro	Ser	Ala	Asp	Ile	Ile	Lys	Ser	Val	His	Ala	Ala	Ala	Ala	Trp
					310						315					320
	Tyr	Glu	Gln	Asn	Lys	Ile	Ile	Gly	Lys	Thr	Trp	Thr	Arg	Gly	Asp	Thr
				325						330					335	
	Val	Leu	Lys	Asp	Asp	Lys	Asp	Ala	Pro	Pro	Ile	Trp	Ala	Arg	Phe	Tyr
40				340					345					350		
	Glu	Ile	Gly	Thr	Asn	Lys	Pro	Leu	Phe	Gly	Asp	Arg	Asp	Asp	Ser	Val
			355					360					365			
	His	Tyr	Asp	Leu	Ala	Lys	Val	Ser	Glu	Glu	Arg	Arg	Thr	Gly	Tyr	Ala
		370					375					380				
45	Trp	Tyr	Thr	Thr	Ser	Pro	Asn	Gln	Val	Leu	Lys	Lys	Tyr	Ala	Arg	Trp
		385			390						395					400
	Ala	Lys	Gln	Tyr	Pro	Gln										
				405												

<210> 75

<211> 1767

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 75

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	atgaccacaa	cccgccgcac	tatcctgaaa	gccgccgccca	gcgccggcgc	gatcgccagc	60
	accggctggc	ccgccttggc	cgccgcacag	gccgcgcaag	ccgccgaccc	gtgggcccgc	120
	gcccagcaga	tcatcgaccg	cttcgccaag	ccgctcagct	tcccgaacag	ggacttcccg	180
5	atcaccgagt	tcggcgccaa	accctgcaag	ctggtcaaag	cccagggcct	ggtcgaagta	240
	agagtcaaag	gcgaactcga	aacgccagca	ccgcaagcgc	cggacgccta	cccggcaatc	300
	aaagccgcca	tcgccgcagc	gagcaaggcc	ggaggagggc	gcgtgctgat	cccggccggc	360
	aactggtact	gcaagggccc	tatcgtgctg	ctgtcgaacg	tgcacgtgca	ccttgccaag	420
	ggcgcgcaag	tctacttcag	cgccaacgcc	aaggacttcg	cccgcgacgg	cgactacgac	480
	tgcggcgcca	acggcaagct	ggtgctctcg	cgctggcaag	gcaacgattg	cctgaacttc	540
	tcgccccatg	tctacgcgcg	cgggcaaaaag	aatatcgcca	ttaccggcga	agactggacc	600
10	agcatcctga	acggccaggc	cggcgtggcg	ttcgaagacg	gcagcggaag	tggctggtgg	660
	ggcatgaacc	ccgccggcgc	gccgcccggc	agcaccacgc	accagggcgc	agccaatccg	720
	aacaacgccg	aggagccaat	cgccagactg	cccacgcgcc	acgcgaactg	gagcgccgac	780
	gacaagtacc	tgccgctgct	gtccgaagcc	ggcgtgcccg	ccgagcgccg	cgtgttcggt	840
	ctggggcact	acctgcggcc	gtcgaatggtc	gaattcgtcg	actgcgggga	tgtgtgatg	900
	cagggtacc	aggtcatcaa	cacgccgttc	tggattcatc	acccggtcaa	ctcacgcaac	960
15	attcacttct	ccaaagtgcg	catggaaaagc	atcggcccca	attcggacgg	tttcgatccc	1020
	gagtcctgcg	acaccatcct	ggtggacggc	tgcctgttca	ataccggcga	cgactgcatc	1080
	gccatcaaat	ccggcaagaa	ccgagactcg	caatacggcc	caacgcgcaa	tatggtggtc	1140
	cagaactgca	tcatgaaccg	cggccacggc	ggcgttacgc	tgggcagcga	aatggcggtg	1200
	ggcatcgagc	atatctacgc	gcagaaaatc	gaattccgca	acgcgttctg	ggaccacgac	1260
	ccgctgggca	cggccatccg	aatgaagacg	aacatgaacc	gcggcggtca	ccttcgtcat	1320
20	ttctacgtgc	gcgacgtgac	gctgccgaat	ggcgtgcgta	ccaagagcgg	cttctacaag	1380
	acgctgccgg	gatctccgct	ggcaggcaag	gtctccacca	gcggcggcgc	tgttatcact	1440
	atcgactgcg	attacgcgcc	gaatgacgac	agcgtgcgcg	tgcggccgcc	gcaggtgtcg	1500
	gacgtgcata	tctcgaacgt	ccgcgtcagc	aatgtgaaaa	cggccgaagg	ctcgttctcc	1560
	tgctaccagg	ccatggtgct	gctcggggccc	gtggcgggcca	gcttcaacgg	cgcgcctggc	1620
	acggccatcc	tgccgatcac	gaatgtcacc	gtcagcgatt	cggacttcgg	cacgccgcgc	1680
25	aacagcgagc	agccctgggt	cgcgttcaac	gtgcagggac	tcaagctgcg	caacgtgcgc	1740
	atcgatggca	aggagtacaa	cgtatga				1767

<210> 76

<211> 588

30 <212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

35

<221> SIGNAL

<222> (1)...(34)

<221> DOMAIN

40 <222> (110)...(555)

<223> Catalytic domain

<400> 76

45

50

55

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	Met	Thr	Thr	Thr	Arg	Arg	Thr	Ile	Leu	Lys	Ala	Ala	Ala	Ser	Ala	Gly
	1				5					10					15	
	Ala	Ile	Ala	Ser	Thr	Gly	Trp	Pro	Ala	Leu	Ala	Ala	Ala	Gln	Ala	Ala
5				20					25					30		
	Gln	Ala	Ala	Asp	Pro	Trp	Ala	Arg	Ala	Gln	Gln	Ile	Ile	Asp	Arg	Phe
			35				40						45			
	Ala	Lys	Pro	Leu	Ser	Phe	Pro	Asn	Arg	Asp	Phe	Pro	Ile	Thr	Glu	Phe
		50					55					60				
	Gly	Ala	Lys	Pro	Cys	Lys	Leu	Val	Lys	Ala	Gln	Gly	Leu	Val	Glu	Val
10		65				70					75					80
	Arg	Val	Lys	Gly	Glu	Leu	Glu	Thr	Pro	Ala	Pro	Gln	Ala	Pro	Asp	Ala
				85						90					95	
	Tyr	Pro	Ala	Ile	Lys	Ala	Ala	Ile	Ala	Ala	Ala	Ser	Lys	Ala	Gly	Gly
				100					105					110		
	Gly	Arg	Val	Leu	Ile	Pro	Ala	Gly	Asn	Trp	Tyr	Cys	Lys	Gly	Pro	Ile
			115					120					125			
15	Val	Leu	Leu	Ser	Asn	Val	His	Val	His	Leu	Ala	Lys	Gly	Ala	Gln	Val
		130					135					140				
	Tyr	Phe	Ser	Ala	Asn	Ala	Lys	Asp	Phe	Ala	Arg	Asp	Gly	Asp	Tyr	Asp
		145				150					155					160
	Cys	Gly	Ala	Asn	Gly	Lys	Leu	Val	Leu	Ser	Arg	Trp	Gln	Gly	Asn	Asp
				165						170					175	
20	Cys	Leu	Asn	Phe	Ser	Pro	Met	Val	Tyr	Ala	Arg	Gly	Gln	Lys	Asn	Ile
				180					185					190		
	Ala	Ile	Thr	Gly	Glu	Asp	Trp	Thr	Ser	Ile	Leu	Asn	Gly	Gln	Ala	Gly
			195					200					205			
	Val	Ala	Phe	Glu	Asp	Gly	Ser	Gly	Asn	Gly	Trp	Trp	Gly	Met	Asn	Pro
		210					215					220				
25	Ala	Gly	Ala	Pro	Pro	Gly	Ser	Thr	Thr	His	Gln	Gly	Ala	Ala	Asn	Pro
		225				230					235					240
	Asn	Asn	Ala	Glu	Glu	Pro	Ile	Ala	Arg	Leu	Pro	Thr	Arg	His	Ala	Asn
				245						250					255	
	Trp	Ser	Ala	Asp	Asp	Lys	Tyr	Leu	Pro	Leu	Leu	Ser	Glu	Ala	Gly	Val
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30	Pro	Ala	Glu	Arg	Arg	Val	Phe	Gly	Leu	Gly	His	Tyr	Leu	Arg	Pro	Ser
			275					280					285			
	Met	Val	Glu	Phe	Val	Asp	Cys	Gly	Asp	Val	Leu	Met	Gln	Gly	Tyr	Gln
		290					295					300				
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		305				310					315					320
35	Ile	His	Phe	Ser	Lys	Val	Arg	Met	Glu	Ser	Ile	Gly	Pro	Asn	Ser	Asp
				325						330					335	
	Gly	Phe	Asp	Pro	Glu	Ser	Cys	Asp	Thr	Ile	Leu	Val	Asp	Gly	Cys	Leu
				340					345					350		
	Phe	Asn	Thr	Gly	Asp	Asp	Cys	Ile	Ala	Ile	Lys	Ser	Gly	Lys	Asn	Arg
			355					360					365			
40	Asp	Ser	Gln	Tyr	Gly	Pro	Thr	Arg	Asn	Met	Val	Val	Gln	Asn	Cys	Ile
		370					375					380				
	Met	Asn	Arg	Gly	His	Gly	Gly	Val	Thr	Leu	Gly	Ser	Glu	Met	Ala	Gly
		385				390					395					400
	Gly	Ile	Glu	His	Ile	Tyr	Ala	Gln	Lys	Ile	Glu	Phe	Arg	Asn	Ala	Phe
				405						410					415	
45	Trp	Asp	His	Asp	Pro	Leu	Gly	Thr	Ala	Ile	Arg	Met	Lys	Thr	Asn	Met
				420					425					430		
	Asn	Arg	Gly	Gly	Tyr	Leu	Arg	His	Phe	Tyr	Val	Arg	Asp	Val	Thr	Leu
			435					440					445			
	Pro	Asn	Gly	Val	Arg	Thr	Lys	Ser	Gly	Phe	Tyr	Lys	Thr	Leu	Pro	Gly
		450					455					460				
50	Ser	Pro	Leu	Ala	Gly	Lys	Val	Ser	Thr	Ser	Gly	Gly	Ala	Val	Ile	Thr
		465				470					475					480

55

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Ile Asp Cys Asp Tyr Ala Pro Asn Asp Asp Ser Val Arg Val Arg Pro
 485 490 495
 Pro Gln Val Ser Asp Val His Ile Ser Asn Val Arg Val Ser Asn Val
 500 505 510
 Lys Thr Ala Glu Gly Ser Phe Ser Cys Tyr Gln Ala Met Val Leu Leu
 515 520 525
 Gly Pro Val Ala Ala Ser Phe Asn Gly Ala Pro Gly Thr Ala Ile Leu
 530 535 540
 Pro Ile Thr Asn Val Thr Val Ser Asp Ser Asp Phe Gly Thr Pro Arg
 545 550 555 560
 Asn Ser Ala Glu Pro Trp Phe Ala Phe Asn Val Gln Gly Leu Lys Leu
 565 570 575
 Arg Asn Val Arg Ile Asp Gly Lys Glu Tyr Asn Val
 580 585

<210> 77
 <211> 2043
 <212> DNA
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<400> 77

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	gtgaacaatt	ggtctgtgtc	atggcaggaa	tccggtgcgg	ctatcaccaa	cgcttggaa	240
	gcaacgctca	gtggctcaaa	cccttacaca	gccgtatccg	ctggttggaa	tggcacactt	300
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	tccagagcga	gcagtagtgc	gccaagcggt	ttctctttta	cgatccagga	agagcaagcg	600
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40	agctcgtcgt	ccgttaaatac	cagcgcaagt	tcttcttcga	gttcatccac	cgctgcaaaa	1080
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	aatggtgcaa	ccattaccga	aatggttttac	ctcgctgaaa	tttataaaaa	cggcggcaac	1320
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	ccgtttgata	acgacatttt	ctctgattct	gatcgggcga	aattcaaaac	cgctgttgcc	1560
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	tcattgagtg	gtagcgagtc	ggtcggcatt	ctcgctttct	tgatgaccca	accacaaacc	1740
50	gcgcaaatcg	aagcggcggt	caaggcgggt	gtcaactggt	tcgccagtcc	aaatacttat	1800
	ttggctaact	acacttacga	ttcatcaaaa	gcgtctacca	acccgattgt	gtataaatcc	1860
	ggaagcagaa	tgtggtatcg	cttctatgac	ctgaacacca	accgtgggtt	ctttagtgat	1920
	cgcgatggca	gcaaattcta	tgatatacacc	caaattgtcag	aagagcgctc	caccggttat	1980
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55	taa						2043

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 <211> 680
 <212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

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<221> SIGNAL

<222> (1)...(33)

<221> BINDING

10

<222> (34)...(126)

<223> Carbohydrate binding module

<221> BINDING

15

<222> (199)...(322)

<223> Carbohydrate binding module

<221> DOMAIN

20

<222> (323)...(680)

<223> Catalytic domain

<400> 78

25

30

35

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	Ala	Leu	Ile	Ala	Ala	Ser	Val	Ser	Met	Ala	Met	Ser	Ser	Ala	Ala	Trp
				20					25					30		
5	Ala	Gly	Cys	Thr	Tyr	Thr	Val	Thr	Asn	Asn	Trp	Gly	Ser	Gly	Phe	Thr
			35					40					45			
	Gly	Glu	Ile	Lys	Val	Thr	Asn	Asn	Thr	Thr	Ala	Ser	Val	Asn	Asn	Trp
		50					55					60				
	Ser	Val	Ser	Trp	Gln	Glu	Ser	Gly	Ala	Ala	Ile	Thr	Asn	Ala	Trp	Asn
	65				70						75				80	
10	Ala	Thr	Leu	Ser	Gly	Ser	Asn	Pro	Tyr	Thr	Ala	Val	Ser	Ala	Gly	Trp
				85						90					95	
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				100					105					110		
	Asn	Gly	Ser	Ala	Gly	Ala	Pro	Lys	Val	Asn	Gly	Ser	Leu	Cys	Gly	Thr
			115					120					125			
15	Asn	Thr	Ser	Ser	Thr	Pro	Ala	Ser	Ser	Ser	Val	Ala	Ser	Ser	Val	Lys
		130					135					140				
	Ser	Ser	Ala	Pro	Val	Ser	Ser	Ser	Ser	Arg	Ser	Ser	Ser	Ser	Ile	Ala
	145					150				155					160	
	Ile	Thr	Ser	Ser	Ser	Leu	Ala	Arg	Ser	Ser	Ile	Ala	Ser	Ser	Ser	Ser
					165					170					175	
20	Leu	Val	Ser	Ser	Arg	Ala	Ser	Ser	Ser	Ala	Pro	Ser	Val	Phe	Ser	
				180					185					190		
	Phe	Thr	Ile	Gln	Glu	Glu	Gln	Ala	Gly	Phe	Cys	Arg	Val	Asp	Gly	Ile
			195					200					205			
	Ala	Thr	Glu	Ser	Thr	Asn	Thr	Gly	Phe	Thr	Gly	Asn	Gly	Tyr	Thr	Asn
		210				215						220				
25	Ala	Asn	Asn	Ala	Gln	Gly	Ala	Ala	Ile	Glu	Trp	Ala	Val	Ser	Ala	Pro
		225				230				235					240	
	Ser	Ser	Gly	Arg	Tyr	Thr	Val	Ala	Phe	Arg	Phe	Ala	Asn	Gly	Gly	Thr
				245					250					255		
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				260					265					270		
30	Tyr	Thr	Val	Glu	Leu	Pro	Leu	Thr	Gly	Ala	Trp	Ala	Thr	Trp	Gln	Ile
			275					280					285			
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		290					295					300				
	Ser	Ala	Leu	Thr	Ala	Asp	Gly	Leu	Ala	Asn	Ile	Asp	Ser	Leu	Lys	Ile
		305				310					315				320	
35	Asp	Gly	Ala	Gln	Thr	Lys	Ala	Gly	Thr	Cys	Ser	Thr	Thr	Ser	Ser	Ser
				325						330				335		
	Ser	Val	Ala	Ser	Ser	Ser	Ser	Ser	Val	Lys	Ser	Ser	Ala	Ser	Ser	Ser
				340					345					350		
	Ser	Ser	Ser	Ser	Thr	Ala	Ala	Lys	Ile	Leu	Thr	Leu	Asp	Gly	Asn	Pro
			355					360					365			
40	Ala	Ala	Ser	Trp	Phe	Asn	Lys	Ser	Arg	Thr	Lys	Trp	Asn	Ser	Ser	Arg
		370					375					380				
	Ala	Asp	Ile	Val	Leu	Ser	Tyr	Gln	Gln	Ser	Asn	Gly	Gly	Trp	Pro	Lys
		385				390					395				400	
45	Asn	Leu	Asp	Tyr	Asn	Ser	Val	Ser	Ala	Gly	Asn	Gly	Gly	Ser	Asp	Ser
					405					410					415	

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	Gly	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Ile	Thr	Glu	Met	Val	Tyr	Leu	Ala
				420					425					430		
	Glu	Ile	Tyr	Lys	Asn	Gly	Gly	Asn	Thr	Lys	Tyr	Arg	Asp	Ala	Val	Arg
			435					440					445			
5	Arg	Ala	Ala	Asn	Phe	Leu	Val	Ser	Ser	Gln	Tyr	Ser	Thr	Gly	Ala	Leu
		450					455					460				
	Pro	Gln	Phe	Tyr	Pro	Leu	Lys	Gly	Gly	Tyr	Ala	Asp	His	Ala	Thr	Phe
	465					470					475					480
	Asn	Asp	Asn	Gly	Met	Ala	Tyr	Ala	Leu	Thr	Val	Leu	Asp	Phe	Ala	Val
					485					490					495	
10	Asn	Lys	Arg	Ala	Pro	Phe	Asp	Asn	Asp	Ile	Phe	Ser	Asp	Ser	Asp	Arg
				500					505					510		
	Ala	Lys	Phe	Lys	Thr	Ala	Val	Ala	Lys	Gly	Val	Asp	Tyr	Ile	Leu	Lys
			515					520					525			
	Ala	Gln	Trp	Lys	Gln	Asn	Gly	Lys	Leu	Thr	Ala	Trp	Cys	Ala	Gln	His
		530					535					540				
15	Gly	Ala	Thr	Asp	Tyr	Gln	Pro	Lys	Lys	Ala	Arg	Ala	Tyr	Glu	Leu	Glu
	545					550					555					560
	Ser	Leu	Ser	Gly	Ser	Glu	Ser	Val	Gly	Ile	Leu	Ala	Phe	Leu	Met	Thr
					565				570						575	
	Gln	Pro	Gln	Thr	Ala	Gln	Ile	Glu	Ala	Ala	Val	Lys	Ala	Gly	Val	Asn
				580					585					590		
20	Trp	Phe	Ala	Ser	Pro	Asn	Thr	Tyr	Leu	Ala	Asn	Tyr	Thr	Tyr	Asp	Ser
			595					600					605			
	Ser	Lys	Ala	Ser	Thr	Asn	Pro	Ile	Val	Tyr	Lys	Ser	Gly	Ser	Arg	Met
		610					615					620				
	Trp	Tyr	Arg	Phe	Tyr	Asp	Leu	Asn	Thr	Asn	Arg	Gly	Phe	Phe	Ser	Asp
	625					630					635					640
25	Arg	Asp	Gly	Ser	Lys	Phe	Tyr	Asp	Ile	Thr	Gln	Met	Ser	Glu	Glu	Arg
					645					650					655	
	Arg	Thr	Gly	Tyr	Ser	Trp	Gly	Gly	Ser	Tyr	Gly	Glu	Ser	Ile	Ile	Ser
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			675					680								

<210> 79

<211> 1746

<212> DNA

35 <213> Unknown

<220>

<223> Obtained from an environmental sample

40 <400> 79

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	atcctcgcac	gcatcacacc	acctcgtttt	ccgaaacgca	ccttctatct	caatcgattc	180
	ggcgccaagg	gcgatggagt	cacagactgc	accgcggctt	ttcatcgcg	gatcgatgaa	240
5	tgcaccaaag	ccggcggtgg	gaaagtcgtc	gtgccggcgg	gcacttatct	caccggcgcg	300
	attcatttga	agagcaacgt	caacctcgaa	gtctcggaa	gcgcgacgat	caagttcagt	360
	caggacccga	aacactacct	gcctgttgct	ttctcgcgtt	gggaagggtg	cgaagtcttc	420
	aactactcgc	ctttcattta	cgcgttcgaa	cagcgaaaaca	tcgcgatcac	cggcaaaggc	480
	acgctcgacg	gacagagtga	ttcggaacac	tgggtggccgt	ggaacggccg	tccgcagtac	540
	ggatggaatg	aagggatgaa	acagcagcgt	cccgatcgca	acgcgttggt	cacaatggcg	600
10	gagaaaggcg	tgccgggtgcg	cgagcgcac	tttggcgaag	gtcattatct	gaggccgcag	660
	ttcattcagc	cgtaccgctg	ccagaacgtg	ctgatccagg	gcgtgacgat	tcggaactcg	720
	ccgatgtggg	agattcatcc	ggtgttggtg	cgcaacgtga	ctattcacga	cgtgcacatc	780
	gatagtcatg	gaccaaacaa	cgacggctgc	aatcccgaat	cgtgcagcga	cgtgttgatt	840
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	gacgggcgcg	ggcttaaagc	gccgactgag	aacatcatcg	ttcaaggatg	tcgcatgaaa	960
15	gacggccacg	gtggaatcac	ggtcggcgag	gagatctcgg	gcggcggtcg	aaacctgttt	1020
	gccgagaatt	gccggctcga	cagtccaaac	ctcgatcacg	ccctgcgcgt	gaagaacaat	1080
	gccatgcgcg	gcggtattac	cgagaacttc	cacttccgta	acatcgaagt	cgggcagggtg	1140
	gcccattgccg	tgattacgat	cgacttcaac	tacgaagagg	gcgcgaaagg	gtcgttcacg	1200
	ccggtcggtc	gcgattacac	ggtcgatcgt	ttgcgcagca	cgaagagcaa	gcacgcactc	1260
	gacgtccagg	gtctgcccgg	cgcgccgggc	atcaaccctg	gattgacaaa	ctgcacattc	1320
20	aacgatgtgc	agcaaccgaa	cattctcaag	aacgtcgaac	aatcaaccct	tgagaacgtc	1380
	acgattaacg	gaaagacgat	cacacaaaca	ggatccatct	cagaaagagc	ggccacgaca	1440

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	aagaagtatc	tgaagtccat	ccaggacagc	atggaccact	tcgtcaacga	cgacggctcc	1620
	attcgcacct	acacgatcga	cgagtacaac	atcgatcacg	ttcttcccgg	acgaaaacctc	1680
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 <212> PRT
 <213> Unknown

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 <222> (81)...(458)
 <223> Catalytic domain

45 <400> 80

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	Ala	Val	Ala	Val	Ala	Pro	Arg	Leu	Ile	Ala	Phe	Gly	Ala	Glu	Ala	Ser
5				20					25					30		
	Pro	Trp	Glu	Thr	Met	Met	Pro	Ser	Ile	Leu	Ala	Arg	Ile	Thr	Pro	Pro
			35					40					45			
	Arg	Phe	Pro	Lys	Arg	Thr	Phe	Tyr	Leu	Asn	Arg	Phe	Gly	Ala	Lys	Gly
	50						55					60				
	Asp	Gly	Val	Thr	Asp	Cys	Thr	Ala	Ala	Phe	His	Arg	Ala	Ile	Asp	Glu
10	65					70					75					80
	Cys	Thr	Lys	Ala	Gly	Gly	Lys	Val	Val	Val	Pro	Ala	Gly	Thr	Tyr	
				85					90					95		
	Leu	Thr	Gly	Ala	Ile	His	Leu	Lys	Ser	Asn	Val	Asn	Leu	Glu	Val	Ser
				100					105					110		
	Glu	Gly	Ala	Thr	Ile	Lys	Phe	Ser	Gln	Asp	Pro	Lys	His	Tyr	Leu	Pro
			115					120					125			
15	Val	Val	Phe	Ser	Arg	Trp	Glu	Gly	Val	Glu	Val	Phe	Asn	Tyr	Ser	Pro
		130					135					140				
	Phe	Ile	Tyr	Ala	Phe	Glu	Gln	Arg	Asn	Ile	Ala	Ile	Thr	Gly	Lys	Gly
	145					150					155					160
	Thr	Leu	Asp	Gly	Gln	Ser	Asp	Ser	Glu	His	Trp	Trp	Pro	Trp	Asn	Gly
				165						170					175	
20	Arg	Pro	Gln	Tyr	Gly	Trp	Asn	Glu	Gly	Met	Lys	Gln	Gln	Arg	Pro	Asp
			180						185					190		
	Arg	Asn	Ala	Leu	Phe	Thr	Met	Ala	Glu	Lys	Gly	Val	Pro	Val	Arg	Glu
		195						200					205			
	Arg	Ile	Phe	Gly	Glu	Gly	His	Tyr	Leu	Arg	Pro	Gln	Phe	Ile	Gln	Pro
	210						215					220				
25	Tyr	Arg	Cys	Gln	Asn	Val	Leu	Ile	Gln	Gly	Val	Thr	Ile	Arg	Asn	Ser
	225					230					235					240
	Pro	Met	Trp	Glu	Ile	His	Pro	Val	Leu	Cys	Arg	Asn	Val	Thr	Ile	His
				245						250					255	
	Asp	Val	His	Ile	Asp	Ser	His	Gly	Pro	Asn	Asn	Asp	Gly	Cys	Asn	Pro
			260						265					270		
30	Glu	Ser	Cys	Ser	Asp	Val	Leu	Ile	Lys	Asp	Ser	Tyr	Phe	Asp	Thr	Gly
			275					280					285			
	Asp	Asp	Cys	Ile	Ala	Ile	Lys	Ser	Gly	Arg	Asn	Ala	Asp	Gly	Arg	Arg
		290					295					300				
	Leu	Lys	Ala	Pro	Thr	Glu	Asn	Ile	Ile	Val	Gln	Gly	Cys	Arg	Met	Lys
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	Asp	Gly	His	Gly	Gly	Ile	Thr	Val	Gly	Ser	Glu	Ile	Ser	Gly	Gly	Val
				325						330					335	
	Arg	Asn	Leu	Phe	Ala	Glu	Asn	Cys	Arg	Leu	Asp	Ser	Pro	Asn	Leu	Asp
			340						345					350		
40	His	Ala	Leu	Arg	Val	Lys	Asn	Asn	Ala	Met	Arg	Gly	Gly	Leu	Leu	Glu

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355 360 365
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 370 375 380
 5 Ile Thr Ile Asp Phe Asn Tyr Glu Glu Gly Ala Lys Gly Ser Phe Thr
 385 390 400
 Pro Val Val Arg Asp Tyr Thr Val Asp Arg Leu Arg Ser Thr Lys Ser
 405 410 415
 Lys His Ala Leu Asp Val Gln Gly Leu Pro Gly Ala Pro Val Ile Asn
 420 425 430
 10 Leu Arg Leu Thr Asn Cys Thr Phe Asn Asp Val Gln Gln Pro Asn Ile
 435 440 445
 Leu Lys Asn Val Glu Gln Ser Thr Phe Glu Asn Val Thr Ile Asn Gly
 450 455 460
 Lys Thr Ile Thr Gln Thr Gly Ser Ile Ser Glu Arg Ala Ala Thr Thr
 465 470 475 480
 15 Ala Met Thr Ala Leu Trp Arg Asp Ala Ser Arg Lys Glu Asn Gly Tyr
 485 490 495
 Pro Ala Lys Trp Thr Tyr Asp His Gly Leu Val Leu Lys Gly Ile Glu
 500 505 510
 Arg Val Trp Asn Asn Thr Gly Asp Lys Lys Tyr Leu Lys Phe Ile Gln
 515 520 525
 20 Asp Ser Met Asp His Phe Val Asn Asp Asp Gly Ser Ile Arg Thr Tyr
 530 535 540
 Thr Ile Asp Glu Tyr Asn Ile Asp His Val Leu Pro Gly Arg Asn Leu
 545 550 555 560
 Leu Phe Leu Tyr Lys Thr Thr Gly Gln Glu Lys Tyr Arg Lys Ala Ala
 565 570 575
 25 Ala Phe Leu Arg Glu Gln
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<210> 81

<211> 1065

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 81

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<213> Unknown

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<221> SIGNAL

<222> (1)...(30)

<221> DOMAIN

<222> (31)...(354)

<223> Catalytic domain

<400> 82

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<221> DOMAIN

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 gatgagtggg atgcgagtgt tgaggcactg cggatggccg ataacgtcgt tctctatcaa 240
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 40 gtcgacgaag ctcggcgcgcg gaaggcgacg agtgcagttg aaaaagggat cgagtgcatt 660
 ttgaaaacgc aggtgggtgg agatgggcgt cgcactgtgt ggagtgcgca acatgatgaa 720
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 <211> 383
 <212> PRT
 <213> Unknown

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 <220>
 <223> Obtained from an environmental sample

<221> DOMAIN

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<222> (1)...(383)

<223> Catalytic domain

<400> 86

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10     Phe Ser Leu Ala Ala Glu His Thr Ser Lys Lys Asn Asp Gln Glu Val
      20      25      30
      Gly Ala Ile Ala Trp Lys Asp Ala His Gly Lys Pro Asp Glu Trp Tyr
      35      40      45
15     Ala Ser Val Glu Ala Leu Arg Met Ala Asp Asn Val Val Leu Tyr Gln
      50      55      60      65      70      75      80
      Arg Asp Ser Gly Gly Trp Pro Lys Asn Ile Asp Met Ala Lys Ala Leu
      85      90      95
      Asn Asp Arg Glu Gln Ala Ala Ile Leu Arg Gln Lys Lys Lys Asn Asp
      100      105      110
20     Ser Thr Ile Asp Asn Gly Ala Thr His Thr Gln Leu Ser Phe Leu Ala
      115      120      125
      Arg Val Tyr Thr Ala Gln Arg Gln Glu Arg His Arg Glu Ser Phe Phe
      130      135      140
25     Lys Gly Leu Asp Tyr Leu Leu Asn Ala Gln Tyr Pro Asn Gly Gly Trp
      145      150      155      160
      Pro Gln Phe Tyr Pro Asn Pro Thr Gly Tyr His Lys His Ile Thr Tyr
      165      170      175
      Asn Asp Gly Ala Met Ile Gly Val Met Lys Val Leu Arg Asp Ile Ala
      180      185      190
30     Ala Ala Lys Pro Leu Tyr Ala Phe Val Asp Glu Ala Arg Ala Lys
      195      200      205
      Ala Thr Ser Ala Val Glu Lys Gly Ile Glu Cys Ile Leu Lys Thr Gln
      210      215      220
      Val Val Val Asp Gly Arg Arg Thr Val Trp Ser Ala Gln His Asp Glu
      225      230      235      240
      Val Thr Leu Ala Pro Ala Pro Ala Arg Thr Phe Glu Leu Thr Ser Leu
      245      250      255
35     Ser Gly Gly Glu Ser Val Asp Ile Val Arg Phe Leu Met Ser Ile Lys
      260      265      270
      Asp Pro Ser Pro Lys Val Val Asp Ala Val Glu Ser Ala Val Lys Trp
      275      280      285
      Phe Glu Gln Ser Glu Leu Lys Gly Val Lys Trp Val Lys Lys Ala Asp
      290      295      300
40     Ala Ser Lys Pro Gly Gly Phe Asp Cys Val Val Val Lys Asp Pro Glu
      305      310      315      320
      Ser Ser Val Trp Ala Arg Phe Tyr Glu Ile Gly Thr Asn Arg Pro Ile
      325      330      335
      Phe Ser Gly Arg Asp Gly Val Val Lys Tyr Asp Val Ala Gln Ile Glu
      340      345      350
45     His Glu Arg Arg Thr Asn Tyr Glu Trp Tyr Val Asp Glu Ala Ala Lys
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<210> 87

<211> 1698

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 87

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5	aacaccattg	ttgccaatgc	gaagagtggc	ggttaccggg	ttgtgattac	ctataccggg	240
	aatgaagaca	gcttgattaa	ccagatgata	aaagaccaca	ccgtggattc	ttcaggcaac	300
	tgcccgaacc	cacgctggag	tgaaacctac	cgcaaggtag	aaattaagga	gatgaccaa	360
	ggtgtcacca	tcacgtgtgc	caatggttct	tcggcaaaact	tcggtattgt	ggtgaacaag	420
	tccagcaatg	tgattatccg	caacatgaaa	atcgggtgcg	tgcccggtgc	cagcaacgac	480
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10	gacatcaaga	aagattcaca	caacatcacc	gtgtctttaca	acctgattcg	cgacagcaaa	660
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15	cacatgtata	acaacctgta	cgacggcatt	accggttccg	gtatcaacgt	acgtcaggcc	840
	ggttatgcgt	tgattgaaag	caactgggtc	caaaatgcgg	ttaacccggg	gacttgccgt	900
	tacgacagca	gcaactgcgg	tttctgggat	ctgcgcaata	acaacgtgaa	gtcgccagca	960
	gatttcgcga	cctataacat	cacctggacc	agcggcggca	ctattgatgc	aaccaactgg	1020
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20	tgctgtaagg	acaagttggc	cagcggttgc	gggtgtgggt	aaaacgggtg	agttctgaac	1140
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	aaatcatcca	gctcggtagc	aaccagcaag	tccagctccg	tcgctacgac	gtccagtaag	1260
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	atcgcgtaa	ccgccactgc	taccggcaat	agcattgtcc	tgagctgggt	gccgaacaac	1380
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	gcttcggcgg	caattggcga	tgcagctcgc	gccatttcgc	catgctgcag	aaaccgagga	1620
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 <211> 565
 <212> PRT
 <213> Unknown

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 <222> (1)...(32)

40 <221> DOMAIN
 <222> (33)...(375)
 <223> Catalytic domain

45 <400> 88

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55

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	Ala	Val	Ala	Ala	Ala	Val	Ala	Met	Phe	Ala	Gly	Ser	Ser	Val	Phe	Ala
				20					25					30		
5	Ala	Ala	Thr	Gly	Gly	Phe	Ser	Thr	Thr	Asp	Gly	Gly	Ala	Ala	Ser	Gly
			35					40					45			
	Ser	Gln	Ser	Phe	Thr	Ala	Ala	Asn	Leu	Asp	Gln	Leu	Asn	Thr	Ile	Val
		50					55					60				
	Ala	Asn	Ala	Lys	Ser	Gly	Gly	Tyr	Pro	Val	Val	Ile	Thr	Tyr	Thr	Gly
	65					70					75					80
10	Asn	Glu	Asp	Ser	Leu	Ile	Asn	Gln	Met	Ile	Lys	Asp	His	Thr	Val	Asp
					85					90					95	
	Ser	Ser	Gly	Asn	Cys	Pro	Asn	Pro	Arg	Trp	Ser	Glu	Thr	Tyr	Arg	Lys
				100					105					110		
	Val	Glu	Ile	Lys	Glu	Met	Thr	Lys	Gly	Val	Thr	Ile	Ile	Gly	Ala	Asn
			115					120					125			
15	Gly	Ser	Ser	Ala	Asn	Phe	Gly	Ile	Val	Val	Asn	Lys	Ser	Ser	Asn	Val
		130					135					140				
	Ile	Ile	Arg	Asn	Met	Lys	Ile	Gly	Ala	Leu	Ala	Gly	Ala	Ser	Asn	Asp
	145					150					155					160
	Ala	Asp	Met	Ile	Arg	Ile	Asp	Ser	Gly	Thr	Asn	Val	Trp	Val	Asp	His
					165					170					175	
20	Asn	Glu	Leu	Phe	Ala	Val	Asn	Asn	Glu	Cys	Lys	Gly	Ser	Pro	Asp	Gly
				180					185					190		
	Asp	Leu	Thr	Phe	Glu	Ser	Ala	Ile	Asp	Ile	Lys	Lys	Asp	Ser	His	Asn
			195					200					205			
	Ile	Thr	Val	Ser	Tyr	Asn	Leu	Ile	Arg	Asp	Ser	Lys	Lys	Val	Gly	Leu
		210					215					220				
25	Asp	Gly	Ser	Ser	Ser	Ser	Asp	Ile	Ala	Gly	Gly	Arg	Glu	Ile	Thr	Phe
	225					230					235					240
	His	His	Asn	Ile	Tyr	Lys	Asn	Val	Asn	Ala	Arg	Leu	Pro	Leu	Gln	Arg
					245					250					255	
	Gly	Gly	Trp	Thr	His	Met	Tyr	Asn	Asn	Leu	Tyr	Asp	Gly	Ile	Thr	Gly
				260					265					270		
30	Ser	Gly	Ile	Asn	Val	Arg	Gln	Ala	Gly	Tyr	Ala	Leu	Ile	Glu	Ser	Asn

35

40

45

50

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5																	
10																	
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25																	
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<210> 89
 <211> 1377
 <212> DNA
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<400> 89

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	atcctcgaac	gcatcaagcc	accgcgtttt	ccgaaacgca	cgtgctatct	caaccggttt	180
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	tgttcgaaaag	cgggcggttg	caaagtgatc	gttccgcagg	gaatgtatct	caccggcgca	300
	attcacttga	agagcaacgt	caatctcgag	atctccgaag	gcgcgacgat	caagttcagt	360
	caaaacccga	aagactatct	cccgggtggt	ttttcgcggt	gggaaggcgt	cgaagtattc	420
	aactactcac	ctttcatcta	cgcatttgaa	cagcagaaca	tcgcgatcac	gggcaagggc	480
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15	gacggccggc	ggcttaaagc	gccgacagag	aacgtgatcg	tgcaagactg	tcaaatagaa	960
	gatggacacg	gcgggatcac	tgtcggcagt	gagatctcag	gcgggtgtgag	aaatctgttt	1020
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	gcgatgcgtg	gagggctgct	cgagaatttg	cacttccgaa	acatcgaagt	tggtcagggtg	1140
	gcgcatgcag	tgatcacgat	cgattttaat	tacgaggaag	gcgcgaaagg	atcgttcacg	1200
	ccggtgggtc	gtgactacac	tgtcgaatgg	ttgcgcagca	cgcaagcaa	atacgcgctc	1260
20							

	gacgttcaag	gtctgtcggg	cgcgccgatc	gtaaactctgc	gtctgacgaa	ttgcacgttc	1320
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 <212> PRT
 <213> Unknown

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 <222> (1)...(31)

35 <221> DOMAIN
 <222> (32)...(459)
 <223> Catalytic domain

40 <400> 90

45

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55

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	Val	Val	Ala	Val	Ala	Pro	Arg	Phe	Leu	Ala	Phe	Ser	Ser	Val	Ala	Ser
				20					25					30		
5	Pro	Trp	Glu	Thr	Val	Met	Pro	Ser	Ile	Leu	Glu	Arg	Ile	Lys	Pro	Pro
			35					40					45			
	Arg	Phe	Pro	Lys	Arg	Thr	Cys	Tyr	Leu	Asn	Arg	Phe	Gly	Ala	Lys	Gly
		50					55					60				
	Asp	Gly	Gln	Thr	Asp	Cys	Thr	Ser	Ala	Phe	Arg	Arg	Ala	Ile	Asp	Gln
	65					70				75					80	
10	Cys	Ser	Lys	Ala	Gly	Gly	Gly	Lys	Val	Ile	Val	Pro	Gln	Gly	Met	Tyr
				85						90					95	
	Leu	Thr	Gly	Ala	Ile	His	Leu	Lys	Ser	Asn	Val	Asn	Leu	Glu	Ile	Ser
				100					105					110		
	Glu	Gly	Ala	Thr	Ile	Lys	Phe	Ser	Gln	Asn	Pro	Lys	Asp	Tyr	Leu	Pro
			115					120					125			
15	Val	Val	Phe	Ser	Arg	Trp	Glu	Gly	Val	Glu	Val	Phe	Asn	Tyr	Ser	Pro
		130					135					140				
	Phe	Ile	Tyr	Ala	Phe	Glu	Gln	Gln	Asn	Ile	Ala	Ile	Thr	Gly	Lys	Gly
	145					150				155						160
	Thr	Leu	Asp	Gly	Gln	Ser	Asp	Asn	Glu	His	Trp	Trp	Pro	Trp	Asn	Gly
				165						170					175	
20	Arg	Ala	Arg	Tyr	Gly	Trp	Lys	Glu	Gly	Met	Ser	His	Gln	Arg	Pro	Asp
				180					185					190		
	Arg	Asn	Ala	Leu	Phe	Ala	Met	Ala	Glu	Lys	Gly	Val	Ser	Val	Arg	Glu
			195					200					205			
	Arg	Val	Phe	Gly	Glu	Gly	His	Tyr	Leu	Arg	Pro	Gln	Phe	Ile	Gln	Pro
		210					215					220				
25	Tyr	Arg	Cys	Gln	Asn	Val	Leu	Ile	Asp	Gly	Val	Thr	Ile	Arg	Asn	Ser
	225					230				235						240
	Pro	Met	Trp	Glu	Ile	His	Pro	Val	Leu	Cys	Arg	Asn	Val	Ile	Val	Gln
				245						250					255	
	Asn	Val	His	Ile	Asn	Ser	His	Gly	Pro	Asn	Asn	Asp	Gly	Cys	Asn	Pro
			260						265					270		
30	Glu	Ser	Cys	Thr	Asp	Val	Leu	Ile	Lys	Asn	Cys	Tyr	Phe	Asp	Thr	Gly
			275					280					285			
	Asp	Asp	Cys	Ile	Ala	Val	Lys	Ser	Gly	Arg	Asn	Ala	Asp	Gly	Arg	Arg
		290					295					300				
	Leu	Lys	Ala	Pro	Thr	Glu	Asn	Val	Ile	Val	Gln	Asp	Cys	Gln	Met	Lys
						310					315					320
35	Asp	Gly	His	Gly	Gly	Ile	Thr	Val	Gly	Ser	Glu	Ile	Ser	Gly	Gly	Val
				325						330					335	
	Arg	Asn	Leu	Phe	Ala	Glu	Asn	Cys	Arg	Leu	Asp	Ser	Pro	Asn	Leu	Asp
				340					345					350		
	His	Ala	Leu	Arg	Val	Lys	Asn	Asn	Ala	Met	Arg	Gly	Gly	Leu	Leu	Glu
			355					360					365			
40	Asn	Leu	His	Phe	Arg	Asn	Ile	Glu	Val	Gly	Gln	Val	Ala	His	Ala	Val
		370					375					380				
	Ile	Thr	Ile	Asp	Phe	Asn	Tyr	Glu	Glu	Gly	Ala	Lys	Gly	Ser	Phe	Thr
45																
	385					390				395						400
	Pro	Val	Val	Arg	Asp	Tyr	Thr	Val	Asp	Gly	Leu	Arg	Ser	Thr	Arg	Ser
					405					410					415	
	Lys	Tyr	Ala	Leu	Asp	Val	Gln	Gly	Leu	Ser	Gly	Ala	Pro	Ile	Val	Asn
				420					425					430		
	Leu	Arg	Leu	Thr	Asn	Cys	Thr	Phe	Asp	Asn	Val	Ala	Glu	Gly	Asn	Val
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		450					455									

<210> 91

<211> 1125

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 91

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20

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gcggaacaac tcctcctcta ccagcgcaac accggcggtt ggccgaagga catagatatg 240
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gccgcccagt acccgaacgg cggctggccc cagtattatc ctaacttgag gggctactac 480
gccaacatca cttataacga caatgccatg gtgaacgtgc tcaccctcct ccagagcatc 540
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gccgtggcga aagggatcga ctgcattctc aagaccaga tccgtgtcaa tggaaaactt 660
accgcctggt gcgccagca tgacgccaag acgctggcgc ccgcgccggc ccgttcgtat 720
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gagaatccga gcccgaaagt catcgaggcg gtagaggccg ccgtgaaatg gttcgaggag 840
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ggcaccaacc gtcccttctt ctgcggccgc gatggtatca aaaaatacag cctggcggag 1020
atcgaacacg aacgcgggtt cggttactcc tggtacacca atgccccggc ctacctcatc 1080
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<210> 92

25 <211> 374

<212> PRT

<213> Unknown

<220>

30 <223> Obtained from an environmental sample

<221> DOMAIN

<222> (1)...(374)

35 <400> 92

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45
50

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20 25 30
Val Gln Ala Pro Val Arg Val Thr Trp Ser Ala Ser Leu Val Gln Arg
35 40 45
Pro Glu Trp Tyr Gly Ser Asp Glu Ala Ile Arg Ile Ala Asp Asn Val
50 55 60
Leu Leu Tyr Gln Arg Asn Thr Gly Gly Trp Pro Lys Asp Ile Asp Met
65 70 75 80
Ala Glu Pro Ile Pro Glu His Arg Lys Ser Phe Phe Leu Thr Glu Lys
85 90 95
Glu Arg Thr Asp Asp Ser Thr Ile Asp Asn Gly Ala Thr Val Thr Gln
100 105 110
Leu Lys Tyr Leu Ala Arg Val Tyr Lys Ala Thr Arg Leu Glu Arg Phe
115 120 125
Lys Glu Gly Phe Leu Lys Gly Leu Asp Tyr Leu Leu Ala Ala Gln Tyr

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55

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	130		135		140	
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5	145 Ala Asn Ile Thr Tyr	150 Asn Asp Asn Ala Met	155 Val Asn Val Leu Thr Leu			
	165 Leu Gln Ser Ile Ala	170 Lys Lys Ala Pro Glu	175 Tyr Asp Phe Val Asp Pro			
	180 Ala Arg Arg Glu Lys	185 Ala Ala Arg Val Ala	190 Lys Gly Ile Asp Cys			
10	195 Ile Leu Lys Thr Gln	200 Ile Arg Val Asn Gly	205 Leu Thr Ala Trp Cys			
	210 Ala Gln His Asp Ala	215 Lys Thr Leu Ala Pro	220 Ala Pro Ala Arg Ser Tyr			
	225 Glu Leu Glu Ser Ile	230 Ser Gly Phe Glu Ser	235 Val Gly Ile Val Arg Phe			
15	245 Leu Met Ser Leu Glu	250 Asn Pro Ser Pro Lys	255 Val Ile Glu Ala Val Glu			
	260 Ala Ala Val Lys Trp	265 Phe Glu Glu Val Lys	270 Leu Thr Gly Ile Lys Val			
	275 Val Glu Lys Pro Asp	280 Pro Ser Ser Pro Gly	285 Gly Tyr Asp Arg Val Val			
20	290 Val Glu Asp Pro Asn	295 Ala Pro Pro Ile Trp	300 Ala Arg Phe Tyr Glu Ile			
	305 Gly Thr Asn Arg Pro	310 Phe Phe Cys Gly Arg	315 Asp Gly Ile Lys Lys Tyr			
	325 Ser Leu Ala Glu Ile	330 Glu His Glu Arg Val	335 Gly Tyr Ser Trp Tyr			
25	340 Thr Asn Ala Pro Ala	345 Tyr Leu Ile Glu Lys	350 Glu Tyr Pro Leu Trp Arg			
	355 Ala Lys His Pro Thr	360 Lys Lys	365			
	370					

30 <210> 93
 <211> 1062
 <212> DNA
 <213> Unknown

35 <220>
 <223> Obtained from an environmental sample

<400> 93

40	gtggatccaa	agaattggaa	cccgaaaaaa	gccgacgatt	catggctcga	aaagacgaaa	60
	cccgattacc	ggctgggtctc	ctggcgcgac	gttttagatc	aaactcagct	ctggtacgcg	120
	gtcgacgaag	cgacgcgcac	cgccaaccag	gttttgctct	ttcagcgcca	taacggcggc	180
	tgggaaaaaa	acgtcgacat	ggcggcgatg	ctcactcaag	ccgaacgaga	aaaactcgtc	240
	aaagaaaaat	ctcacaccga	tacgaccatc	gacaacggcg	cgacgaccac	gcagctgcgt	300
45	tatctggcaa	aagtcatcac	ggcgaaaaac	atcgaagctc	ataaacagtc	gtttctcaag	360
	ggattggatt	ttctgctcgc	gatgcagtat	gaaaacggag	gatttccgca	atattatcct	420
	ttgaaaaacg	attattcgcg	cgagattact	ttcaacgacg	acgcgatgat	caatgttctt	480
	aaattgctgc	gcgacgtggc	aaaaaagaag	gaagattatt	tattcgtcga	cgaagaccgg	540
	cgcgccagag	cggaaaggcg	ggtcgaaaaa	ggcgtccgcc	tgatcttgaa	aacacagggtc	600
	gccatcgacg	gcaaaaaaac	gatctgggcg	gcgcagtagc	acgaaaacac	tttgaaaccg	660
50	gcaaatgcga	gaaagtgtga	gcccgcctcg	ctcgcttcgc	gcgaatcggt	cagcgtggtc	720
	agatttttga	tgctcgacgc	caaaccgcac	gaggaaaaaa	tcggagcgat	cgaatcggcg	780
	atcgaatggt	ttcaaaaaaa	caaactgagc	ggcattcgct	gggaatcgaa	aagcggagaa	840
	aacctggctg	tcaaagacaa	agcggcgccg	ccgatctggg	gaaggtttta	tcaattcgaa	900
	accatgcgcc	ccatttttat	cgggcgcgac	gcggtgattc	gctacgatgt	catgcaaadc	960
	gaagccgaac	gccgcaacgg	ctacggctgg	tacacgaacg	agccgaacga	gcttttggac	1020
55	aaagattatc	cgaaatggaa	agagaaaaatt	aagaaaaatt	ag		1062

<210> 94
 <211> 353

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<212> PRT

<213> Unknown

<220>

5 <223> Obtained from an environmental sample

<221> DOMAIN

<222> (1)...(353)

10 <400> 94

	Met	Asp	Pro	Lys	Asn	Trp	Asn	Pro	Lys	Lys	Ala	Asp	Asp	Ser	Trp	Leu
	1				5					10					15	
15	Glu	Lys	Thr	Lys	Pro	Asp	Tyr	Arg	Leu	Val	Ser	Trp	Arg	Asp	Val	Leu
				20					25					30		
	Asp	Gln	Thr	Gln	Leu	Trp	Tyr	Ala	Val	Asp	Glu	Ala	Thr	Arg	Ile	Ala
			35					40					45			
	Asn	Gln	Val	Leu	Leu	Phe	Gln	Arg	Asp	Asn	Gly	Gly	Trp	Glu	Lys	Asn
		50					55					60				
20	Val	Asp	Met	Ala	Ala	Met	Leu	Thr	Gln	Ala	Glu	Arg	Glu	Lys	Leu	Val
	65					70					75					80
	Lys	Glu	Lys	Ser	His	Thr	Asp	Thr	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Thr
					85					90					95	
	Thr	Gln	Leu	Arg	Tyr	Leu	Ala	Lys	Val	Ile	Thr	Ala	Lys	Asn	Ile	Glu
				100					105					110		
25	Ala	His	Lys	Gln	Ser	Phe	Leu	Lys	Gly	Leu	Asp	Phe	Leu	Leu	Ala	Met
			115					120					125			
	Gln	Tyr	Glu	Asn	Gly	Gly	Phe	Pro	Gln	Tyr	Tyr	Pro	Leu	Lys	Asn	Asp
		130					135					140				
	Tyr	Ser	Arg	Glu	Ile	Thr	Phe	Asn	Asp	Asp	Ala	Met	Ile	Asn	Val	Leu
	145					150					155					160
30	Lys	Leu	Leu	Arg	Asp	Val	Ala	Lys	Lys	Lys	Glu	Asp	Tyr	Leu	Phe	Val
					165					170					175	
	Asp	Glu	Asp	Arg	Arg	Ala	Arg	Ala	Glu	Gly	Ala	Val	Glu	Lys	Gly	Val
				180					185					190		
	Arg	Leu	Ile	Leu	Lys	Thr	Gln	Val	Ala	Ile	Asp	Gly	Lys	Lys	Thr	Ile
			195					200					205			
35	Trp	Ala	Ala	Gln	Tyr	Asp	Glu	Asn	Thr	Leu	Lys	Pro	Ala	Asn	Ala	Arg
		210					215					220				
	Lys	Phe	Glu	Pro	Ala	Ser	Leu	Ala	Ser	Arg	Glu	Ser	Val	Ser	Val	Val
					230						235					240
	Arg	Phe	Leu	Met	Leu	Asp	Ala	Lys	Pro	Asp	Glu	Glu	Lys	Ile	Gly	Ala
				245						250					255	
40	Ile	Glu	Ser	Ala	Ile	Glu	Trp	Phe	Gln	Lys	Asn	Lys	Leu	Ser	Gly	Ile
				260					265					270		
	Arg	Trp	Glu	Ser	Lys	Ser	Gly	Glu	Asn	Leu	Val	Val	Lys	Asp	Lys	Ala
			275					280					285			
	Ala	Pro	Pro	Ile	Trp	Gly	Arg	Phe	Tyr	Gln	Phe	Glu	Thr	Met	Arg	Pro
		290					295					300				
45	Ile	Phe	Ile	Gly	Arg	Asp	Ala	Val	Ile	Arg	Tyr	Asp	Val	Met	Gln	Ile
					310						315					320
	Glu	Ala	Glu	Arg	Arg	Asn	Gly	Tyr	Gly	Trp	Tyr	Thr	Asn	Glu	Pro	Asn
					325					330					335	
	Glu	Leu	Leu	Asp	Lys	Asp	Tyr	Pro	Lys	Trp	Lys	Glu	Lys	Ile	Lys	Lys
				340					345					350		
50	Asn															

<210> 95

<211> 1074

<212> DNA

55 <213> Unknown

<220>

<223> Obtained from an environmental sample

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<400> 95

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	gcctgcgcgg	gcgccgcggc	acccgcgact	gcgaccgatc	cggtcgccga	gaacatgctg	120
	cttctgcaga	ccgcctccgg	cggttggtcc	aagcactacc	gcgagaagaa	ggtcgactac	180
	gcgcgcgact	acgacgccgc	cgagcgcgcc	gcgctgcgcg	cgcccgaccg	gcatgacgat	240
	gcgacgatcg	acaacaaggc	cacgaccacc	gagatcgctt	acctgggtga	ggcacatgcc	300
	aggacgggca	atccggccta	cctcgacggc	gcgcgcgcgc	gcgtcgagta	cctgctgcgc	360
	gcccagttac	cgaacggcgg	ctggccgcag	ttctaccccg	accattcgct	ctaccggcac	420
10	cagatcacgc	tcaacgacga	tgcgatggtg	cacgccatca	ccgtgctgca	ggacatcgcc	480

15	gcggggccgca	acggcatgca	ggtgctggcg	ccggagttcg	gcgtccgcgc	cgccgcggcc	540
	gcgcagcgcg	gcatcgga	cctgctcgag	ttgcaggtgc	ggatcgccgg	ggtgccgacg	600
	atatgggccc	cgagtacga	cgagaccagc	ctgcaaccgg	ccaaggcccc	cgctacgaa	660
	ctgccttcgc	tggccgtggc	cgaatcggtc	ggcgtggtgc	gcctgctgat	gcgccagccg	720
	gcgcctgatg	cgcgacgggt	cgccgcgac	gaggcgccgg	ccgactggct	ggaggcgac	780
	cgctgcccgc	acctcgccct	ggaacgcac	gaagcccccg	ccgaggaaac	cggcaaggac	840
	gtccgcgtcg	tggccagacc	ggcgcgctcg	ttgtgggcgc	gcttctacga	cctcgagcgg	900
	caggtgccgc	tggtcgtcga	tcgcaacagc	cgtccggtgc	ccttcgccga	gcttcccaac	960
20	gagcgtcgta	ccggctatgg	ctgggtatgg	acctggccgg	aaaagctgct	ggcacaggaa	1020
	ctcccgcgct	ggcgcaagggt	ccatgcggcc	agcgcgggcg	ctccggcccc	ttga	1074

<210> 96

<211> 357

25 <212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

30

<221> SIGNAL

<222> (1)...(31)

<221> DOMAIN

35 <222> (32)...(357)

<223> Catalytic domain

<400> 96

40

45

50

55

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5 Met Thr Leu Pro Val Val Ser Leu Arg Val Leu Leu Ala Leu Leu Ala
 1 5 10
 Thr Ser Pro Val Ala Cys Ala Gly Ala Ala Ala Pro Ala Thr Ala Thr
 20 25 30
 Asp Pro Val Ala Glu Asn Met Leu Leu Leu Gln Thr Ala Ser Gly Gly
 35 40 45
 Trp Ser Lys His Tyr Arg Glu Lys Lys Val Asp Tyr Ala Arg Asp Tyr
 50 55 60
 Asp Ala Ala Glu Arg Ala Ala Leu Arg Ala Pro Asp Arg His Asp Asp
 65 70 75 80
 10 Ala Thr Ile Asp Asn Lys Ala Thr Thr Thr Glu Ile Ala Tyr Leu Val
 85 90 95
 Gln Ala His Ala Arg Thr Gly Asn Pro Ala Tyr Leu Asp Gly Ala Arg
 100 105 110
 Arg Gly Val Glu Tyr Leu Leu Arg Ala Gln Tyr Pro Asn Gly Gly Trp
 115 120 125
 15 Pro Gln Phe Tyr Pro Asp His Ser Ser Tyr Arg His Gln Ile Thr Leu
 130 135 140
 Asn Asp Asp Ala Met Val His Ala Ile Thr Val Leu Gln Asp Ile Ala
 145 150 155 160
 20 Ala Gly Arg Asn Gly Met Gln Val Leu Ala Pro Glu Phe Gly Val Arg
 165 170 175
 Ala Ala Ala Ala Ala Gln Arg Gly Ile Gly Asn Leu Leu Glu Leu Gln
 180 185 190
 Val Arg Ile Ala Gly Val Pro Thr Ile Trp Ala Ala Gln Tyr Asp Glu
 195 200 205
 Thr Ser Leu Gln Pro Ala Lys Ala Arg Ala Tyr Glu Leu Pro Ser Leu
 210 215 220
 25 Ala Val Ala Glu Ser Val Gly Val Val Arg Leu Leu Met Arg Gln Pro
 225 230 235 240
 Ala Pro Asp Ala Arg Thr Val Ala Ala Ile Glu Ala Ala Ala Asp Trp
 245 250 255
 Leu Glu Ala His Arg Leu Pro Asp Leu Ala Leu Glu Arg Ile Glu Ala
 260 265 270
 30 Pro Ala Glu Thr Gly Lys Asp Val Arg Val Val Ala Arg Pro Gly
 275 280 285
 Ala Ser Leu Trp Ala Arg Phe Tyr Asp Leu Glu Arg Gln Val Pro Leu
 290 295 300
 35 Phe Val Asp Arg Asn Ser Arg Pro Val Pro Phe Ala Glu Leu Pro Asn
 305 310 315 320
 Glu Arg Arg Thr Gly Tyr Gly Trp Tyr Gly Thr Trp Pro Glu Lys Leu

40 Leu Ala Gln Glu Leu Pro Arg Trp Arg Lys Val His Ala Ala Ser Ala
 325 330 335
 Gly Ala Pro Ala Arg
 340 345 350
 355

<210> 97
 <211> 2097
 <212> DNA
 <213> Unknown

<220>
 <223> Obtained from an environmental sample

<400> 97

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	gtgcccgcgc	tggcgccctt	ctttcgcgac	gagccgctgg	cgggaggagt	cgccgcgctc	120
	cagcgcagcg	tcgatgcat	cgtcgccg	gacggcaccg	gacagtttgc	gacggtgcag	180
	gaggcgatca	acgccgcgcc	gcagaacacc	agcacgacca	gccgctggat	catcctcgtc	240
5	aaaccaggca	cgtatcgcg	ggtcgtctac	gtgcagcg	agaagcgctt	cgtcacgctg	300
	atcggcgaag	acccggcacg	gacgacgac	acgtaccacc	tcaaagcg	tgacgtgggg	360
	ctcgacggca	agcccac	cacgtttcgc	acgccgacga	tggtggtgga	tgccgacgat	420
	ttcacgatcg	agaacctcac	catcgagaac	ggggcagggc	cggtcgggtca	agcgctggcc	480
	ttgcgagtgg	acggcgatcg	cgtgacggtg	aggaacagcc	gcctgctggg	ctggcaggac	540
	acgatctttc	tcaaccgtgg	gcgccactac	ttcgaggact	cgttcatcgg	cgggcacgtg	600
10	gattttcattt	tcggcggcgc	gacggcggtg	ttcgagcgat	gccatcttcg	cgctggcg	660
	gacggctacc	tcacggccgc	gtccacgccc	gcggagcaac	gattcggctt	cgtgttcctg	720
	aacagcatcg	tcagtggaga	agctggcgcc	cgcacgtacc	tcggtcgacc	gtggcggg	780
	ttcgcgacg	tggccttcat	caagacgacg	atggg	tggtgcgcc	ggtgggctgg	840
	aacaactggg	accggccgga	gcgtgagaag	accgtgcgtt	ttctcgaagc	aggcaccagc	900
	ggcgcgggcg	gcagcgctgc	tgcgcgctgc	gcctggggcg	gcgtcgccac	gccagccgag	960
15	ctcgctgatc	tgacgaccga	ggtggtgctt	ggcggcaccg	acggctggga	cccgcgtcgc	1020
	gtcgccccgt	acccgctggc	cgttcgcgcc	aacgcggcg	cgctgccgcg	gccgcccggg	1080
	cccga	ctggcccgc	gagcccgc	gccttgacgt	gggaccaggt	cgcgcgccag	1140
	ccagcgctcg	ggctggccac	acccgaagcg	ctgcggattg	ccgagaacgt	gcgcctctat	1200
	caacggcaca	ctggcggtcg	gccccaaaac	ctcgacatgg	cgagccgtt	gacggacgcc	1260
	gatcgcgcg	gtctcacggc	cgatcgcg	ctcgacgact	cgaccatcga	caatggcgcc	1320
20	acgacgcggc	agatcgagtt	tctcgcccg	atcgccgc	ccaaccgcga	cgagcgcg	1380
	caggcgctga	tgctgggtgg	gatcgactac	ctgctcgcg	cccagtatcc	aaacggcg	1440
	tggccgcagt	atttcccgt	ccgcaacgac	tactcgcgcc	acatcacgtt	caacgacgac	1500
	gcgatgatcg	cggccgcgac	gacctgcag	tcggtcgcgc	tgccccgtcc	gccgttcg	1560
	ggcgctcgacg	cgactcgccg	ccggcgggcg	gcggaggccg	tcgcgcgcgc	ccatcgcg	1620
	attctggcct	cgcagattcg	cgtcaacggc	cagctcactg	gctggtgcca	gcagcacgat	1680
25	gcacgcacgc	tggagccagc	gcgcgggcgc	acctacgagc	atccatcgat	cagtggccgc	1740
	gaaaccgtga	cgatcgtcaa	tttcctgcgg	tcgatcgaac	cgcgcgaccg	ccagacccaa	1800
	gccgcatcg	atgccgcgat	ggagtggctc	aaggccgtgc	agatccgcgg	ctggcgacg	1860
	gagcgggcg	ccgatccctc	aggaccgggc	ggttacgacg	tggtgatggt	ggaggacccc	1920
	aacgcggcg	cgtctgggc	ccgtttctac	gagattggca	ccaatcgctc	gatctactcg	1980
	ggccgggacg	gcgtcatcaa	gtaccggctc	gccgagatcg	aaattgaacg	gcggaccggc	2040
30	tacagctggg	tcggaccgta	cgcgcaggcg	ctgctcgatg	aagagcgag	gaagtaa	2097

<210> 98

<211> 698

<212> PRT

35 <213> Unknown

<220>

<223> Obtained from an environmental sample

40 <221> DOMAIN

<222> (45)...(333)

<223> Pectin methyl esterase domain

<221> DOMAIN

45 <222> (336)...(698)

<223> Catalytic domain

<400> 98

50

55

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	1				5					10					15	
	Leu	Arg	Arg	Leu	Val	Pro	Ala	Leu	Ala	Pro	Phe	Phe	Arg	Asp	Glu	Pro
5				20					25					30		
	Leu	Ala	Gly	Gly	Val	Ala	Ala	Leu	Gln	Arg	Ser	Val	Asp	Ala	Ile	Val
			35					40					45			
	Ala	Ala	Asp	Gly	Thr	Gly	Gln	Phe	Ala	Thr	Val	Gln	Glu	Ala	Ile	Asn
		50				55					60					
	Ala	Ala	Pro	Gln	Asn	Thr	Ser	Thr	Thr	Ser	Arg	Trp	Ile	Ile	Leu	Val
10	65				70						75				80	
	Lys	Pro	Gly	Thr	Tyr	Arg	Glu	Val	Val	Tyr	Val	Gln	Arg	Glu	Lys	Arg
				85						90					95	
	Phe	Val	Thr	Leu	Ile	Gly	Glu	Asp	Pro	Ala	Arg	Thr	Thr	Ile	Thr	Tyr
				100					105					110		
	His	Leu	Lys	Ala	Ser	Asp	Val	Gly	Leu	Asp	Gly	Lys	Pro	Ile	Gly	Thr
15			115					120					125			
	Phe	Arg	Thr	Pro	Thr	Met	Val	Val	Asp	Ala	Asp	Asp	Phe	Thr	Ile	Glu
		130					135				140					
	Asn	Leu	Thr	Ile	Glu	Asn	Gly	Ala	Gly	Pro	Val	Gly	Gln	Ala	Leu	Ala
	145					150					155				160	
	Leu	Arg	Val	Asp	Gly	Asp	Arg	Val	Thr	Val	Arg	Asn	Ser	Arg	Leu	Leu
				165						170					175	
20	Gly	Trp	Gln	Asp	Thr	Ile	Phe	Leu	Asn	Arg	Gly	Arg	His	Tyr	Phe	Glu
			180						185					190		
	Asp	Ser	Phe	Ile	Gly	Gly	His	Val	Asp	Phe	Ile	Phe	Gly	Gly	Ala	Thr
			195					200					205			
	Ala	Val	Phe	Glu	Arg	Cys	His	Leu	Arg	Ala	Trp	Arg	Asp	Gly	Tyr	Leu
		210					215					220				
25	Thr	Ala	Ala	Ser	Thr	Pro	Ala	Glu	Gln	Arg	Phe	Gly	Phe	Val	Phe	Leu
	225					230					235				240	
	Asn	Ser	Ile	Val	Ser	Gly	Glu	Ala	Gly	Ala	Arg	Thr	Tyr	Leu	Gly	Arg
				245						250					255	
	Pro	Trp	Arg	Ala	Phe	Ala	His	Val	Ala	Phe	Ile	Lys	Thr	Thr	Met	Gly
				260					265					270		
30	Glu	Val	Val	Arg	Pro	Val	Gly	Trp	Asn	Asn	Trp	Asp	Arg	Pro	Glu	Arg
			275					280					285			
	Glu	Lys	Thr	Val	Arg	Phe	Leu	Glu	Ala	Gly	Thr	Ser	Gly	Ala	Gly	Gly
		290					295					300				
	Ser	Val	Ala	Ala	Arg	Val	Ala	Trp	Ala	Arg	Val	Ala	Thr	Pro	Ala	Glu
	305					310					315				320	
35	Leu	Ala	Asp	Leu	Thr	Thr	Glu	Val	Val	Leu	Gly	Gly	Thr	Asp	Gly	Trp
				325						330					335	
	Asp	Pro	Arg	Arg	Val	Ala	Pro	Tyr	Pro	Ser	Ala	Val	Arg	Ala	Asn	Ala
				340					345					350		
	Ala	Pro	Leu	Pro	Arg	Pro	Pro	Gly	Pro	Asp	Val	Ala	Gly	Pro	Gln	Ser
			355					360					365			
40	Pro	Pro	Ala	Leu	Thr	Trp	Asp	Gln	Val	Ala	Arg	Gln	Pro	Ala	Ser	Trp
		370					375					380				
	Leu	Ala	Thr	Pro	Glu	Ala	Leu	Arg	Ile	Ala	Glu	Asn	Val	Arg	Leu	Tyr
	385					390					395				400	
	Gln	Arg	His	Thr	Gly	Gly	Trp	Pro	Lys	Asn	Leu	Asp	Met	Ala	Gln	Pro
				405						410				415		
45	Leu	Thr	Asp	Ala	Asp	Arg	Ala	Arg	Leu	Thr	Ala	Asp	Arg	Ala	Leu	Asp
				420					425					430		
	Asp	Ser	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Thr	Arg	Gln	Ile	Glu	Phe	Leu
			435					440					445			
	Ala	Arg	Ile	Ala	Ala	Ala	Asn	Arg	Asp	Glu	Arg	Ala	Gln	Ala	Ser	Met
		450					455					460				
50	Leu	Ala	Gly	Ile	Asp	Tyr	Leu	Leu	Ala	Ala	Gln	Tyr	Pro	Asn	Gly	Gly
	465					470					475				480	
	Trp	Pro	Gln	Tyr	Phe	Pro	Leu	Arg	Asn	Asp	Tyr	Ser	Arg	His	Ile	Thr
				485						490				495		
	Phe	Asn	Asp	Asp	Ala	Met	Ile	Ala	Ala	Ala	Thr	Ile	Leu	Gln	Ser	Val
				500					505					510		
55	Ala	Leu	Ala	Arg	Pro	Pro	Phe	Ala	Gly	Val	Asp	Ala	Thr	Arg	Arg	Arg
		515						520					525			
	Arg	Ala	Ala	Glu	Ala	Val	Ala	Arg	Ala	His	Arg	Val	Ile	Leu	Ala	Ser
		530					535					540				

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Gln Ile Arg Val Asn Gly Gln Leu Thr Gly Trp Cys Gln Gln His Asp
 545 550 555 560
 Ala Arg Thr Leu Glu Pro Ala Arg Gly Arg Thr Tyr Glu His Pro Ser
 565 570 575
 5 Ile Ser Gly Arg Glu Thr Val Thr Ile Val Asn Phe Leu Arg Ser Ile
 580 585 590
 Glu Pro Arg Asp Arg Gln Thr Gln Ala Ala Ile Asp Ala Met Glu
 595 600 605
 Trp Leu Lys Ala Val Gln Ile Arg Gly Trp Arg Thr Glu Arg Arg Pro
 610 615 620
 10 Asp Pro Ser Gly Pro Gly Gly Tyr Asp Val Val Met Val Glu Asp Pro
 625 630 635 640
 Asn Ala Ala Pro Leu Trp Ala Arg Phe Tyr Glu Ile Gly Thr Asn Arg
 645 650 655
 Pro Ile Tyr Ser Gly Arg Asp Gly Val Ile Lys Tyr Arg Leu Ala Glu
 660 665 670
 15 Ile Glu Ile Glu Arg Arg Thr Gly Tyr Ser Trp Val Gly Pro Tyr Ala
 675 680 685
 Gln Ala Leu Leu Asp Glu Glu Arg Arg Lys
 690 695

20 <210> 99
 <211> 1782
 <212> DNA
 <213> Unknown

25 <220>
 <223> Obtained from an environmental sample

<400> 99

30 atgttcacta ctaacagctc tattttgcgcc cggaaatccg cgcgtttttc actgactgcc 60
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 ttttcgacca cggatggcgg caatgtgtca ggggtcaaat cctttaccgc ctcaagccac 180
 acccaaattcc agcaaatcct tgaggatgcc aaagatggca attatccggt ggtgatcacc 240
 tacaccggca atgaggattc actgattaac caagtcgtcc gggatcacac cgtcgattct 300
 35 tcaggcaact gccctaaagc gcgttggaat gatgcctacc gcaaagtcga aatcaaagaa 360
 atgaccaagg gtgtcaccat tcagggtgcc aatggttcgt cggcgaattt cggaatcgtg 420
 gtgaataaat ccagcaacgt gattatttcgc aacatgaaga ttggtgcaact gggcggcgct 480
 aataacgatg cggatatgat ccgtgtggac agcgggtgtga acgtctggat cgatcacaac 540
 gaattattcg ccgtgaacaa cgagtgttaag gggtcacccg atggcgatct gacctttgaa 600
 agcgcgattg atatcaaaaa agcctcgcaa gatataccg tgcctacaa cgtgattcgc 660
 40 gacagtaaaa aagtcggttt ggatggctcc agcagcagcg atatcgccgg cggccgcaaa 720
 attactttcc accacaatat ctaccgcaac gtaggtgctc gcttaccttt gcagcgccgc 780
 ggttggaagc acatgtacaa caacctgtac gacggcatta ccagctcggg catcaacgtg 840
 cgccaaaacg gttatgcgtt aattgaaagc aactggttcc aaaacgcggg taaccgggtc 900
 acctgccgtt ttgacagcag caactgcggc aagtgggata tgcgcaacaa taacatccgc 960
 aaccggggtg attttgcgac ttacaacatc acctggacca gtggcggcac catcgacgcc 1020
 45 accaactgga ccaccactgc gcccttcctc atcagcattc cctacagcta ttcaccgggt 1080
 actccgcaat gtgtgaaaga tcgtctggcg agttacgcgg gtgtgggttaa aaacggcgcg 1140
 cagctgactg cctcggcctg cgggtgggtgc gcatcgctca cacctgcatc gtccacacct 1200
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 agtgccggta atgcatcggg tgcacttaac tggaccgtga atgccaacat taatgcgctg 1320
 gaaatttatc aggatacggg ttctgatccc gccggacgtg tgcgattgc gtcgctgcca 1380
 50 accagcgcca ccaactacac cgcaacaggt ctgagcaacg gcaactaccta ttacttctgg 1440
 gtgaaatatc gcaccaccaa taatgtgtgg agcaactcca atgtgttcag cgccaagcca 1500
 agttcaggta caaccccgtc atcatccagc agcgcggctt catcaacgcc aagtgggtgca 1560
 ccggtgttaa gtgttacagg tgattaccca agcggcttct ccaagtgtgc tgatctgggt 1620
 ggcacctgct cagtcgcctc gggcgatggg ttgggtgcct ttggtcgcaa aggcaagtgg 1680
 55 gtcacaaaaa aagtgtcagt cggtagctct attgcctgta ccgttgccgc gtttgatctt 1740
 gatccacaag gcaatcccaa taagtgttct tataaaaagt aa 1782

<210> 100

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<211> 593
<212> PRT
<213> Unknown

5 <220>
 <223> Obtained from an environmental sample

 <221> SIGNAL
10 <222> (1)...(35)

 <221> DOMAIN
 <222> (36)...(593)
 <223> Catalytic domain

15 <400> 100

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	Met	Phe	Thr	Thr	Asn	Ser	Ser	Ile	Cys	Ala	Arg	Lys	Ser	Ala	Arg	Phe
	1				5					10					15	
	Ser	Leu	Thr	Ala	Met	Ala	Ala	Ala	Val	Ala	Met	Ile	Ala	Gly	Thr	Ser
5				20					25					30		
	Ala	Phe	Ala	Ala	Ser	Thr	Gly	Gly	Phe	Ser	Thr	Thr	Asp	Gly	Gly	Asn
			35					40					45			
	Val	Ser	Gly	Ser	Lys	Ser	Phe	Thr	Ala	Ser	Ser	His	Thr	Gln	Ile	Gln
		50					55					60				
	Gln	Ile	Leu	Glu	Asp	Ala	Lys	Asp	Gly	Asn	Tyr	Pro	Val	Val	Ile	Thr
10	65				70					75					80	
	Tyr	Thr	Gly	Asn	Glu	Asp	Ser	Leu	Ile	Asn	Gln	Val	Val	Arg	Asp	His
				85						90					95	
	Thr	Val	Asp	Ser	Ser	Gly	Asn	Cys	Pro	Lys	Ala	Arg	Trp	Asn	Asp	Ala
				100					105					110		
	Tyr	Arg	Lys	Val	Glu	Ile	Lys	Glu	Met	Thr	Lys	Gly	Val	Thr	Ile	Gln
			115					120					125			
15	Gly	Ala	Asn	Gly	Ser	Ser	Ala	Asn	Phe	Gly	Ile	Val	Val	Asn	Lys	Ser
							135					140				
	Ser	Asn	Val	Ile	Ile	Arg	Asn	Met	Lys	Ile	Gly	Ala	Leu	Gly	Gly	Ala
	145				150					155						160
	Asn	Asn	Asp	Ala	Asp	Met	Ile	Arg	Val	Asp	Ser	Gly	Val	Asn	Val	Trp
20				165					170					175		
	Ile	Asp	His	Asn	Glu	Leu	Phe	Ala	Val	Asn	Asn	Glu	Cys	Lys	Gly	Ser
				180				185					190			
	Pro	Asp	Gly	Asp	Leu	Thr	Phe	Glu	Ser	Ala	Ile	Asp	Ile	Lys	Lys	Ala
			195				200					205				
	Ser	Gln	Asp	Ile	Thr	Val	Ser	Tyr	Asn	Val	Ile	Arg	Asp	Ser	Lys	Lys
25		210					215					220				
	Val	Gly	Leu	Asp	Gly	Ser	Ser	Ser	Ser	Asp	Ile	Ala	Gly	Gly	Arg	Lys
		225			230					235						240
	Ile	Thr	Phe	His	His	Asn	Ile	Tyr	Arg	Asn	Val	Gly	Ala	Arg	Leu	Pro
				245					250					255		
	Leu	Gln	Arg	Gly	Gly	Trp	Thr	His	Met	Tyr	Asn	Asn	Leu	Tyr	Asp	Gly
30				260				265					270			
	Ile	Thr	Ser	Ser	Gly	Ile	Asn	Val	Arg	Gln	Asn	Gly	Tyr	Ala	Leu	Ile
			275				280					285				
	Glu	Ser	Asn	Trp	Phe	Gln	Asn	Ala	Val	Asn	Pro	Val	Thr	Cys	Arg	Phe
		290				295					300					
	Asp	Ser	Ser	Asn	Cys	Gly	Lys	Trp	Asp	Leu	Arg	Asn	Asn	Asn	Ile	Arg
35	305				310					315						320
	Asn	Pro	Gly	Asp	Phe	Ala	Thr	Tyr	Asn	Ile	Thr	Trp	Thr	Ser	Gly	Gly
				325					330					335		
	Thr	Ile	Asp	Ala	Thr	Asn	Trp	Thr	Thr	Thr	Ala	Pro	Phe	Pro	Ile	Ser
				340				345					350			
	Ile	Pro	Tyr	Ser	Tyr	Ser	Pro	Val	Thr	Pro	Gln	Cys	Val	Lys	Asp	Arg
40			355				360						365			
	Leu	Ala	Ser	Tyr	Ala	Gly	Val	Gly	Lys	Asn	Gly	Ala	Gln	Leu	Thr	Ala
		370				375						380				
	Ser	Ala	Cys	Gly	Gly	Ala	Ala	Ser	Ser	Thr	Pro	Ala	Ser	Ser	Thr	Pro
	385				390					395						400
	Ala	Ser	Ser	Ser	Ser	Ala	Ala	Asn	Ser	Ser	Ala	Ala	Ser	Gly	Ser	Val
45				405					410					415		
	Ser	Leu	Gly	Gly	Ser	Ala	Gly	Asn	Ala	Ser	Val	Ala	Leu	Asn	Trp	Thr
			420					425					430			
	Val	Asn	Ala	Asn	Ile	Asn	Ala	Leu	Glu	Ile	Tyr	Gln	Asp	Thr	Asp	Ser
		435					440					445				
	Asp	Pro	Ala	Gly	Arg	Val	Arg	Ile	Ala	Ser	Leu	Pro	Thr	Ser	Ala	Thr
50		450			455						460					
	Asn	Tyr	Thr	Ala	Thr	Gly	Leu	Ser	Asn	Gly	Thr	Thr	Tyr	Tyr	Phe	Trp

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465 470 475 480
 Val Lys Tyr Arg Thr Thr Asn Asn Val Trp Ser Asn Ser Asn Val Phe
 485 490 495
 5 Ser Ala Lys Pro Ser Ser Gly Thr Thr Pro Ser Ser Ser Ser Ser Ala
 500 505 510
 Ala Ser Ser Thr Pro Ser Gly Ala Pro Val Leu Ser Gly Thr Gly Asp
 515 520 525
 Tyr Pro Ser Gly Phe Ser Lys Cys Ala Asp Leu Gly Gly Thr Cys Ser
 530 535 540
 10 Val Ala Ser Gly Asp Gly Trp Val Ala Phe Gly Arg Lys Gly Lys Trp
 545 550 555 560
 Val Thr Lys Lys Val Ser Val Gly Ser Ser Ile Ala Cys Thr Val Ala
 565 570 575
 Ala Phe Gly Ser Asp Pro Gln Gly Asn Pro Asn Lys Cys Ser Tyr Lys
 580 585 590
 15 Lys

<210> 101

<211> 1404

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 101

atgactatag accgtcgaga attccttata gacctcatta tcggcaccgc cggcttcgca 60
 atcgaccga gtgatgcgtt cggccaagct gatccatgga aaaccgtcta tccgcaaatac 120
 ctcgctcgca tacggccacc gaaatttccg aagcgagatt tcatcatcac tagattcggc 180
 30 gcaaagccgg gaaccgacag cgccgctgcg atcgcaaaag ctatcaccgc gtgcagcaag 240
 gcaggcggag gacgtgttct cgttcccgca ggagagtttc tcaccggagc gatccatctg 300
 aaatcgaacg taaactttca cgtgtcaaaa ggcgcgacgc tgaaattctc gaccgaccgc 360
 aaggcatatc tcccgattgt acatacgcca tgggaaggaa tggagctgat gcatctgtca 420
 ccgttcatct acgcttatga gcagacgaac atcgctatca cgggtcaggg aacgctcgac 480
 ggccagggaa aatcattctt ctggaaatgg catggcaatc cggcttatgg cggcgatccg 540
 35 aacacgctca gccaacggcc cgctcgtgcg cggctttacg agatgatgga taagaatgtg 600
 ccggtcgccg aacgtgtctt cgggtctcgga cattatctgc ggccgcagtt tattcagccg 660
 tacaaatgca ggaacgtttt gatcgaagat gtgacgatcg tcgattcgcc gatgtgggaa 720
 gttcatccgg tgctttgcga gaacgtcacg gtccgaaatg ttcacatttc atcgcatggt 780
 ccgaacaatg acggatgcga tccggagtcg tgcaaggacg tactgatcga caactgtttt 840
 40 ttcgacaccg gcgacgattg catcgcgatc aagtccggcc gcaacaatga cggtcgctcg 900
 atcaatgtcc cgaccgagaa catcatcgtc cgcaactgca caatgaaaga cggtcatggc 960
 ggcacacgg tcggcagtga gatttcggga ggcgtgcaaa atctttttgc gcacgattgt 1020
 cgactcgaca gtgcggatct ctggaccgcg cttcgcgtca agaacaatgc gtcgcgaggc 1080
 ggcaagctcg agaattttta ttttcggaat ataacggtcg gccaggctcg acgcgctgtg 1140
 gtcgagatcg attttaatta cgaggaaggt gcgaaaggct cgtatattcc tgcgttcga 1200
 45 aattatgttg ttgaaggact gacatgcgcc acaggcaatc gcgccgtcga tctgcaagga 1260
 ttggacaacg cgccgatcta caatgtaacg ctgcgaaact gtacgtttgg ttctgtccga 1320
 aatcgtagtg ttgtgaaaaa cgttcgtgga cttcggctcg agaatgtgaa gatcggcggc 1380
 aggatcgtaa acgaactggt atga 1404

<210> 102

<211> 467

<212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

<221> SIGNAL

<222> (1)...(28)

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<221> DOMAIN
<222> (78)...(459)
<223> Catalytic domain

5 <400> 102

	Met	Thr	Ile	Asp	Arg	Arg	Glu	Phe	Leu	Ile	Asp	Leu	Ile	Ile	Gly	Thr
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10	Ala	Gly	Phe	Ala	Ile	Ala	Pro	Ser	Asp	Ala	Phe	Gly	Gln	Ala	Asp	Pro
				20					25					30		
	Trp	Lys	Thr	Val	Tyr	Pro	Gln	Ile	Leu	Ala	Arg	Ile	Arg	Pro	Pro	Lys
			35					40					45			
	Phe	Pro	Lys	Arg	Asp	Phe	Ile	Ile	Thr	Arg	Phe	Gly	Ala	Lys	Pro	Gly
		50					55					60				
15	Thr	Asp	Ser	Ala	Ala	Ala	Ile	Ala	Lys	Ala	Ile	Thr	Ala	Cys	Ser	Lys
	65				70						75				80	
	Ala	Gly	Gly	Gly	Arg	Val	Leu	Val	Pro	Ala	Gly	Glu	Phe	Leu	Thr	Gly
					85					90					95	
	Ala	Ile	His	Leu	Lys	Ser	Asn	Val	Asn	Phe	His	Val	Ser	Lys	Gly	Ala
				100					105					110		
20	Thr	Leu	Lys	Phe	Ser	Thr	Asp	Pro	Lys	Ala	Tyr	Leu	Pro	Ile	Val	His
			115					120					125			
	Thr	Arg	Trp	Glu	Gly	Met	Glu	Leu	Met	His	Leu	Ser	Pro	Phe	Ile	Tyr
		130					135					140				
	Ala	Tyr	Glu	Gln	Thr	Asn	Ile	Ala	Ile	Thr	Gly	Gln	Gly	Thr	Leu	Asp
	145					150					155					160
25	Gly	Gln	Gly	Lys	Ser	Phe	Phe	Trp	Lys	Trp	His	Gly	Asn	Pro	Ala	Tyr
					165					170					175	
	Gly	Gly	Asp	Pro	Asn	Thr	Leu	Ser	Gln	Arg	Pro	Ala	Arg	Ala	Arg	Leu
				180					185					190		
	Tyr	Glu	Met	Met	Asp	Lys	Asn	Val	Pro	Val	Ala	Glu	Arg	Val	Phe	Gly
			195					200					205			
30	Leu	Gly	His	Tyr	Leu	Arg	Pro	Gln	Phe	Ile	Gln	Pro	Tyr	Lys	Cys	Arg
		210					215					220				
	Asn	Val	Leu	Ile	Glu	Asp	Val	Thr	Ile	Val	Asp	Ser	Pro	Met	Trp	Glu
	225					230					235					240
	Val	His	Pro	Val	Leu	Cys	Glu	Asn	Val	Thr	Val	Arg	Asn	Val	His	Ile
					245					250					255	
35	Ser	Ser	His	Gly	Pro	Asn	Asn	Asp	Gly	Cys	Asp	Pro	Glu	Ser	Cys	Lys
				260					265					270		
	Asp	Val	Leu	Ile	Asp	Asn	Cys	Phe	Phe	Asp	Thr	Gly	Asp	Asp	Cys	Ile
			275					280					285			
	Ala	Ile	Lys	Ser	Gly	Arg	Asn	Asn	Asp	Gly	Arg	Arg	Ile	Asn	Val	Pro
		290					295					300				
40	Thr	Glu	Asn	Ile	Ile	Val	Arg	Asn	Cys	Thr	Met	Lys	Asp	Gly	His	Gly
	305					310					315					320
	Gly	Ile	Thr	Val	Gly	Ser	Glu	Ile	Ser	Gly	Gly	Val	Arg	Asn	Leu	Phe
					325					330					335	
	Ala	His	Asp	Cys	Arg	Leu	Asp	Ser	Ala	Asp	Leu	Trp	Thr	Ala	Leu	Arg
				340					345					350		
45	Val	Lys	Asn	Asn	Ala	Ser	Arg	Gly	Gly	Lys	Leu	Glu	Asn	Phe	Tyr	Phe
				355				360								
	Arg	Asn	Ile	Thr	Val	Gly	Gln	Val	Ala	Arg	Ala	Val	Val	Glu	Ile	Asp
		370				375						380				
	Phe	Asn	Tyr	Glu	Glu	Gly	Ala	Lys	Gly	Ser	Tyr	Ile	Pro	Val	Val	Arg
	385					390				395						400
50	Asn	Tyr	Val	Val	Glu	Gly	Leu	Thr	Cys	Ala	Thr	Gly	Asn	Arg	Ala	Val
					405					410					415	
	Asp	Leu	Gln	Gly	Leu	Asp	Asn	Ala	Pro	Ile	Tyr	Asn	Val	Thr	Leu	Arg
				420				425						430		
	Asn	Cys	Thr	Phe	Gly	Ser	Val	Arg	Asn	Arg	Ser	Val	Val	Lys	Asn	Val
			435					440					445			
55	Arg	Gly	Leu	Arg	Leu	Glu	Asn	Val	Lys	Ile	Gly	Gly	Arg	Ile	Val	Asn
		450					455					460				
	Glu	Leu	Val													
	465															

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<210> 103
<211> 1101
<212> DNA
<213> Unknown

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<220>
<223> Obtained from an environmental sample

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<400> 103

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25

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gcgctgcagg	cacaggccac	gcagaccgag	cccgtcgccg	agaacatgct	gctgctgcag	120
accgcgtccg	gcggctgggtc	caagcaccac	cagggcaagg	cggtcgacta	cggccacacg	180
ttcaccgatg	ccgaacgtgc	ggcgctgcgc	gcgcccgaac	gcagggacga	tgcgacgatc	240
gacaacaagg	cgaccacgct	tgagatcgtc	gcgctgctgg	aagcccacca	gcgcaccggc	300
aatgccgcct	atctggcggtc	tgcgagcggc	ggcgctggact	acctgctggc	cgcgaggtac	360
ccgaacggcg	gctggccgca	gtactaccgc	gaccgttcgc	tgtaccggca	ccaggtcacc	420
ttcaacgatg	atgcgatgac	ccgcgtgctg	gagctgctgc	aggacatcgt	cgagggcaag	480
ggcgcgctgg	cgagctgac	acccacgcac	ggcgaacgcg	ccagggccgc	gctcgacagg	540
ggcatcgcc	gcgtgctcgc	cacccaggta	cggatcgatg	gcgagctcac	gctctgggcc	600
gcgcagtagc	acgaagccac	gctgcagccg	gcgaaggcgc	gctcctacga	gctgccatcg	660
ctggcggtcg	ccgaatcggt	cggggtgatg	cggctgctga	tgcgccagcc	acagccgctc	720
ccgcaggtgc	tgacggcggt	cgaggccggc	gcacgctggc	tgagggcgca	ccgcagtcgc	780
gacctggccc	ggcgaaagat	cgacgcgccc	ggcgaagaaa	ccggccagga	cggtggtgatc	840
gtcgccgagc	ccggcgcgctc	gctgtgggca	cgcttctacg	acctgcagca	ccagcagccg	900
atgttcgtga	accgcgaagg	cgagcaggtg	gcccgccttc	ccgacatgcc	caacgaacgc	960
cgcgctcggt	acgcctggta	tggcggtgtg	ccggagaagc	tgctgcagca	ggagctgcca	1020
cgctggtaca	acacccatgc	cgaggcattg	cgcgcgatta	cgctgcgca	tgccgagcca	1080
aggccgcccga	agcggccctg	a				1101

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<210> 104
<211> 366
<212> PRT
<213> Unknown

35

<220>
<223> Obtained from an environmental sample

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<221> SIGNAL
<222> (1)...(26)

<221> DOMAIN
<222> (27)...(366)
<223> Catalytic domain

45

<400> 104

50

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Met Asn Thr Ala Leu His Arg Val Ile Arg Leu Pro Leu Leu Leu Ala
 1 5 10 15
 Leu Cys Leu Pro Ala Leu Gln Ala Gln Ala Thr Gln Thr Glu Pro Val
 20 25 30
 Ala Glu Asn Met Leu Leu Leu Gln Thr Ala Ser Gly Gly Trp Ser Lys
 35 40 45
 His His Gln Gly Lys Ala Val Asp Tyr Gly His Thr Phe Thr Asp Ala
 50 55 60
 Glu Arg Ala Ala Leu Arg Ala Pro Asp Arg Arg Asp Asp Ala Thr Ile
 65 70 75 80
 Asp Asn Lys Ala Thr Leu Glu Ile Val Ala Leu Leu Glu Ala His
 85 90 95
 Gln Arg Thr Gly Asn Ala Ala Tyr Leu Ala Ala Ala Gln Arg Gly Val
 100 105 110
 Asp Tyr Leu Leu Ala Ala Gln Tyr Pro Asn Gly Gly Trp Pro Gln Tyr
 115 120 125
 Tyr Pro Asp Arg Ser Leu Tyr Arg His Gln Val Thr Phe Asn Asp Asp
 130 135 140
 Ala Met Thr Arg Val Leu Glu Leu Leu Gln Asp Ile Val Glu Gly Lys
 145 150 155 160
 Gly Ala Leu Ala Gln Leu Thr Pro Thr His Gly Glu Arg Ala Arg Ala
 165 170 175
 Ala Leu Asp Arg Gly Ile Ala Cys Val Leu Ala Thr Gln Val Arg Ile
 180 185 190
 Asp Gly Glu Leu Thr Leu Trp Ala Ala Gln Tyr Asp Glu Ala Thr Leu
 195 200 205
 Gln Pro Ala Lys Ala Arg Ser Tyr Glu Leu Pro Ser Leu Ala Val Ala
 210 215 220
 Glu Ser Val Gly Val Met Arg Leu Leu Met Arg Gln Pro Gln Pro Ser
 225 230 235 240

 Pro Gln Val Leu Thr Ala Val Glu Ala Gly Ala Arg Trp Leu Glu Ala
 245 250 255
 His Arg Met Arg Asp Leu Ala Arg Arg Lys Ile Asp Ala Pro Gly Glu
 260 265 270
 Glu Thr Gly Gln Asp Val Val Ile Val Ala Glu Pro Gly Ala Ser Leu
 275 280 285
 Trp Ala Arg Phe Tyr Asp Leu Gln His Gln Gln Pro Met Phe Val Asn
 290 295 300
 Arg Glu Gly Glu Gln Val Ala Arg Phe Ala Asp Met Pro Asn Glu Arg
 305 310 315 320
 Arg Val Gly Tyr Ala Trp Tyr Gly Val Trp Pro Glu Lys Leu Leu Gln
 325 330 335
 Gln Glu Leu Pro Arg Trp Tyr Asn Thr His Ala Glu Ala Leu Arg Ala
 340 345 350
 Ile Thr Pro Ala His Ala Glu Pro Arg Pro Pro Lys Arg Pro
 355 360 365

<210> 105

<211> 1203

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 105

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	ggcgacaggc	gctttccgcg	ggatcatgccc	gccgtttgcg	cgggccttgc	cctcgccgtg	120
	tcgtcggccg	agccgggtccg	ggcgagggc	gcggatgcgg	atgcggatgg	cccactgccc	180
5	aggtggaaca	ggaggctggt	ggatcgcccc	gaggactggt	tcgcctccga	cgagggacag	240
	cgcgttgccg	ccaacgtcct	ccgctacca	tcggcggaag	gagcctggcc	caaaaacacc	300
	aatctggccg	ccactcccc	tcgccccgag	gacattccct	cctcgacctc	cggggtggcc	360
	aacacgatcg	acaatgaagc	caccaccgtg	cccattcggt	ttttggcccg	tttcgcgcaa	420
	atcaacgagg	acacggccag	ccgcgaggcg	gtccagcgcg	gattggacta	tctcctcaag	480
	gcgcaatatc	cgaacggtgg	ctggccgcag	tatttcccg	tccgccgcgg	ctaccactcg	540
10	cacatcacct	acaacgacga	cgccatggtg	aatgtgctcg	acctgctgct	ggacgtgctg	600
	ctgggagagg	agccgttcga	ttttgtggac	gaggatcgcc	gccagcgggc	cgcgaccgcc	660
	gtggagcggg	ggatcgaatg	catectccgc	acccaaatcc	ggcaggagga	ccaacccacc	720
	ggctgggtgcg	cgcagtatga	ccccgaaacc	ttggccccgg	cgtggggacg	ggcgtagcag	780
	ccgccgtcga	tttcgggagc	cgagaccgtc	ggcgtggcg	ggtttctgat	gcggctggag	840
	tcgccatcgc	cggaagccgt	cgaagccatc	gagggcgcca	tcgcctggct	cgacacggtg	900
15	ggcatcgagg	aattgcgtct	cgaatggttc	accaacagcg	agggcaagcg	tgaccggcgc	960
	gtggtcgagg	acgcttccgt	gggcaccctt	tgggcgcgct	tttacgaact	cgaacgaac	1020
	cgccccctgt	tcgtggaccg	cgacggggtg	ctccgctacg	acttcgcgga	actgacggcg	1080
	gagcgccgcc	aaggttacag	ctactacggc	acttggccgg	cgccattgct	ggccacggaa	1140
	tatccgcgct	ggcgcaggat	gaacgagtc	gccctgctcg	agtcgtcctt	catctcgcat	1200
	tga						1203

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<210> 106
<211> 400
<212> PRT
<213> Unknown

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<220>
<223> Obtained from an environmental sample

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<221> SIGNAL
<222> (1)...(43)

<221> DOMAIN
<222> (44)...(400)
<223> Catalytic domain

35

<400> 106

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Met	Gln	Phe	Ile	Glu	Thr	Gln	Gln	Leu	Gly	Thr	Ala	Ala	Lys	Pro	Val
1				5					10				15		
Ala	Gly	Arg	Gly	Gly	Asp	Arg	Arg	Phe	Pro	Arg	Val	Met	Pro	Ala	Val
			20					25					30		

45

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	Cys	Ala	Gly	Leu	Ala	Leu	Ala	Val	Ser	Ser	Ala	Glu	Pro	Val	Arg	Ala
			35					40					45			
5	Gln	Gly	Ala	Asp	Ala	Asp	Ala	Asp	Gly	Pro	Leu	Pro	Arg	Trp	Asn	Arg
		50					55					60				
	Arg	Leu	Val	Asp	Arg	Pro	Glu	Asp	Trp	Phe	Ala	Ser	Asp	Glu	Gly	Gln
	65					70					75				80	
	Arg	Val	Ala	Ala	Asn	Val	Leu	Arg	Tyr	Gln	Ser	Ala	Glu	Gly	Ala	Trp
					85					90					95	
10	Pro	Lys	Asn	Thr	Asn	Leu	Ala	Ala	Thr	Pro	Leu	Arg	Pro	Glu	Asp	Ile
			100						105					110		
	Pro	Ser	Ser	Thr	Ser	Gly	Val	Ala	Asn	Thr	Ile	Asp	Asn	Glu	Ala	Thr
			115					120					125			
	Thr	Val	Pro	Ile	Arg	Phe	Leu	Ala	Arg	Phe	Ala	Gln	Ile	Asn	Glu	Asp
	130						135					140				
15	Thr	Ala	Ser	Arg	Glu	Ala	Val	Gln	Arg	Gly	Leu	Asp	Tyr	Leu	Leu	Lys
	145					150					155					160
	Ala	Gln	Tyr	Pro	Asn	Gly	Gly	Trp	Pro	Gln	Tyr	Phe	Pro	Leu	Arg	Arg
					165					170					175	
	Gly	Tyr	His	Ser	His	Ile	Thr	Tyr	Asn	Asp	Asp	Ala	Met	Val	Asn	Val
				180					185					190		
20	Leu	Asp	Leu	Leu	Leu	Asp	Val	Ser	Leu	Gly	Glu	Glu	Pro	Phe	Asp	Phe
			195					200					205			
	Val	Asp	Glu	Asp	Arg	Arg	Gln	Arg	Ala	Ala	Thr	Ala	Val	Glu	Arg	Gly
		210					215					220				
	Ile	Glu	Cys	Ile	Leu	Arg	Thr	Gln	Ile	Arg	Gln	Glu	Asp	Gln	Pro	Thr
	225					230					235					240
25	Gly	Trp	Cys	Ala	Gln	Tyr	Asp	Pro	Glu	Thr	Leu	Ala	Pro	Ala	Trp	Gly
				245						250					255	
	Arg	Ala	Tyr	Glu	Pro	Pro	Ser	Ile	Ser	Gly	Ala	Glu	Thr	Val	Gly	Val
				260					265					270		
	Ala	Arg	Phe	Leu	Met	Arg	Leu	Glu	Ser	Pro	Ser	Pro	Glu	Ala	Val	Glu
			275					280					285			
30	Ala	Ile	Glu	Gly	Ala	Ile	Ala	Trp	Leu	Asp	Thr	Val	Gly	Ile	Glu	Glu
		290					295					300				
	Leu	Arg	Leu	Glu	Trp	Phe	Thr	Asn	Ser	Glu	Gly	Lys	Arg	Asp	Arg	Arg
	305					310					315					320
	Val	Val	Glu	Asp	Ala	Ser	Val	Gly	Thr	Leu	Trp	Ala	Arg	Phe	Tyr	Glu
					325					330					335	
35	Leu	Glu	Thr	Asn	Arg	Pro	Leu	Phe	Val	Asp	Arg	Asp	Gly	Val	Leu	Arg
				340					345					350		
	Tyr	Asp	Phe	Ala	Glu	Leu	Thr	Ala	Glu	Arg	Arg	Gln	Gly	Tyr	Ser	Tyr
			355					360					365			
	Tyr	Gly	Thr	Trp	Pro	Ala	Pro	Leu	Leu	Ala	Thr	Glu	Tyr	Pro	Arg	Trp
		370					375					380				
40	Arg	Arg	Met	Asn	Glu	Ser	Ala	Leu	Leu	Glu	Ser	Ser	Phe	Ile	Ser	His
	385					390					395					400

<210> 107

<211> 1074

45 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

50

<400> 107

55

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	cttctgcaga	ccgcctccgg	cggttggtcc	aagcactacc	gcgagaagaa	ggtcgactac	180
	gcgcgcgact	acgacgccgc	cgagcgcgcc	gcgctgcgcg	cgcccgaccg	gcatgacgat	240
5	gccacgatcg	acaacaaggc	cacgaccacc	gagatcgcat	acctggtgca	ggcacatgcc	300
	aggacggggca	atccgggccta	cctcgacggc	gcgcgcgcgc	gcgtcgagta	cctgctgcgc	360
	gcgcagtaac	cgaacggcgg	ctggccgcag	ttctaccccg	accattcgtc	ctaccggcac	420
	cagatcacgc	tcaacgacga	tgcgatggtg	cacgccatca	ccgtgctgca	ggacatcgcc	480
	gcggggccgca	acggcatgca	ggtgctggcg	ccggagttcg	gcgtccgcgc	cgccgcggcc	540
	gcgcagcgcg	gcatcggaaa	cctgctcgag	ttgcaggtgc	ggatcgacgg	ggtgccgacg	600
10	atctggggccg	cgcgatcgga	cgagaccacc	ctgcaaccgg	ccaaggcccg	tgctgacgag	660
	ttgccctcgc	tggccgtggc	cgaatcgggtg	ggcgtgatgc	gcctgctgat	gcgccagccg	720

	gggcctgatg	cgcgcacgat	cgccgcgata	gaggcggcgg	cggactggct	ggaggcgcac	780
	cgccctgccgg	acctcgccct	ggaacgcata	gaagcccccg	ccgaggaaac	cggcaaggac	840
15	gtccgcgctcg	tggccagacc	gggcgcgctcg	ttgtgggcgc	gcttctacga	cctcgagcgg	900
	caggtgcccgc	tgttcgtcga	tcgcaacagc	cgtccgggttc	cattcgccga	gcttcccaac	960
	gagcgtcgta	ccggctatgg	ctgggtatggc	acctggccgg	aaaagctgct	ggcacaggaa	1020
	ctcccgcgct	ggcgcaaggt	ccatgcggcc	agcgcggggcg	ctccggcccg	ttga	1074

20 <210> 108
 <211> 357
 <212> PRT
 <213> Unknown

25 <220>
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<221> SIGNAL
 <222> (1)...(31)

30 <221> DOMAIN
 <222> (32)...(357)
 <223> Catalytic domain

35 <400> 108

40

45

50

55

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	1				5					10				15		
	Thr	Ser	Pro	Val	Ala	Cys	Ala	Gly	Ala	Ala	Ala	Pro	Ala	Thr	Ala	Thr
5				20					25					30		
	Asp	Pro	Val	Ala	Glu	Asn	Met	Leu	Leu	Gln	Thr	Ala	Ser	Gly	Gly	
			35					40					45			
	Trp	Ser	Lys	His	Tyr	Arg	Glu	Lys	Lys	Val	Asp	Tyr	Ala	Arg	Asp	Tyr
		50					55					60				
	Asp	Ala	Ala	Glu	Arg	Ala	Ala	Leu	Arg	Ala	Pro	Asp	Arg	His	Asp	Asp
10	65					70					75				80	
	Ala	Thr	Ile	Asp	Asn	Lys	Ala	Thr	Thr	Thr	Glu	Ile	Ala	Tyr	Leu	Val
					85					90					95	
	Gln	Ala	His	Ala	Arg	Thr	Gly	Asn	Pro	Ala	Tyr	Leu	Asp	Gly	Ala	Arg
				100					105					110		
	Arg	Gly	Val	Glu	Tyr	Leu	Leu	Arg	Ala	Gln	Tyr	Pro	Asn	Gly	Gly	Trp
			115					120					125			
15	Pro	Gln	Phe	Tyr	Pro	Asp	His	Ser	Ser	Tyr	Arg	His	Gln	Ile	Thr	Leu
		130					135					140				
	Asn	Asp	Asp	Ala	Met	Val	His	Ala	Ile	Thr	Val	Leu	Gln	Asp	Ile	Ala
	145					150					155					160
	Ala	Gly	Arg	Asn	Gly	Met	Gln	Val	Leu	Ala	Pro	Glu	Phe	Gly	Val	Arg
					165					170					175	
20	Ala	Ala	Ala	Ala	Ala	Gln	Arg	Gly	Ile	Gly	Asn	Leu	Leu	Glu	Leu	Gln
				180					185					190		
	Val	Arg	Ile	Asp	Gly	Val	Pro	Thr	Ile	Trp	Ala	Ala	Gln	Tyr	Asp	Glu
			195					200					205			
	Thr	Thr	Leu	Gln	Pro	Ala	Lys	Ala	Arg	Ala	Tyr	Glu	Leu	Pro	Ser	Leu
		210					215					220				
25	Ala	Val	Ala	Glu	Ser	Val	Gly	Val	Met	Arg	Leu	Leu	Met	Arg	Gln	Pro
	225					230					235					240
	Gly	Pro	Asp	Ala	Arg	Thr	Ile	Ala	Ala	Ile	Glu	Ala	Ala	Ala	Asp	Trp
					245					250					255	
	Leu	Glu	Ala	His	Arg	Leu	Pro	Asp	Leu	Ala	Leu	Glu	Arg	Ile	Glu	Ala
				260					265					270		
30	Pro	Ala	Glu	Thr	Gly	Lys	Asp	Val	Arg	Val	Val	Ala	Arg	Pro	Gly	
			275				280					285				
	Ala	Ser	Leu	Trp	Ala	Arg	Phe	Tyr	Asp	Leu	Glu	Arg	Gln	Val	Pro	Leu
		290					295					300				
	Phe	Val	Asp	Arg	Asn	Ser	Arg	Pro	Val	Pro	Phe	Ala	Glu	Leu	Pro	Asn
35	305					310					315					320
	Glu	Arg	Arg	Thr	Gly	Tyr	Gly	Trp	Tyr	Gly	Thr	Trp	Pro	Glu	Lys	Leu
					325					330					335	
	Leu	Ala	Gln	Glu	Leu	Pro	Arg	Trp	Arg	Lys	Val	His	Ala	Ala	Ser	Ala
				340					345					350		
40	Gly	Ala	Pro	Ala	Arg											

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45 <210> 109
 <211> 1422
 <212> DNA
 <213> Unknown

50 <220>
 <223> Obtained from an environmental sample

<400> 109

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    atcctcgcac gcatcagacc acctcgtttt ccgaaacgca ccttctatct caatcgattc 180
    ggcgccaagg gtgatggagt cacagactgc accgcggctt ttcatcgcgc gatcgatgaa 240
    tgcaccaaag ccggcggtgg gaaagtcgtc gtgccggcgg gcacttatct caccggcgcg 300
    attcatttga agagcaacgt caacctcgaa gtctcggaag gcgcgacgat caagttcagt 360
    caggacccga aacactacct gcctgtttgtc ttctcgcggt gggaagggtgt cgaagtcttc 420
    aactactcgc ctttcattta cgcgttcgaa cagcgaacaa tcgcatcac cggcaaaggc 480
    acgctcgacg gacagagtga ttcggaacac tgggtggccg ggaacggccg tccgcagtac 540
    ggatggaaaag aagggatgaa acagcagcgt cccgatcgca acgcgttggt cacaatggcg 600
    gagaaaggcg tgccgggtgcg cgagcgcac tttggcgaag gtcattattt gaggccgcag 660
    ttcattcagc cgtaccgctg ccagaacgtg ctgatccagg gcgtgacgat tcggaactcg 720
    ccgatgtggg agattcatcc ggtgtttgtgc cgtaacgtga ctattcacga cgtgcacatc 780
    gatagtcatg gaccaaacaa cgacggctgc aatcccgaat cgtgcagcga cgtgttgatt 840
    aaggatagct acttcgatac cggcgacgac tgcacgcgca tcaaatacggg acgcaacgcc 900
    gacgggcggc ggcttaaagc gccgactgag aacatcatcg ttcaaggatg tcgcatgaaa 960
    gacggccacg gtggaatcac ggtcggcagc gagatctcgg gcggcggtgc aaacctgttt 1020
    gccgagaatt gccggctcga cagtccaaac ctcgatcacg ccctgcgcgt gaagaacaat 1080
    gccatgcgcg gcggattact cgagaacttc cacttccgta acatcgaagt cgggcaggtg 1140
    gcccatgccg tgattacgat cgacttcaac tacgaagagg gcgcgaaagg gtcgttcacg 1200
    ccggtcggtc gcgattacac ggtcgatcgt ttgcgcagca cgaagagcaa gcacgcactc 1260
    gacgtccagg gtctgcccgg cgcgccggtc atcaacctgc gattgacgaa ctgcacattc 1320
    aacgatgtgc agcaaccgaa cattctcaag aacgtcgaac aatcaacctt tgaaaacgtc 1380
    acgattaacg gaaagacgat cacacaaaca ggatccaaag aa 1422

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25  <210> 110
    <211> 474
    <212> PRT
    <213> Unknown

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30  <220>
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35  <221> SIGNAL
    <222> (1)...(21)

    <221> DOMAIN
    <222> (28)...(308)
    <223> Pectin methyl esterase domain

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40  <221> DOMAIN
    <222> (309)...(637)
    <223> Catalytic domain

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    <400> 110

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    Ala Val Ala Val Ala Pro Arg Leu Leu Ala Phe Ala Ala Glu Ala Ser
    20      25      30
    Pro Trp Glu Thr Met Met Pro Ser Ile Leu Ala Arg Ile Arg Pro Pro
    35      40      45
    Arg Phe Pro Lys Arg Thr Phe Tyr Leu Asn Arg Phe Gly Ala Lys Gly
    50      55      60
    Asp Gly Val Thr Asp Cys Thr Ala Ala Phe His Arg Ala Ile Asp Glu
    65      70      75      80
    Cys Thr Lys Ala Gly Gly Gly Lys Val Val Val Pro Ala Gly Thr Tyr
    85      90      95

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	Leu	Thr	Gly	Ala	Ile	His	Leu	Lys	Ser	Asn	Val	Asn	Leu	Glu	Val	Ser
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	Glu	Gly	Ala	Thr	Ile	Lys	Phe	Ser	Gln	Asp	Pro	Lys	His	Tyr	Leu	Pro
			115					120					125			
5	Val	Val	Phe	Ser	Arg	Trp	Glu	Gly	Val	Glu	Val	Phe	Asn	Tyr	Ser	Pro
		130					135					140				
	Phe	Ile	Tyr	Ala	Phe	Glu	Gln	Arg	Asn	Ile	Ala	Ile	Thr	Gly	Lys	Gly
	145					150					155					160
	Thr	Leu	Asp	Gly	Gln	Ser	Asp	Ser	Glu	His	Trp	Trp	Pro	Trp	Asn	Gly
					165					170					175	
10	Arg	Pro	Gln	Tyr	Gly	Trp	Lys	Glu	Gly	Met	Lys	Gln	Gln	Arg	Pro	Asp
			180						185					190		
	Arg	Asn	Ala	Leu	Phe	Thr	Met	Ala	Glu	Lys	Gly	Val	Pro	Val	Arg	Glu
			195					200					205			
	Arg	Ile	Phe	Gly	Glu	Gly	His	Tyr	Leu	Arg	Pro	Gln	Phe	Ile	Gln	Pro
		210					215					220				
15	Tyr	Arg	Cys	Gln	Asn	Val	Leu	Ile	Gln	Gly	Val	Thr	Ile	Arg	Asn	Ser
	225					230					235					240
	Pro	Met	Trp	Glu	Ile	His	Pro	Val	Leu	Cys	Arg	Asn	Val	Thr	Ile	His
					245					250					255	
	Asp	Val	His	Ile	Asp	Ser	His	Gly	Pro	Asn	Asn	Asp	Gly	Cys	Asn	Pro
				260					265					270		
20	Glu	Ser	Cys	Ser	Asp	Val	Leu	Ile	Lys	Asp	Ser	Tyr	Phe	Asp	Thr	Gly
			275					280					285			
	Asp	Asp	Cys	Ile	Ala	Ile	Lys	Ser	Gly	Arg	Asn	Ala	Asp	Gly	Arg	Arg
		290					295					300				
	Leu	Lys	Ala	Pro	Thr	Glu	Asn	Ile	Ile	Val	Gln	Gly	Cys	Arg	Met	Lys
	305					310					315					320
25	Asp	Gly	His	Gly	Gly	Ile	Thr	Val	Gly	Ser	Glu	Ile	Ser	Gly	Gly	Val
					325					330					335	
	Arg	Asn	Leu	Phe	Ala	Glu	Asn	Cys	Arg	Leu	Asp	Ser	Pro	Asn	Leu	Asp
				340					345					350		
	His	Ala	Leu	Arg	Val	Lys	Asn	Asn	Ala	Met	Arg	Gly	Gly	Leu	Leu	Glu
			355					360					365			
30	Asn	Phe	His	Phe	Arg	Asn	Ile	Glu	Val	Gly	Gln	Val	Ala	His	Ala	Val
		370					375					380				
	Ile	Thr	Ile	Asp	Phe	Asn	Tyr	Glu	Glu	Gly	Ala	Lys	Gly	Ser	Phe	Thr
	385					390					395					400
	Pro	Val	Val	Arg	Asp	Tyr	Thr	Val	Asp	Arg	Leu	Arg	Ser	Thr	Lys	Ser
					405					410					415	
35	Lys	His	Ala	Leu	Asp	Val	Gln	Gly	Leu	Pro	Gly	Ala	Pro	Val	Ile	Asn
				420					425					430		
	Leu	Arg	Leu	Thr	Asn	Cys	Thr	Phe	Asn	Asp	Val	Gln	Gln	Pro	Asn	Ile
			435					440					445			
	Leu	Lys	Asn	Val	Glu	Gln	Ser	Thr	Phe	Glu	Asn	Val	Thr	Ile	Asn	Gly
		450					455					460				
40	Lys	Thr	Ile	Thr	Gln	Thr	Gly	Ser	Lys	Glu						
	465					470										

<210> 111

45 <211> 1440

<212> DNA

<213> Unknown

<220>

50 <223> Obtained from an environmental sample

<400> 111

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	aagatttttag	ccagaatcaa	accgccgaaa	tttcgcaaaa	aagattttcc	gatcaccaaa	180
5	tatggagccg	ttgcggacgg	gaaaaccctg	gcgaccgaaa	gcatcaaaaa	agccatcgaa	240
	gcggtgcgcca	aatcggggcg	cgggcgcgtc	gtcgtgcccc	agggagaatt	tttgaccggc	300
	gcgattcatt	tgaaatcaaa	cgtcaatctg	cacatcacga	aaggcgcgac	cgtcaaat	360
	tccaccaacc	cgaaagatta	tctgccgatc	gttcacacgc	gctgggaagg	gatggaattg	420
	atgcataatt	cgcccttaaat	ttatgcctac	gagcaaacca	acatcgccgt	caccggcgag	480
	ggaacgctcg	acgggcaggg	caaggctttt	ttctggaaat	ggcacggaaa	cccgcgctac	540
10	ggcggaaaatc	cggatgtgat	cagccagcgt	ccggcgcgcg	cccggctgta	tgaaatgatg	600

	gaaaaaggcg	tgctgtggc	ggagcggatt	ttcggcgaaa	ctcagtatct	tcgcccgcag	660
	tttatccagc	cctataaatg	caaaaatggt	ttgatcgaag	gcgttaaaat	catcgattcg	720
15	ccgatgtggg	aagttcaccc	cgttttgtgc	gaaaacgtga	cgatccgaaa	acttcatatt	780
	tctaccacag	gaccgaacaa	cgacgggtgc	gatccggaaa	gctgcaagga	cgttttgatc	840
	gaagactgct	atttcgacac	cggcgacgat	tgcatcgcca	tcaaggcggg	gcgcaatgaa	900
	gacgggacgac	gcatcaatgt	tccgaccgaa	aacgtcgctg	tgcgcggtg	cgtgatgaag	960
	gacggctcac	gcggaatcac	catcggaagc	gagatttccg	gcggcggtgc	aaatgttttc	1020
	gcggaaaaca	accggctcga	cagcgcggtg	ttgtggactg	cgctgagagt	gaaaaacaac	1080
20	gcttcgcgcg	gcggaaaact	ggagaatttt	tacttccgcg	atatcaccgt	cgggcaggtc	1140
	tcgcgcgcgg	tcgtcgaaat	agattttta	tacgaggaag	gcgctaaagg	aaaacacacg	1200
	ccggtcgttc	gcaattacgt	ggtcgaaaat	ctaacctgca	ataaaggcaa	tcgagcggtc	1260
	gatctgcagg	gcttggaaca	cgccccgatt	tacgacatca	cgatgaaaaa	ctgtacgttt	1320
	aacgtggtcg	aaaagccgag	cgtcgtgaaa	aacgtcaaag	gcgtcaaact	ggaaaacgtg	1380
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<210> 112
 <211> 479
 <212> PRT
 <213> Unknown

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 <223> Obtained from an environmental sample

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 <222> (1)...(27)

 <221> DOMAIN
 <222> (82)...(461)
 <223> Catalytic domain

40

<400> 112

45

50

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 20 25 30
 Asp Ala Trp Thr Thr Glu Tyr Pro Lys Ile Leu Ala Arg Ile Lys Pro
 35 40 45
 Pro Lys Phe Arg Lys Lys Asp Phe Pro Ile Thr Lys Tyr Gly Ala Val
 50 55 60
 Ala Asp Gly Lys Thr Leu Ala Thr Glu Ser Ile Lys Lys Ala Ile Glu
 65 70 75 80
 10 Ala Cys Ala Lys Ser Gly Gly Gly Arg Val Val Val Pro Gln Gly Glu
 85 90 95
 Phe Leu Thr Gly Ala Ile His Leu Lys Ser Asn Val Asn Leu His Ile
 100 105 110
 Thr Lys Gly Ala Thr Val Lys Phe Ser Thr Asn Pro Lys Asp Tyr Leu
 115 120 125
 15 Pro Ile Val His Thr Arg Trp Glu Gly Met Glu Leu Met His Ile Ser
 130 135 140
 Pro Leu Ile Tyr Ala Tyr Glu Gln Thr Asn Ile Ala Val Thr Gly Glu
 145 150 155 160
 Gly Thr Leu Asp Gly Gln Gly Lys Ala Phe Phe Trp Lys Trp His Gly
 165 170 175
 20 Asn Pro Arg Tyr Gly Gly Asn Pro Asp Val Ile Ser Gln Arg Pro Ala
 180 185 190
 Arg Ala Arg Leu Tyr Glu Met Met Glu Lys Gly Val Pro Val Ala Glu
 195 200 205
 Arg Ile Phe Gly Glu Thr Gln Tyr Leu Arg Pro Gln Phe Ile Gln Pro
 210 215 220
 25 Tyr Lys Cys Lys Asn Val Leu Ile Glu Gly Val Lys Ile Ile Asp Ser
 225 230 235 240
 Pro Met Trp Glu Val His Pro Val Leu Cys Glu Asn Val Thr Ile Arg
 245 250 255
 Lys Leu His Ile Ser Thr His Gly Pro Asn Asn Asp Gly Cys Asp Pro
 260 265 270
 30 Glu Ser Cys Lys Asp Val Leu Ile Glu Asp Cys Tyr Phe Asp Thr Gly
 275 280 285
 Asp Asp Cys Ile Ala Ile Lys Ala Gly Arg Asn Glu Asp Gly Arg Arg
 290 295 300
 35 Ile Asn Val Pro Thr Glu Asn Val Val Val Arg Gly Cys Val Met Lys
 305 310 315 320
 Asp Gly His Gly Gly Ile Thr Ile Gly Ser Glu Ile Ser Gly Gly Val
 325 330 335
 40 Arg Asn Val Phe Ala Glu Asn Asn Arg Leu Asp Ser Ala Asp Leu Trp
 340 345 350
 Thr Ala Leu Arg Val Lys Asn Asn Ala Ser Arg Gly Gly Lys Leu Glu
 355 360 365
 Asn Phe Tyr Phe Arg Asp Ile Thr Val Gly Gln Val Ser Arg Ala Val
 370 375 380
 45 Val Glu Ile Asp Phe Asn Tyr Glu Glu Gly Ala Lys Gly Lys His Thr
 385 390 395 400
 Pro Val Val Arg Asn Tyr Val Val Glu Asn Leu Thr Cys Asn Lys Gly
 405 410 415
 Asn Arg Ala Val Asp Leu Gln Gly Leu Asp Asn Ala Pro Ile Tyr Asp
 420 425 430
 50 Ile Thr Met Lys Asn Cys Thr Phe Asn Val Val Glu Lys Pro Ser Val
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<210> 113

<211> 1017

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<212> DNA
<213> Unknown

<220>

5 <223> Obtained from an environmental sample

<400> 113

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	aagcacttgg	ccgataaatc	tggttgtaac	tattcaaaac	ctctctcacc	tgctttgcaa	180
	aaagtcacgc	atcaatcgac	cgaaaagtct	gcgacaattg	ataataatgc	aaccacacgt	240
	gagataaacc	atcttctcct	cgcttattcc	aaaaccaaca	atgacaagta	tcttcaagcg	300
	gcgacaaaag	gtgttgagta	tatcctgagt	gctcaaaatg	acaaaggagg	atggcctcaa	360
15	tattatccag	acagtagctc	atatcggtgt	cagatcacct	acaatgacgg	cgcgatgatt	420
	aatgtattgg	aaattttact	ttccatatca	acaaaacaag	agccctatgc	tggttctaacg	480
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	acccagggtta	aacaaggaga	taaactaacc	atctgggccc	cacagtacga	tcagaaaaca	600
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20	aaccacgctg	tagaatggtt	ttcctcccat	aaagaggtag	gctatgatta	cgttaaaacg	780
	gaaaaaacg	gaaaactttt	gcgggatttg	gtttcttcgc	cggcctctac	cgatatgggca	840
	agattttatg	acatcaggac	gaatcaaccc	atctttgggt	atcgcgataa	tacgataaag	900
	tattcgctga	atgaaataag	cgaggaacga	caaatggcct	actcttggtg	tggtaactgg	960
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25 <210> 114
<211> 338
<212> PRT
<213> Unknown

30 <220>
<223> Obtained from an environmental sample

<221> SIGNAL
<222> (1)...(18)

35 <221> DOMAIN
<222> (19)...(388)
<223> Catalytic domain

40 <400> 114

Met Lys Ile Phe Leu Thr Ile Leu Leu Ser Ala Leu Phe Ser Ile Ser

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	1		5		10		15
	Asn	Ala	Gln	Val	Leu	Ser	Tyr
			20				
	Gln	Leu	Lys	Asn	Gly	Gly	Trp
5			35				
	Val	Asn	Tyr	Ser	Lys	Pro	Leu
			50				
	Gln	Ser	Thr	Glu	Lys	Ser	Ala
			65				
	Glu	Ile	Asn	His	Leu	Leu	Ala
10			85				
	Tyr	Leu	Gln	Ala	Ala	Thr	Lys
			100				
	Asn	Asp	Lys	Gly	Gly	Trp	Pro
			115				
	Arg	Gly	Gln	Ile	Thr	Tyr	Asn
15			130				
	Ile	Leu	Leu	Ser	Ile	Ser	Thr
			145				
	Asn	Lys	Phe	Asn	Glu	Arg	Ile
			165				
	Cys	Ile	Leu	Gln	Thr	Gln	Val
20			180				
	Ala	Ala	Gln	Tyr	Asp	Gln	Lys
			195				
	Phe	Glu	Pro	Val	Ala	Leu	Ala
			210				
	Phe	Leu	Met	Arg	Leu	Asp	His
25			225				
	Asn	His	Ala	Val	Glu	Trp	Phe
			245				
	Tyr	Val	Lys	Thr	Glu	Lys	Asn
			260				
	Ser	Pro	Ala	Ser	Thr	Val	Trp
30			275				
	Gln	Pro	Ile	Phe	Gly	Asp	Arg
			290				
	Glu	Ile	Ser	Glu	Glu	Arg	Gln
			305				
	Pro	Glu	Lys	Ile	Ile	Thr	Lys
35			325				
	Asn	Glu					

<210> 115

<211> 996

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample

<400> 115

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	tgggcgacga	ttctccggca	gccatcgccg	tggtacgcgt	ccgcggacgc	gaaggcgggt	120
	gccgaaaccg	tgcgcgcgag	ccagagagcc	accggcgggt	ggccgaagaa	cacggattgg	180
5	acggcgctcc	agagcgacgc	tgagcggcag	gcgctgcgaa	atgcccgcgc	cgagaccgat	240
	tcgacgatcg	acaatggcgc	cacggtcacc	gagcttcgct	ttctcaccgc	cggtgatgtc	300
	gccacgcgcg	acgagctttt	acgggaggcc	gtgcttcgcg	gcctcgacta	cctgctggcg	360
	tcgcagtaca	gcaacggcgg	ctggccacaa	tactttccgt	tgcggaccga	ttactcgcg	420
	gacatcacgt	tcaacgacga	cgcgatgacc	ggcgtggtgc	tgctgctgaa	ggatgccgcg	480
	gacgggtcag	caggttttcga	attcgtcgac	aaggcgagac	gtgaccgcgc	tgccgcggcc	540
10	gtgacgcgcg	ccatcgcggt	gatcctccgc	acgcagattc	gggtcaacgg	tacgctgacc	600
	ggctgggtgcc	agcagtacga	cgccgacgcg	ctgacgccgg	cgcgcgggcg	ctcgtacgag	660
	catccgtcga	ttgcgagccg	cgagacggtc	gggatcgcg	ggctgctgat	gggcgtgccg	720
	aatccgtcgc	cagagatcgt	ggctgccgtt	gacgcggctg	ccgcatgggt	gggtaaatcg	780
	gaactgaagg	gtgtgcccga	ggcgacggcg	ccaggacttt	gggcgcgctt	ctacgacatc	840
	gctacgaatc	ggccgatcta	ttcgggcccgc	gacggcgctca	tcaagtaccg	gctcgacgag	900

15	atcgagctcg	agcggcgcac	aggctacagc	tggtttggcc	cgtacgccgc	ggcattttctg	960
	acgaccgaat	atccgaaatg	gcgggcggca	cgatga			996

20 <210> 116
<211> 331
<212> PRT
<213> Unknown

25 <220>
<223> Obtained from an environmental sample

<221> DOMAIN
<222> (1)...(331)
30 <223> Catalytic domain

<400> 116

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	Met	Ala	Lys	Ala	Ile	Gly	Gly	Pro	Leu	Pro	Pro	Ala	Pro	Gly	Gln	Gly
	1				5					10					15	
	Ser	Pro	Val	Thr	Trp	Ala	Thr	Ile	Leu	Arg	Gln	Pro	Ser	Pro	Trp	Tyr
				20					25					30		
5	Ala	Ser	Ala	Asp	Ala	Lys	Ala	Val	Ala	Glu	Thr	Val	Arg	Ala	Ser	Gln
			35					40					45			
	Arg	Ala	Thr	Gly	Gly	Trp	Pro	Lys	Asn	Thr	Asp	Trp	Thr	Ala	Leu	Gln
		50					55					60				
	Ser	Asp	Ala	Glu	Arg	Gln	Ala	Leu	Arg	Asn	Ala	Arg	Ala	Glu	Thr	Asp
	65					70					75					80
10	Ser	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Val	Thr	Glu	Leu	Arg	Phe	Leu	Thr
				85						90					95	
	Arg	Val	Tyr	Val	Ala	Thr	Arg	Asp	Glu	Leu	Leu	Arg	Glu	Ala	Val	Leu
				100					105					110		
	Arg	Gly	Leu	Asp	Tyr	Leu	Leu	Ala	Ser	Gln	Tyr	Ser	Asn	Gly	Gly	Trp
			115					120					125			
15	Pro	Gln	Tyr	Phe	Pro	Leu	Arg	Thr	Asp	Tyr	Ser	Arg	Asp	Ile	Thr	Phe
		130					135					140				
	Asn	Asp	Asp	Ala	Met	Thr	Gly	Val	Val	Leu	Leu	Leu	Lys	Asp	Ala	Ala
	145					150				155						160
	Asp	Gly	Ser	Ala	Gly	Phe	Glu	Phe	Val	Asp	Lys	Ala	Arg	Arg	Asp	Arg
				165						170					175	
20	Ala	Ala	Ala	Ala	Val	Thr	Arg	Ala	Ile	Ala	Val	Ile	Leu	Arg	Thr	Gln
				180					185					190		
	Ile	Arg	Val	Asn	Gly	Thr	Leu	Thr	Gly	Trp	Cys	Gln	Gln	Tyr	Asp	Ala
			195					200					205			
	Asp	Ala	Leu	Thr	Pro	Ala	Arg	Gly	Arg	Ser	Tyr	Glu	His	Pro	Ser	Ile
		210					215					220				
25	Ala	Ser	Arg	Glu	Thr	Val	Gly	Ile	Ala	Arg	Leu	Leu	Met	Gly	Val	Pro
						230					235					240
	Asn	Pro	Ser	Pro	Glu	Ile	Val	Ala	Ala	Val	Asp	Ala	Ala	Ala	Ala	Trp
				245						250					255	
	Leu	Gly	Lys	Ser	Glu	Leu	Lys	Gly	Val	Pro	Glu	Ala	Thr	Ala	Pro	Gly
				260					265							
30	Leu	Trp	Ala	Arg	Phe	Tyr	Asp	Ile	Ala	Thr	Asn	Arg	Pro	Ile	Tyr	Ser
			275					280					285			
	Gly	Arg	Asp	Gly	Val	Ile	Lys	Tyr	Arg	Leu	Asp	Glu	Ile	Glu	Leu	Glu
		290					295					300				
	Arg	Arg	Thr	Gly	Tyr	Ser	Trp	Val	Gly	Pro	Tyr	Ala	Ala	Ala	Phe	Leu
		305				310					315					320
35	Thr	Thr	Glu	Tyr	Pro	Lys	Trp	Arg	Ala	Ala	Arg					
					325					330						

<210> 117

<211> 1725

40 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample.

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<400> 117

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	ttttcgtatg	cggcaagcta	tacacccccg	tcaacagcag	tttcgaaaat	caacagctat	120
	cgaggctatt	cggagctgac	ttcagctgca	tccggcatgg	atatcgacca	gtacacctac	180
	aacatgacca	cttggcaaat	cgcaaacggc	ggttttttaca	aagccatggc	cgacaagtat	240
5	aaaagcgcgt	atggcggcgg	tcaaaaatcc	gaatggcaag	ctaaaggcgg	tggcgacctc	300
	ggcactatag	acaacaacgc	caccatccag	gaaatgcgtt	tgctcgccgt	gcgttacaaa	360
	gaaacgacga	acaacaatta	caaataccga	tttaagacaa	gtttcaacaa	ggcgggtcaat	420
	ttttcttttga	ccatgcagcg	ctccaaaggc	ggactcccac	aagtttggcc	caaacgcggc	480
	aactattctg	accaaatac	gctaaatgac	aacgccatga	tccgcgccat	ggtcacgatg	540
	atggatatcg	ccaacaagac	gagtcacatt	gattcggata	tcacgcagca	cgccacccgc	600
10	agcaaaaatga	aatcggctct	cgacaaagcg	gtcgattact	tgctcaaggc	gcaaatcgtg	660
	aacgacggaa	agggtcacgg	atggtgcg	cagcacgaca	ccaacagcct	cgcccccgta	720
	ggcgacagag	cctacgaact	cccagacaaa	tccggcaacg	aatccatggg	cgttgtgtgg	780
	tttttgatga	actggccaga	ccaaaacgaa	gcaatccaga	aggcgggtcaa	aggcgcaatc	840
	gcttgggtaca	aaaagaataa	actaaaagac	aaggcggtta	gcaagaccgc	aggcggttgtg	900
	gacaaggcgg	gttcacgcgt	gtggttccgc	ttttacgaag	tcaacaacga	caactacttt	960
15	ttctgcgacc	gcgatgggtg	tagcaccaag	acgcaggact	tcataaaaat	cagcgaagaa	1020
	cgtcgcaagg	gctaccagtg	ggcaggcgat	tacggctctg	caattctagg	caccgaaaat	1080
	gcataccttg	aagcactcgc	caagatggac	gacaactatg	ttccacctcc	gccagacca	1140
	gctatgtgcg	gaaacgacac	ttgcaaaacg	tacatcgatg	gcgttgactt	tattgacatt	1200
	caaggcgcta	aggaaacaac	caacacggga	ttcgtttggc	aaggttacgc	caacgttgac	1260
	aactccaccg	gaagctatgt	gacctacggc	gtcaccgcat	tcaaggaagg	caaatacact	1320
20	ttgttcatca	gctttgcaaa	cggcgggtgg	tccgcacgcg	gttacagcgt	ttctgcagga	1380
	gacaagacgt	tacttgacga	cggcagcatg	gaatctacag	ccgcatggac	cacttgga	1440
	atgcaatcca	tcgaaatcga	attgccaatg	ggctatagcg	aactcaagtt	cacaagcctt	1500
	tcgaaagacg	gtatggcgaa	catcgattac	atcggctgga	tgaacgatga	tttgaaagtt	1560
	ggcgaagttg	aagtaccacg	ctcatccatt	gaagcaatac	gcgccatccg	caaagcccag	1620
	caggacaacc	gctactttgt	ggactttggc	ggcaacaata	atagcgcagg	ggcttacttt	1680
25	aagcgtggca	tcaacacggt	ccgcgtgaat	gggaagatga	ggtaa		1725

<210> 118

<211> 574

<212> PRT

30 <213> Unknown

<220>

<223> Obtained from an environmental sample.

35 <221> SIGNAL

<222> (1)...(24)

<221> DOMAIN

<222> (25)...(574)

40 <223> Catalytic domain

<400> 118

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	1				5					10					15	
	Ser	Val	Ala	Ser	Phe	Ser	Tyr	Ala	Ala	Ser	Tyr	Thr	Pro	Pro	Ser	Thr
				20				25						30		
5	Ala	Val	Ser	Lys	Ile	Asn	Ser	Tyr	Arg	Gly	Tyr	Ser	Glu	Leu	Thr	Ser
			35					40					45			
	Ala	Ala	Ser	Gly	Met	Asp	Ile	Asp	Gln	Tyr	Thr	Tyr	Asn	Met	Thr	Thr
		50					55					60				
	Trp	Gln	Ile	Ala	Asn	Gly	Gly	Phe	Tyr	Lys	Ala	Met	Ala	Asp	Lys	Tyr
	65					70					75				80	
10	Lys	Ser	Ala	Tyr	Gly	Gly	Gly	Gln	Lys	Ser	Glu	Trp	Gln	Ala	Lys	Gly
					85					90					95	
	Gly	Gly	Asp	Leu	Gly	Thr	Ile	Asp	Asn	Asn	Ala	Thr	Ile	Gln	Glu	Met
				100					105					110		
	Arg	Leu	Leu	Ala	Val	Arg	Tyr	Lys	Glu	Thr	Thr	Asn	Asn	Asn	Tyr	Lys
			115					120					125			
15	Ser	Ala	Phe	Lys	Thr	Ser	Phe	Asn	Lys	Ala	Val	Asn	Phe	Leu	Leu	Thr
		130					135					140				
	Met	Gln	Arg	Ser	Lys	Gly	Gly	Leu	Pro	Gln	Val	Trp	Pro	Lys	Arg	Gly
	145					150					155				160	
	Asn	Tyr	Ser	Asp	Gln	Ile	Thr	Leu	Asn	Asp	Asn	Ala	Met	Ile	Arg	Ala
20					165					170					175	

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	Met	Val	Thr	Met	Met	Asp	Ile	Ala	Asn	Lys	Thr	Ser	Pro	Phe	Asp	Ser
	Asp	Ile	Ile	180	Asp	Ala	Thr	Arg	185	Lys	Met	Lys	Ser	190	Ala	Leu
5	Lys	Ala	Val	195	Asp	Tyr	Leu	Leu	200	Lys	Ala	Gln	Ile	Val	Asn	Asp
	Val	Thr	Val	210	Trp	Cys	Ala	Gln	215	His	Asp	Thr	Asn	Ser	Leu	Ala
	Gly	Ala	Arg	225	Ala	Tyr	Glu	Leu	230	Pro	Ser	Lys	Ser	Gly	Asn	Glu
10	Gly	Val	Val	245	Trp	Phe	Leu	Met	250	Asn	Trp	Pro	Asp	Gln	Asn	Glu
	Gln	Lys	Ala	260	Val	Lys	Gly	Ala	265	Ile	Ala	Trp	Tyr	Lys	Lys	Asn
	Lys	Asp	Lys	275	Ala	Phe	Ser	Lys	280	Thr	Ala	Gly	Val	Val	Asp	Lys
15	Ser	Ser	Leu	290	Trp	Phe	Arg	Phe	295	Tyr	Glu	Val	Asn	Asn	Asp	Asn
	Phe	Cys	Asp	305	Arg	Asp	Gly	Ala	310	Ser	Thr	Lys	Thr	Gln	Asp	Phe
	Ile	Ser	Glu	325	Glu	Arg	Arg	Lys	330	Gly	Tyr	Gln	Trp	Ala	Gly	Asp
20	Ser	Ala	Ile	340	Leu	Gly	Thr	Glu	345	Asn	Ala	Tyr	Leu	Glu	Ala	Leu
	Met	Asp	Asn	355	Tyr	Val	Pro	Pro	360	Pro	Pro	Pro	Ala	Pro	Ala	Met
	Asn	Asp	Thr	370	Cys	Lys	Thr	Tyr	375	Ile	Asp	Gly	Val	Asp	Phe	Ile
25	Gln	Gly	Val	385	Lys	Glu	Thr	Thr	390	Asn	Thr	Gly	Phe	Val	Gly	Glu
	Ala	Asn	Val	405	Asn	Ser	Thr	Gly	410	Ser	Tyr	Val	Thr	Tyr	Gly	Val
	Ala	Phe	Lys	420	Glu	Gly	Lys	Tyr	425	Thr	Leu	Phe	Ile	Ser	Phe	Ala
30	Gly	Gly	Ser	435	Ala	Arg	Gly	Tyr	440	Ser	Val	Ser	Ala	Gly	Asp	Lys
	Leu	Ala	Asp	450	Gly	Ser	Met	Glu	455	Ser	Thr	Ala	Ala	Trp	Thr	Thr
	Met	Gln	Ser	465	Ile	Glu	Ile	Glu	470	Leu	Pro	Met	Gly	Tyr	Ser	Glu
35	Phe	Thr	Ser	485	Leu	Ser	Lys	Asp	490	Gly	Met	Ala	Asn	Ile	Asp	Tyr
	Trp	Met	Asn	500	Asp	Asp	Leu	Lys	505	Val	Gly	Glu	Val	Glu	Val	Pro
	Ser	Ile	Glu	515	Ala	Ile	Arg	Ala	520	Ile	Arg	Lys	Ala	Gln	Gln	Asp
40	Tyr	Phe	Val	530	Asp	Phe	Gly	Gly	535	Asn	Asn	Asn	Ser	Ala	Gly	Ala
	Lys	Arg	Gly	545	Ile	Asn	Thr	Phe	550	Arg	Val	Asn	Gly	Lys	Met	Arg
				565					570							

<210> 119

<211> 1848

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample.

<400> 119

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	tcaaacccct	acacagccgt	atccgctggg	tggaatggca	cacttgcccc	caatgcatcg	120
	gccacttttg	gtttccaggc	aaacgggttct	gccgggtgcac	ctaaaagtga	tggcagcttg	180
	tgcggcacca	acacttcatc	aacaccggca	tccagcagtg	ttgccagctc	ggttaaatca	240
5	agcgcgccc	tatcgtccag	cagcagatca	tccagttcaa	tcgctatcac	tagcagctct	300
	ttagcgagaa	gttctattgc	ctccagcagc	tcactagtta	gtagctccag	agcgagcagt	360
	agtgcgcaa	gcgttttctc	ttttacgatc	caggaagagc	aagcgggctt	ctgtcgtgtt	420
	gatggcattg	cgacagaaa	caccaacacc	ggttttaccg	gcaatggcta	caccaatgcg	480
10							
	aacaacgcgc	aaggcgcagc	gattgaatgg	gcagtcagcg	cācctagcag	tggccgttat	540
	acagtagcct	tccgcttcgc	caatggcggc	acagcagcgc	gcaacggctc	gttggttaatc	600
	aatggcggta	gcaatggtaa	ttacactgtg	gagttacccc	tgaccggcgc	atgggcaacc	660
	tggcaaattg	ccagcgtgga	aattgattta	gtgcaaggca	ataatatatt	aaaactctcg	720
15	gcgttaaccg	ctgacggttt	ggccaatatc	gactcattaa	aaatagacgg	cgcgcaaacc	780
	aaagcaggta	cttgacgac	tacatcaagc	agcagcggtg	ccagcagctc	gtcgtccggt	840
	aaatccagcg	caagttcttc	ttcgaagttc	tccaccgctg	caaaaatact	gacattagac	900
	ggtaaccg	ccgccagctg	gttcaacaaa	tccaggacca	agtgggaatg	cagccgcgcc	960
	gatattgtgt	tgtcttacca	gcaatccaac	ggcgggttgg	caaaaaacct	ggattacaac	1020
	tcagtgcg	caggcaatgg	cgaggagcgc	agcggcacca	tcgacaatgg	tgcaaccatt	1080
20	accgaaatgg	tttacctcgc	tgaattttat	aaaaacggcg	gcaacaccaa	atatcgcgat	1140
	gcagtgcgca	gagcagcaaa	cttttttagtg	agctcgcaat	acagcacagg	cgccttgcca	1200
	caattttatc	cggtgaaagg	cggctatg	gatcatgcga	cctttaacga	taacggcatg	1260
	gcgtacgcgt	tgacgggtatt	ggatttcgca	gtaaacaac	gcgcaccggt	tgataacgac	1320
	attttctctg	attctgatcg	ggcgaaattc	aaaaccgctg	ttgccaaagg	tgtggattac	1380
	attttaaaag	cgcagtggaa	acaaaatgga	aaactcactg	catggtgtgc	acaacacggt	1440
25	gctacggatt	accaaccgaa	aaaagcgcgc	gcttatgaat	tggaaatcatt	gagtggttagc	1500
	gagtcggctg	gcattctcgc	cttcttgatg	acccaaccac	aaaccgcgca	aatcgaagcg	1560
	gcgggtcaagg	cgggtgtcaa	ctggttcgcc	agtccaata	cttatttggc	taactacact	1620
	tacgattcat	caaaagcgtc	taccaaccgc	atttgtgtata	aatccggaag	cagaatgtgg	1680
	tatcgcttct	atgacctgaa	caccaaccgt	ggtttcttta	gtgatcgca	tggcagcaaa	1740
	ttctatgata	tcacccaaat	gtcagaagag	cgtcgcaccg	gttatagctg	gggtggctct	1800
30	tacggtgaat	ctattatttc	cttcgcgcaa	aaagtgggtt	atctgtaa		1848
	<210> 120						
	<211> 615						
	<212> PRT						
35	<213> Unknown						
	<220>						
	<223> Obtained from an environmental sample.						
40	<221> BINDING						
	<222> (1)...(61)						
	<223> Carbohydrate binding module						
	<221> BINDING						
45	<222> (134)...(257)						
	<223> Carbohydrate binding module						
	<221> DOMAIN						
	<222> (258)...(615)						
50	<223> Catalytic domain						
	<400> 120						
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	Met	Ser	Trp	Gln	Glu	Ser	Gly	Ala	Ala	Ile	Thr	Asn	Ala	Trp	Asn	Ala
	1				5					10					15	
	Thr	Leu	Ser	Gly	Ser	Asn	Pro	Tyr	Thr	Ala	Val	Ser	Ala	Gly	Trp	Asn
				20					25					30		
5	Gly	Thr	Leu	Ala	Pro	Asn	Ala	Ser	Ala	Thr	Phe	Gly	Phe	Gln	Ala	Asn
			35					40					45			
	Gly	Ser	Ala	Gly	Ala	Pro	Lys	Val	Asn	Gly	Ser	Leu	Cys	Gly	Thr	Asn
		50					55					60				
	Thr	Ser	Ser	Thr	Pro	Ala	Ser	Ser	Ser	Val	Ala	Ser	Ser	Val	Lys	Ser
	65				70					75					80	
10	Ser	Ala	Pro	Val	Ser	Ser	Ser	Arg	Ser	Ser	Ser	Ser	Ser	Ile	Ala	Ile
				85					90					95		
	Thr	Ser	Ser	Ser	Leu	Ala	Arg	Ser	Ser	Ile	Ala	Ser	Ser	Ser	Ser	Leu
				100					105					110		
	Val	Ser	Ser	Ser	Arg	Ala	Ser	Ser	Ser	Ala	Pro	Ser	Val	Phe	Ser	Phe
			115					120					125			
15	Thr	Ile	Gln	Glu	Glu	Gln	Ala	Gly	Phe	Cys	Arg	Val	Asp	Gly	Ile	Ala
		130					135					140				
	Thr	Glu	Ser	Thr	Asn	Thr	Gly	Phe	Thr	Gly	Asn	Gly	Tyr	Thr	Asn	Ala
	145				150					155					160	
	Asn	Asn	Ala	Gln	Gly	Ala	Ala	Ile	Glu	Trp	Ala	Val	Ser	Ala	Pro	Ser
				165					170					175		
20	Ser	Gly	Arg	Tyr	Thr	Val	Ala	Phe	Arg	Phe	Ala	Asn	Gly	Gly	Thr	Ala

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				180					185				190			
	Ala	Arg	Asn	Gly	Ser	Leu	Leu	Ile	Asn	Gly	Gly	Ser	Asn	Gly	Asn	Tyr
	Thr	Val	Glu	Leu	Pro	Leu	Thr	Gly	Ala	Trp	Ala	Thr	Trp	Gln	Ile	Ala
5		210					215					220				
	Ser	Val	Glu	Ile	Asp	Leu	Val	Gln	Gly	Asn	Asn	Ile	Leu	Lys	Leu	Ser
	225					230					235					240
	Ala	Leu	Thr	Ala	Asp	Gly	Leu	Ala	Asn	Ile	Asp	Ser	Leu	Lys	Ile	Asp
				245						250					255	
10	Gly	Ala	Gln	Thr	Lys	Ala	Gly	Thr	Cys	Ser	Thr	Thr	Ser	Ser	Ser	Ser
				260					265					270		
	Val	Ala	Ser	Ser	Ser	Ser	Ser	Val	Lys	Ser	Ser	Ala	Ser	Ser	Ser	Ser
			275					280					285			
	Ser	Ser	Ser	Thr	Ala	Ala	Lys	Ile	Leu	Thr	Leu	Asp	Gly	Asn	Pro	Ala
		290					295					300				
15	Ala	Ser	Trp	Phe	Asn	Lys	Ser	Arg	Thr	Lys	Trp	Asn	Ser	Ser	Arg	Ala
	305					310					315					320
	Asp	Ile	Val	Leu	Ser	Tyr	Gln	Gln	Ser	Asn	Gly	Gly	Trp	Pro	Lys	Asn
				325						330					335	
	Leu	Asp	Tyr	Asn	Ser	Val	Ser	Ala	Gly	Asn	Gly	Gly	Ser	Asp	Ser	Gly
				340					345					350		
20	Thr	Ile	Asp	Asn	Gly	Ala	Thr	Ile	Thr	Glu	Met	Val	Tyr	Leu	Ala	Glu
			355					360					365			
	Ile	Tyr	Lys	Asn	Gly	Gly	Asn	Thr	Lys	Tyr	Arg	Asp	Ala	Val	Arg	Arg
		370					375					380				
	Ala	Ala	Asn	Phe	Leu	Val	Ser	Ser	Gln	Tyr	Ser	Thr	Gly	Ala	Leu	Pro
	385					390					395					400
25	Gln	Phe	Tyr	Pro	Leu	Lys	Gly	Gly	Tyr	Ala	Asp	His	Ala	Thr	Phe	Asn
				405						410					415	
	Asp	Asn	Gly	Met	Ala	Tyr	Ala	Leu	Thr	Val	Leu	Asp	Phe	Ala	Val	Asn
				420					425					430		
	Lys	Arg	Ala	Pro	Phe	Asp	Asn	Asp	Ile	Phe	Ser	Asp	Ser	Asp	Arg	Ala
			435					440					445			
30	Lys	Phe	Lys	Thr	Ala	Val	Ala	Lys	Gly	Val	Asp	Tyr	Ile	Leu	Lys	Ala
		450					455					460				
	Gln	Trp	Lys	Gln	Asn	Gly	Lys	Leu	Thr	Ala	Trp	Cys	Ala	Gln	His	Gly
	465					470					475					480
	Ala	Thr	Asp	Tyr	Gln	Pro	Lys	Lys	Ala	Arg	Ala	Tyr	Glu	Leu	Glu	Ser
				485						490					495	
35	Leu	Ser	Gly	Ser	Glu	Ser	Val	Gly	Ile	Leu	Ala	Phe	Leu	Met	Thr	Gln
				500					505					510		
	Pro	Gln	Thr	Ala	Gln	Ile	Glu	Ala	Ala	Val	Lys	Ala	Gly	Val	Asn	Trp
			515					520					525			
	Phe	Ala	Ser	Pro	Asn	Thr	Tyr	Leu	Ala	Asn	Tyr	Thr	Tyr	Asp	Ser	Ser
		530					535					540				
40	Lys	Ala	Ser	Thr	Asn	Pro	Ile	Val	Tyr	Lys	Ser	Gly	Ser	Arg	Met	Trp
		545				550					555					560
	Tyr	Arg	Phe	Tyr	Asp	Leu	Asn	Thr	Asn	Arg	Gly	Phe	Phe	Ser	Asp	Arg
				565						570					575	
	Asp	Gly	Ser	Lys	Phe	Tyr	Asp	Ile	Thr	Gln	Met	Ser	Glu	Glu	Arg	Arg
				580					585					590		
45	Thr	Gly	Tyr	Ser	Trp	Gly	Gly	Ser	Tyr	Gly	Glu	Ser	Ile	Ile	Ser	Phe
			595					600					605			
	Ala	Gln	Lys	Val	Gly	Tyr	Leu									
		610					615									

50 <210> 121
 <211> 1047
 <212> DNA
 <213> Bacteria

55 <400> 121

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	acgtttaaata	ggggaacaac	tggtgggtgca	ggtggagatg	tagtgacggg	tcgtacaggg	180
5	aatgaattaa	taaacgcttt	gaagtccaaa	aaccctaatac	gtccggttaac	aatttatgta	240
	aacggtacga	taacacctag	taatacgtct	gatagtaaga	tcgatattaa	ggatgtttcc	300
	aatgtatcga	tttttaggggt	tggtacaaat	ggacgattaa	atgggatcgg	tattaaagta	360

	tggcgagcga	ataatatcat	cattcgcaac	ttgacgatcc	atgaagtcca	tacaggtgat	420
10	aaagatgcga	ttagcattga	agggccctct	cggaacattt	ggattgacca	taacgagctt	480
	tatgccagct	tgaacgttca	taaagaccac	tatgacggct	tgtttgacgt	aaagcgcgat	540
	gcttacaata	ttaccttctc	ttggaattat	gtccatgatg	gctggaaagc	gatgctcatg	600
	gggaactctg	atagtgataa	ctacgaccga	aacataacat	tccaccataa	ctacttcaaa	660
	aacttaaact	ctcgcgtacc	tgcggtaccgt	tttggaaagg	cgcacttggt	tagcaattac	720
	tttgagaaca	ttttagaaac	aggcattaat	tcacggatgg	gagcggaaat	gctcgttgaa	780
15	cataacgttt	ttgagaatgc	caccaacccg	ttaggattct	ggcatagcag	tcgaacaggt	840
	tattggaatg	ttgccaataa	ccgctataat	aatagcacgg	gtagcatgcc	gaccacttcc	900
	acgaccaatt	atcgacctcc	ttatccctat	acggtcacac	cagttgggtga	tgtgaaatcg	960
	gttgtcacac	gttatgcggg	agttgggtgc	atccagccgt	atgcaagaaa	gccatccgag	1020
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20 <210> 122
 <211> 348
 <212> PRT
 <213> Bacteria

25 <220>
 <221> SIGNAL
 <222> (1)...(29)

30 <221> DOMAIN
 <222> (30)...(348)
 <223> Catalytic domain

35 <400> 122

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 Met Leu Trp Leu Gly Val Ser Phe Gln Thr Ala Glu Ala Asn Thr Pro
 20 25 30
 Asn Phe Asn Leu Gln Gly Phe Ala Thr Leu Asn Gly Gly Thr Thr Gly
 35 40 45
 Gly Ala Gly Gly Asp Val Val Thr Val Arg Thr Gly Asn Glu Leu Ile
 50 55 60
 Asn Ala Leu Lys Ser Lys Asn Pro Asn Arg Pro Leu Thr Ile Tyr Val
 65 70 75 80
 Asn Gly Thr Ile Thr Pro Ser Asn Thr Ser Asp Ser Lys Ile Asp Ile
 85 90 95
 Lys Asp Val Ser Asn Val Ser Ile Leu Gly Val Gly Thr Asn Gly Arg
 100 105 110
 Leu Asn Gly Ile Gly Ile Lys Val Trp Arg Ala Asn Asn Ile Ile Ile
 115 120 125
 Arg Asn Leu Thr Ile His Glu Val His Thr Gly Asp Lys Asp Ala Ile
 130 135 140
 Ser Ile Glu Gly Pro Ser Arg Asn Ile Trp Ile Asp His Asn Glu Leu
 145 150 155 160
 Tyr Ala Ser Leu Asn Val His Lys Asp His Tyr Asp Gly Leu Phe Asp
 165 170 175
 Val Lys Arg Asp Ala Tyr Asn Ile Thr Phe Ser Trp Asn Tyr Val His
 180 185 190
 Asp Gly Trp Lys Ala Met Leu Met Gly Asn Ser Asp Ser Asn Tyr
 195 200 205
 Asp Arg Asn Ile Thr Phe His His Asn Tyr Phe Lys Asn Leu Asn Ser
 210 215 220
 Arg Val Pro Ala Tyr Arg Phe Gly Lys Ala His Leu Phe Ser Asn Tyr
 225 230 235 240
 Phe Glu Asn Ile Leu Glu Thr Gly Ile Asn Ser Arg Met Gly Ala Glu
 245 250 255
 Met Leu Val Glu His Asn Val Phe Glu Asn Ala Thr Asn Pro Leu Gly
 260 265 270
 Phe Trp His Ser Ser Arg Thr Gly Tyr Trp Asn Val Ala Asn Asn Arg
 275 280 285
 Tyr Ile Asn Ser Thr Gly Ser Met Pro Thr Thr Ser Thr Thr Asn Tyr
 290 295 300
 Arg Pro Pro Tyr Pro Tyr Thr Val Thr Pro Val Gly Asp Val Lys Ser
 305 310 315 320

 Val Val Thr Arg Tyr Ala Gly Val Gly Val Ile Gln Pro Tyr Ala Arg
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 340 345

<210> 123

<211> 1830

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample.

<400> 123

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	tggggctggg	ccacctgctc	cgatgagtc	gcgacagctt	atactctgaa	cggaggttgc	120
	ttttctgatg	catcttccgt	tactctgaaa	gctcttggca	atgaacaaac	agatgacaaa	180
	caaatcaaac	aggctatcgc	tcagaaagac	atcattatct	tagatgggtc	caatggcgat	240
5	ttcatcccta	atgaatacat	caagatttcg	accaaaaaca	aaaccatcat	tggatatcaac	300
	aacgcccggc	tgtgtacaaa	gttctaccta	accgctgatg	atattacgta	ccttaaagca	360
	caaggactgg	agggactgag	tagtacaaat	caacatacag	gaactctgcc	tgatggcaca	420
	acagtgacct	gtgacgagcg	tgcctttttc	accaagaaaag	ccatcatgga	actccaatat	480
	cagaaaacag	gatcctatac	cctacccaat	aatcaggtta	tcttttattt	agatgccgct	540
	tctgagaata	tcatcatccg	aaatatttcg	ctgatagggc	caggagccgt	agatatagac	600
10	ggagctggacc	tgattaccaa	tcagggttaag	cacgtctgga	ttgaccattg	cacgtttgtg	660
	gactctcaag	atgggtgccct	ggacagcaag	gtatgcgact	gggccaccta	tacctataac	720
	cacttctact	atacagaccg	cagttactca	catgcctaca	cttgcggttg	cggatgggtc	780
	agcaatcatg	aaatgggtgat	tcacatgacc	tttgcatgta	atatctgggg	agcaaaatgt	840
	atgcgctcgtc	tgccgcaagc	agatgactgt	ttcatacacc	ttgtgaacaa	ctatcacaac	900
	tgtcctggca	atagtgtcgg	tatgaccatt	aacagttaca	gcaaagcatt	ggttgagggt	960
15	aactatgctg	ctgcagggtgt	caacaagcca	ttagatggca	gtggggccaa	ccgtaatgta	1020
	acagctaagg	ataatagttt	tgcaaactca	caagccggtt	ctgttgtgtc	tgtgccatac	1080
	gactatacca	agattgcagc	cgccgacgtt	ccagctacgc	tgactggaac	agaggggtgca	1140
	ggcgccacat	taggcaacga	tgcaacatac	attctgtcta	ctattccaac	tgtcgaccga	1200
	caagaaggcg	aatcttcact	ctactatttc	attgatggcc	tggtgggaac	taatagttaa	1260
	ggctattcca	ttatagagtt	taatgatggc	gcaacattgc	tgctgaacaa	taaagagaaa	1320
20	gcatgggtcta	atggtagtgc	aattcaactt	ggtgacgata	attatacgag	tattaaactt	1380
	tctaattggag	cagaaaacat	cttcacagca	cctactggca	aaaaagtaag	tggtattacc	1440
	ttctattctt	atatcaatat	aaaagaagaa	aaactcgact	tcaccaaata	tccagaatat	1500
	ggtttccgca	cctgtttctg	gcagaaagtt	gccaacctca	cttattctgc	gacttctgat	1560
	gacgtacaaa	tcttgaaatc	tcgtgatcca	cagaatactg	acgtggcatc	attccatttc	1620
	actccaacaa	atggttgaag	tttcaaaaat	tcaggtgaac	agctttgttt	cttaatgaaa	1680
25	gtcacctata	gtgatgaaag	cacaggtatc	tctgctatcc	agaaaaaaat	gcctatcgat	1740
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 <213> Unknown

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40 <221> DOMAIN
 <222> (22)...(390)
 <223> Catalytic domain

45 <400> 124

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				20					25					30		
50	Ala	Tyr	Thr	Leu	Asn	Gly	Gly	Cys	Phe	Ser	Asp	Ala	Ser	Ser	Val	Thr
			35					40					45			

55

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	Leu	Lys	Ala	Leu	Gly	Asn	Glu	Gln	Thr	Asp	Asp	Lys	Gln	Ile	Lys	Gln
	50						55					60				
	Ala	Ile	Ala	Gln	Lys	Asp	Ile	Ile	Ile	Leu	Asp	Gly	Ser	Asn	Gly	Asp
5	65					70					75					80
	Phe	Ile	Leu	Asn	Glu	Tyr	Ile	Lys	Ile	Ser	Thr	Lys	Asn	Lys	Thr	Ile
					85					90					95	
	Ile	Gly	Ile	Asn	Asn	Ala	Arg	Leu	Cys	Thr	Lys	Phe	Tyr	Leu	Thr	Ala
				100					105					110		
	Asp	Asp	Ile	Thr	Tyr	Leu	Lys	Ala	Gln	Gly	Leu	Glu	Gly	Leu	Ser	Ser
			115					120					125			
10	Thr	Asn	Gln	His	Thr	Gly	Thr	Leu	Pro	Asp	Gly	Thr	Thr	Val	Thr	Cys
		130					135					140				
	Asp	Glu	Arg	Ala	Phe	Phe	Thr	Lys	Lys	Ala	Ile	Met	Glu	Leu	Gln	Tyr
	145					150					155					160
	Gln	Lys	Thr	Gly	Ser	Tyr	Thr	Leu	Pro	Asn	Lys	Ser	Gly	Ile	Phe	Tyr
					165					170					175	
15	Leu	Asp	Ala	Ala	Ser	Glu	Asn	Ile	Ile	Ile	Arg	Asn	Ile	Ser	Leu	Ile
				180					185					190		
	Gly	Pro	Gly	Ala	Val	Asp	Ile	Asp	Gly	Ala	Asp	Leu	Ile	Thr	Asn	Gln
			195					200					205			
	Gly	Lys	His	Val	Trp	Ile	Asp	His	Cys	Thr	Phe	Val	Asp	Ser	Gln	Asp
		210					215					220				
20	Gly	Ala	Leu	Asp	Ser	Lys	Val	Cys	Asp	Trp	Ala	Thr	Tyr	Thr	Tyr	Asn
	225					230					235					240
	His	Phe	Tyr	Tyr	Thr	Asp	Arg	Ser	Tyr	Ser	His	Ala	Tyr	Thr	Cys	Gly
					245					250					255	
	Cys	Gly	Trp	Val	Ser	Asn	His	Glu	Met	Val	Ile	His	Met	Thr	Phe	Ala
				260					265					270		
25	Cys	Asn	Ile	Trp	Gly	Ala	Lys	Cys	Met	Arg	Arg	Leu	Pro	Gln	Ala	Asp
			275					280					285			
	Asp	Cys	Phe	Ile	His	Leu	Val	Asn	Asn	Tyr	His	Asn	Cys	Pro	Gly	Asn
		290					295					300				
	Ser	Val	Gly	Met	Thr	Ile	Asn	Ser	Tyr	Ser	Lys	Ala	Leu	Val	Glu	Gly
	305					310					315					320
30	Asn	Tyr	Ala	Ala	Ala	Gly	Val	Asn	Lys	Pro	Leu	Asp	Gly	Ser	Gly	Ala
					325					330					335	
	Asn	Arg	Asn	Val	Thr	Ala	Lys	Asp	Asn	Ser	Phe	Ala	Asn	Ser	Gln	Ala
				340					345					350		
	Gly	Ser	Val	Val	Ser	Val	Pro	Tyr	Asp	Tyr	Thr	Lys	Ile	Ala	Ala	Ala
			355					360					365			
35	Asp	Val	Pro	Ala	Thr	Leu	Thr	Gly	Thr	Glu	Gly	Ala	Gly	Ala	Thr	Leu
		370					375					380				
	Gly	Asn	Asp	Ala	Thr	Tyr	Ile	Leu	Ser	Thr	Ile	Pro	Thr	Val	Asp	Arg
	385					390					395					400
	Gln	Glu	Gly	Glu	Ser	Leu	Tyr	Tyr	Phe	Ile	Asp	Gly	Leu	Val	Gly	
					405				410					415		
40	Thr	Asn	Ser	Glu	Gly	Tyr	Ser	Ile	Ile	Glu	Phe	Asn	Asp	Gly	Ala	Thr
				420					425					430		
	Leu	Leu	Leu	Asn	Asn	Lys	Glu	Lys	Ala	Trp	Ser	Asn	Gly	Ser	Ala	Ile
			435					440					445			
	Gln	Leu	Gly	Asp	Asp	Asn	Tyr	Thr	Ser	Ile	Lys	Leu	Ser	Asn	Gly	Ala
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45	Glu	Asn	Ile	Phe	Thr	Ala	Pro	Thr	Gly	Lys	Lys	Val	Ser	Gly	Ile	Thr
	465					470					475					480
	Phe	Tyr	Ser	Tyr	Ile	Asn	Ile	Lys	Glu	Glu	Lys	Leu	Asp	Phe	Thr	Lys
					485					490					495	
	Tyr	Pro	Glu	Tyr	Gly	Phe	Arg	Thr	Cys	Phe	Trp	Gln	Lys	Val	Ala	Asn
				500					505					510		
50	Leu	Thr	Tyr	Ser	Ala	Thr	Ser	Asp	Asp	Val	Gln	Ile	Leu	Lys	Ser	Arg
			515					520					525			
	Asp	Pro	Gln	Asn	Thr	Asp	Val	Ala	Ser	Phe	His	Phe	Thr	Pro	Thr	Asn
		530					535					540				
	Val	Val	Ser	Phe	Lys	Asn	Ser	Gly	Glu	Gln	Leu	Cys	Phe	Leu	Met	Lys
	545					550					555					560
55	Val	Thr	Tyr	Ser	Asp	Glu	Ser	Thr	Gly	Ile	Ser	Ala	Ile	Gln	Lys	Lys
					565					570					575	
	Met	Pro	Ile	Asp	Gly	Val	Thr	Tyr	Asn	Leu	Gln	Gly	Ile	Arg	Ile	Asp
				580					585					590		

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Asn Pro Thr Lys Gly Ile Tyr Ile Gln Asn Gly Lys Lys Ile Ile Ile
 595 600 605
 Lys

5
 <210> 125
 <211> 1170
 <212> DNA
 <213> Unknown

10
 <220>
 <223> Obtained from an environmental sample.

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 <400> 125

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gttgggtgtaa	ataatgctcg	cctatgcacc	aagttctatc	taaccgatga	aataaagact	180
gcgctcgatg	ctgctaattg	aaaatcagca	agttcaacca	gtggagggtg	tacactctca	240
aatggggaaat	cagtgtcaga	acaacgtgaa	taccttactc	gtcaaacaat	tatcgatcta	300
actggcgatg	cttcggaatc	gtgtcagaaa	gcgggcatct	ttagcttcag	tagttgtacc	360
aatatcatca	tgcgaaacct	cgttttgggt	ggccctggcc	catgcatgtg	aggtggcaac	420
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gatagcgaag	atgctgccta	tttgaacact	actatggcat	gcaatatttg	gggctacaag	660
tgcaatcagc	gaatgccaat	ggctcgtgct	ggtaatatct	accttggtga	caacttttac	720
gattgctgctg	gcaatagtg	ggctgttaac	cctcgtaaaa	attctgagtt	cttagtcgag	780
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tttattgatt	gctatacaga	agattcatac	acttttcagc	agagtgggtac	agtgtctgtg	900
ccatacgttt	actctaagtt	tgatgtgcaa	ttagtaccgc	agcaactcaa	taaatatgct	960
ggcgcaacgc	ttacttctcc	gcttgtcata	ggtcgggaag	aggggtgtgt	tactcctatt	1020
agtgtgtgt	ctgttgatag	cgatgtttgt	ttggtcgaat	actattcgct	gactggtaat	1080
cgtgttaaca	cgctcaatag	aggcatcaat	atcgtttagaa	ctattttacgc	caacggcaaa	1140
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 <210> 126
 <211> 389
 <212> PRT
 <213> Unknown

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 <220>
 <223> Obtained from an environmental sample.

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 <221> DOMAIN
 <222> (24)...(325)
 <223> Catalytic domain

<400> 126

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 Leu Asp Gly Ser Asn Gly Asp Phe Thr Ile Ser Ala Thr Met Ser Phe
 20 25 30
 Ser Ser Lys Ser Asn Lys Thr Ile Val Gly Val Asn Asn Ala Arg Leu
 35 40 45
 Cys Thr Lys Phe Tyr Leu Thr Asp Glu Ile Lys Thr Ala Leu Asp Ala
 50 55 60
 Ala Asn Val Lys Ser Ala Ser Ser Thr Ser Gly Gly Gly Thr Leu Ser
 65 70 75 80
 Asn Gly Lys Ser Val Ser Glu Gln Arg Glu Tyr Leu Thr Arg Gln Thr
 85 90 95
 Ile Ile Asp Leu Thr Gly Asp Ala Ser Glu Ser Cys Gln Lys Ala Gly
 100 105 110
 Ile Phe Ser Phe Ser Ser Cys Thr Asn Ile Ile Met Arg Asn Leu Val
 115 120 125
 Leu Val Gly Pro Gly Pro Cys Asp Val Gly Gly Asn Asp Leu Leu Ser
 130 135 140
 Leu Thr Gly Ser Lys His Phe Trp Val Asp His Cys Glu Leu Thr Asp
 145 150 155 160

 Gly Ile Asp Gly Asn Phe Asp Ile Thr Lys Ser Ser Asp Phe Asn Thr
 165 170 175
 Val Thr Trp Cys Ile Phe Asn Tyr Thr Asp Arg Ala Tyr Asp His Met
 180 185 190
 Asn Ser Asn Leu Ile Gly Ser Ser Asp Ser Glu Asp Ala Ala Tyr Leu
 195 200 205
 Asn Thr Thr Met Ala Cys Asn Ile Trp Gly Tyr Lys Cys Asn Gln Arg
 210 215 220
 Met Pro Met Ala Arg Ala Gly Asn Ile His Leu Val Asn Asn Phe Tyr
 225 230 235 240
 Asp Cys Ala Gly Asn Ser Val Ala Val Asn Pro Arg Lys Asn Ser Glu
 245 250 255
 Phe Leu Val Glu Asn Cys Tyr Phe Ala Thr Gly Val Lys Pro Phe Ser
 260 265 270
 Gln Ser Gly Ala Leu Gly Tyr Asn Phe Ile Asp Cys Tyr Thr Glu Asp
 275 280 285
 Ser Tyr Thr Phe Gln Gln Ser Gly Thr Val Ser Val Pro Tyr Val Tyr
 290 295 300
 Ser Lys Phe Asp Val Gln Leu Val Pro Glu Gln Leu Asn Lys Tyr Ala
 305 310 315 320
 Gly Ala Thr Leu Thr Ser Pro Leu Val Ile Gly Arg Glu Glu Gly Val
 325 330 335
 Val Thr Pro Ile Ser Ala Val Ser Val Asp Ser Asp Val Val Leu Val
 340 345 350
 Glu Tyr Tyr Ser Leu Thr Gly Asn Arg Val Asn Thr Leu Asn Arg Gly
 355 360 365
 Ile Asn Ile Val Arg Thr Ile Tyr Ala Asn Gly Lys Val Thr Thr Gln
 370 375 380
 Lys Val Leu Val Lys
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<210> 127

<211> 1449

<212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample.

<400> 127

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	ctggcacagg	caaataacct	ggcaattaca	ggccccggag	ccggggctga	tggttccagc	120
	aaagccagtg	gcagtagcta	cggcgatgta	aaagacgccg	atctgcaaag	ctactggcaa	180
	ccgcctgcta	ataacggcca	aagagtgtcg	gttaagtgga	gcagcgctat	cagcgttaat	240
5	caggtaatac	tgcgtgaaca	gggcagtaat	gtaaccagct	ggcggctggt	aaataatgac	300
	aacggcgcag	tattggcaac	gggcaccagc	attggcagca	acagaacggt	taacttcagc	360
	actgtaagca	cgaaaaaact	caatctggaa	atactaactg	ccagcggtgc	cccgcgcatt	420
	gctgagtttg	aagttttattt	aaataaccaat	ggcggcaacc	cgccaaatcc	tactgaccgg	480
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<400> 128

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	Gln	Val	Ile	Leu	Arg	Glu	Gln	Gly	Ser	Asn	Val	Thr	Ser	Trp	Arg	Leu
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<213> Bacillus halodurans ATCC 27557

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	tctaattgga	cgtcggctcag	tgaggctcgc	gaattggcta	ctcgtcaaac	gttgattgat	300
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15	gatggcatcg	atggcaactt	cgacatcacc	aagagtagcg	acttcaacac	cgtttcgtgg	540
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	tccgattcag	agaatgcggc	ttaccttaat	actactatgg	cttccaacgt	ctggggcaat	660
	aagtgcaatc	agcgtatgcc	tatggctcgt	gccggtaata	ttcacctcgt	aaataattat	720
	tacaactgcc	ctggcaatag	cgtggctgtg	aatcctcgca	aaaactcaga	atTTTTTggtg	780
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20	ctattttatcg	attgctacac	cgaagatact	tacaccttcc	agaaatctgg	ctctactacg	900
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	gtctcagtc	ttgctgcaaa	tagcgatgtc	atatctgtag	aatactattc	gctcactggc	1080
	aagcgcac	gcgaaccaac	taaaggcatc	aatatcgtaa	gaactattta	tactaacggc	1140
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<213> Bacillus halodurans ATCC 27557

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			35					40				45				
45	Cys	Thr	Lys	Phe	Tyr	Ile	Thr	Pro	Glu	Ile	Lys	Glu	Ala	Leu	Asp	Ala
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	65					70					75				80	
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	Gly	Ile	Phe	Gly	Phe	Ser	Asn	Cys	Thr	Asn	Ile	Ile	Met	Arg	Asn	Ile
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 Asp Gly Ile Asp Gly Asn Phe Asp Ile Thr Lys Ser Ser Asp Phe Asn
 165 170 175
 5 Thr Val Ser Trp Cys Thr Phe Ser Tyr Thr Asp Arg Ala Tyr Asp His
 180 185 190
 Met Asn Ser Asn Leu Ile Gly Ser Asp Ser Glu Asn Ala Ala Tyr
 195 200 205
 10 Leu Asn Thr Thr Met Ala Ser Asn Val Trp Gly Asn Lys Cys Asn Gln
 210 215 220
 Arg Met Pro Met Ala Arg Ala Gly Asn Ile His Leu Val Asn Asn Tyr
 225 230 235 240
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 245 250 255
 15 Glu Phe Leu Val Glu Asn Cys Tyr Phe Ala Ser Gly Val Lys Pro Phe
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 275 280 285
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 290 295 300
 20 Tyr Ser Lys Phe Asp Ala Gln Leu Val Pro Glu Gln Leu Thr Gln Phe
 305 310 315 320
 Ala Gly Ala Thr Leu Thr Ser Pro Leu Val Ile Gly Arg Glu Ser Glu
 325 330 335
 Asn Val Thr Pro Val Ser Val Ile Ala Ala Asn Ser Asp Val Ile Ser
 340 345 350
 25 Val Glu Tyr Tyr Ser Leu Thr Gly Lys Arg Ile Ser Glu Pro Thr Lys
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caatacacga	caggcgcctt	gccacaattt	tatccggttg	aaggcggcta	tgcggatcat	360
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aaacgcgcac	cgtttgataa	cgacattttc	tctgattctg	atcgggcgaa	attcaaaacc	480
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15      50      55      60
      Gly Ala Thr Ile Thr Glu Met Val Tyr Leu Ala Glu Ile Tyr Lys Asn
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      Leu Val Ser Ser Gln Tyr Ser Thr Gly Ala Leu Pro Gln Phe Tyr Pro
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      Ala Tyr Ala Leu Thr Val Leu Asp Phe Ala Val Asn Lys Arg Ala Pro
      130      135      140
      Phe Asp Asn Asp Ile Phe Ser Asp Ser Asp Arg Ala Lys Phe Lys Thr
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      Ala Val Ala Lys Gly Val Asp Tyr Ile Leu Lys Ala Gln Trp Lys Gln
      165      170      175
      Asn Gly Lys Leu Thr Ala Trp Cys Ala Gln His Gly Ala Thr Asp Tyr
      180      185      190
      Gln Pro Lys Lys Ala Arg Ala Tyr Glu Leu Glu Ser Leu Ser Gly Ser
30      195      200      205
      Glu Ser Val Gly Ile Leu Ala Phe Leu Met Thr Gln Pro Gln Thr Ala
      210      215      220
      Gln Ile Glu Ala Ala Val Lys Ala Gly Val Asn Trp Phe Ala Ser Pro
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      Asn Pro Ile Val Tyr Lys Ser Gly Ser Arg Met Trp Tyr Arg Phe Tyr
      260      265      270
      Asp Leu Asn Thr Asn Arg Gly Phe Phe Ser Asp Arg Asp Gly Ser Lys
      275      280      285
      Phe Tyr Asp Ile Thr Gln Met Ser Glu Glu Arg Arg Thr Gly Tyr Ser
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<211> 972

<212> DNA

<213> Artificial Sequence

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<223> Synthetically generated polynucleotide

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	ggcggcaaca	ccaaatatcg	cgatgcagtg	cgcagagcag	caaacttttt	agtgagctcg	300
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	tttagtgatc	gcgatggcag	caaattctat	gatatcacc	aatgtcaga	agagcgtcgc	900

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<210> 134
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<220>
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<400> 134

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 Ser Tyr Gln Gln Ser Asn Gly Gly Trp Pro Lys Asn Leu Asp Tyr Asn
 35 40 45
 Ser Val Ser Ala Gly Asn Gly Gly Ser Asp Ser Gly Thr Ile Asp Asn
 50 55 60
 Gly Ala Thr Ile Thr Glu Met Val Tyr Leu Ala Glu Ile Tyr Lys Asn
 65 70 75 80
 Gly Gly Asn Thr Lys Tyr Arg Asp Ala Val Arg Arg Ala Ala Asn Phe
 85 90 95
 Leu Val Ser Ser Gln Tyr Ser Thr Gly Ala Leu Pro Gln Phe Tyr Pro
 100 105 110
 Leu Lys Gly Gly Tyr His Asp His Ala Thr Phe Asn Asp Asn Gly Met
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 Ala Tyr Ala Leu Thr Val Leu Asp Phe Ala Val Asn Lys Arg Ala Pro
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 Phe Asp Asn Asp Ile Phe Ser Asp Ser Asp Arg Ala Lys Phe Lys Thr
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 Asn Gly Lys Leu Thr Ala Trp Cys Ala Gln His Gly Ala Leu Asp Tyr
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 Gln Pro Lys Lys Gly Arg Ala Tyr Glu Leu Glu Ser Leu Ser Gly Lys
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 Phe Tyr Asp Ile Thr Gln Met Ser Glu Glu Arg Arg Thr Gly Tyr Ser
 290 295 300
 Trp Gly Gly Ser Trp Gly Glu Val Ile Ile Ser Phe Ala Gln Lys Val
 305 310 315 320
 Gly Tyr Leu

Claims

1. An isolated, synthetic or recombinant nucleic acid comprising

(a) a nucleic acid sequence as set forth in SEQ ID NO:99;

(b) a nucleic acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:99,

wherein the nucleic acid encodes at least one polypeptide having a pectate lyase activity,

(c) a nucleic acid that hybridizes under stringent hybridization conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:99, wherein the stringent hybridization conditions comprise a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes, and wherein the nucleic acid encodes at least one polypeptide having a pectate lyase activity;

(d) a nucleic acid encoding a polypeptide having a sequence as set forth in SEQ ID NO:100, wherein the nucleic acid encodes a polypeptide having a pectate lyase activity;

(e) the nucleic acid sequence of (a), (b), (c) or (d), but lacking nucleic acid sequence encoding a signal sequence;

(f) the nucleic acid sequence of any of (a) to (e), further comprising a nucleic acid sequence coding for a heterologous peptide or polypeptide; or

(g) a nucleic acid sequence completely complementary to any of (a) through (f).

2. An expression cassette or a vector comprising the nucleic acid of claim 1.

3. An isolated or transformed cell comprising (a) the nucleic acid of claim 1 or (b) the expression cassette or vector of claim 2.
- 5 4. A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid of claim 1; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.
- 10 5. A transgenic non-human animal comprising (a) the nucleic acid of claim 1 or (b) the expression cassette or vector of claim 2.
6. An isolated, synthetic or recombinant polypeptide comprising:
 - (a) an amino acid sequence as set forth in SEQ ID NO:100;
 - 15 (b) an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:100, wherein the polypeptide has a pectate lyase activity,
 - (c) a polypeptide having a pectate lyase activity encoded by the nucleic acid of claim 1;
 - (d) an amino acid sequence of (a), (b) or (c) having a pectate lyase activity, and having at least one conservative amino acid residue substitution which comprises substituting one amino acid for another of like characteristics,
 - 20 (e) the amino acid sequence of any of (a) through (d), but lacking a signal sequence;
 - (f) the amino acid sequence of any of (a) through (e) further comprising a heterologous peptide or polypeptide; or
 - (g) the polypeptide of any of (a) through (f), wherein the polypeptide comprises at least one glycosylation site or is glycosylated.
- 25 7. A composition comprising a polypeptide as set forth in claim 6, wherein the composition is selected from: a detergent composition, a feed, a food, a feed composition, a food composition, a fiber, thread, textile, fabric, paper, paper product, paper pulp, wood pulp and cellulosic material.
- 30 8. An isolated or recombinant antibody that specifically binds to a polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO:100.
9. A method for hydrolyzing, breaking up or disrupting a pectin-, pectate (polygalacturonic acid)-, homogalacturonan-, rhamnogalacturonan- or plant cell wall-comprising composition comprising the following steps:
 - 35 (a) providing the polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
 - (b) providing a composition comprising a pectin, a pectate, homogalacturonan, rhamnogalacturonan or plant cell wall; and
 - (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide hydrolyzes, breaks up or disrupts the said composition.
- 40 10. A method for liquefying or removing a pectin, a pectate (polygalacturonic acid), a homogalacturonan or a rhamnogalacturonan from a composition comprising the following steps:
 - 45 (a) providing the polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
 - (b) providing a composition comprising a pectin, a pectate (polygalacturonic acid), a homogalacturonan or a rhamnogalacturonan; and
 - (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide removes or liquefies the pectin, pectate (polygalacturonic acid), homogalacturonan or a rhamnogalacturonan.
- 50 11. A formulation for treating a material with a pectate lyase comprising a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1, wherein the formulation comprises a dosage of pectate lyase between about 0.1 ml to about 1 ml per kg of material.
- 55 12. A bioscouring process comprising the following steps:
 - (a) providing a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
 - (b) providing a pectin-, polygalacturonic acid- (pectate-), homogalacturonan-, rhamnogalacturonan- or plant cell

wall-comprising material;

(c) contacting the pectate lyase of (a) with the material of (b) under conditions comprising

- i) between about pH 8.5 to about pH 10.0,
- ii) a bicarbonate buffer
- iii) a non-ionic wetting agent at, about 1 g/L,
- iv) a liquor ratio between about 10:1 to 50:1 L liquor:kg of material,
- v) a dose between about 0.1 and about 1 ml per kg of material, or equivalent,
- vi) a temperature range between about 70°C to about 95°C,
- vii) a treatment time of about 20 min, or
- viii) a combination of any of i) through vii);

further comprising the use of a chelant, wherein the chelant is excluded from the enzyme bath and is added after about 20 minutes of enzyme treatment and retained for about 10 minutes before discharging bath.

13. A method of washing an object comprising:

- (a) providing a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
- (b) providing an object; and
- (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

14. A method of preparing a fruit or vegetable juice, syrup, puree or extract comprising the following steps:

- (a) providing a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
- (b) providing a composition or a liquid comprising a fruit or vegetable material; and
- (c) contacting the polypeptide of step (a) and the composition, thereby preparing the fruit or vegetable juice, syrup, puree or extract.

15. A method of treating a paper, a paper product, a wood pulp or a cellulosic material comprising the following steps:

- (a) providing a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
- (b) providing a composition comprising a paper, a paper product, a wood pulp or a cellulosic material; and
- (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the pectate lyase can treat the paper, paper product, paper pulp, wood pulp or cellulosic material.

16. A method of improving the extraction of oil from an oil-rich plant material comprising the following steps:

- (a) providing a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
- (b) providing an oil-rich plant material; and
- (c) contacting the polypeptide of step (a) and the oil-rich plant material.

17. A method of fiber, thread, textile, fabric or cellulosic material scouring comprising the following steps:

- (a) providing a polypeptide of claim 6, or a polypeptide encoded by the nucleic acid of claim 1;
- (b) providing a fiber, a thread, a textile, a fabric or a cellulosic material; and
- (c) contacting the polypeptide of step (a) and the fiber, thread, textile, fabric or cellulosic material of step (b) under conditions wherein the pectate lyase can scour the fiber, thread, textile, fabric or cellulosic material.

Patentansprüche

1. Isolierte, synthetische oder rekombinante Nucleinsäure, die Folgendes umfasst:

- (a) eine Nucleinsäuresequenz nach SEQ ID Nr. 99;
- (b) eine Nucleinsäuresequenz mit wenigstens 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % oder mehr Sequenzidentität mit SEQ ID Nr. 99, wobei die Nucleinsäure für wenigstens ein Polypeptid mit einer Pektatlyaseaktivität kodiert,

- (c) eine Nucleinsäure, die unter strengen Hybridisierungsbedingungen in eine Nucleinsäure hybridisiert, welche eine Sequenz nach SEQ ID Nr. 99 umfasst, wobei die strengen Hybridisierungsbedingungen einen Waschschrift umfassen, der eine Wäsche in 0,2X SSC, bei einer Temperatur von etwa 65 °C über etwa 15 Minuten umfasst und wobei die Nucleinsäure für wenigstens ein Polypeptid mit einer Pektatlyaseaktivität kodiert;
- 5 (d) eine Nucleinsäure, die für ein Polypeptid mit einer Sequenz nach SEQ ID Nr. 100 kodiert, wobei die Nucleinsäure für ein Polypeptid mit einer Pektatlyaseaktivität kodiert;
- (e) die Nucleinsäuresequenz nach (a), (b), (c) oder (d), aber ohne eine Nucleinsäuresequenz, die für eine Signalsequenz kodiert;
- 10 (f) die Nucleinsäuresequenz nach einem aus (a) bis (e), die ferner eine Nucleinsäuresequenz umfasst, die für ein heterologes Peptid oder Polypeptid kodiert; oder
- (g) eine Nucleinsäuresequenz, die zu einem beliebigen aus (a) bis einschließlich (f) vollständig komplementär ist.
2. Expressionskassette oder Vektor, umfassend die Nucleinsäure nach Anspruch 1.
- 15 3. Isolierte oder transformierte Zelle, umfassend (a) die Nucleinsäure nach Anspruch 1 oder (b) die Expressionskassette oder den Vektor nach Anspruch 2.
4. Verfahren zum Produzieren eines rekombinanten Polypeptids, das die folgenden Schritte umfasst: (a) Bereitstellen einer Nucleinsäure nach Anspruch 1; und (b) Expressieren der Nucleinsäure aus Schritt (a) unter Bedingungen, die
- 20 eine Expression des Polypeptids zulassen, so dass ein rekombinantes Polypeptid produziert wird.
5. Transgenes nicht-humanes Tier, umfassend (a) die Nucleinsäure nach Anspruch 1 oder (b) die Expressionskassette oder den Vektor nach Anspruch 2.
- 25 6. Isoliertes, synthetisches oder rekombinantes Polypeptid, Folgendes umfassend:
- (a) eine Aminosäuresequenz nach SEQ ID Nr. 100;
- (b) eine Aminosäuresequenz mit wenigstens 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % oder mehr Sequenzidentität mit SEQ ID Nr. 100,
- 30 wobei das Polypeptid eine Pektatlyaseaktivität hat,
- (c) ein Polypeptid mit einer Pektatlyaseaktivität, für die die Nucleinsäure nach Anspruch 1 kodiert;
- (d) eine Aminosäuresequenz nach (a), (b) oder (c) mit einer Pektatlyaseaktivität und mit wenigstens einer konservativen Aminosäurerests substitution, umfassend das Substituieren einer Aminosäure für eine andere mit ähnlichen Eigenschaften, wobei alle Substitutionen konservativ sind und das Polypeptid im Wesentlichen Pektatlyaseaktivität beibehält;
- 35 (e) die Aminosäuresequenz nach einem beliebigen von (a) bis einschließlich (d), aber ohne eine Signalsequenz;
- (f) die Aminosäuresequenz nach einem beliebigen von (a) bis einschließlich (e), ferner ein heterologes Peptid oder Polypeptid umfassend; oder
- (g) das Polypeptid nach einem beliebigen von (a) bis einschließlich (f), wobei das Polypeptid wenigstens eine
- 40 Glykosylierungsstelle umfasst oder glykosyliert ist.
7. Zusammensetzung, umfassend ein Polypeptid nach Anspruch 6, wobei die Zusammensetzung ausgewählt ist aus: einer Reinigungsmittelzusammensetzung, einem Futter, einem Nahrungsmittel, einer Futterzusammensetzung, einer Nahrungsmittelzusammensetzung, einer Faser, Faden, Textilie, Stoff, Papier, Papierprodukt, Papierzellstoff, Holzzellstoff und Zellulosematerial.
- 45 8. Isolierter oder rekombinanter Antikörper, der spezifisch an das Polypeptid bindet, das aus der Aminosäuresequenz nach SEQ ID Nr. 100 besteht.
- 50 9. Verfahren zum Hydrolysieren, Aufbrechen oder Disruptieren einer Pektin-, Pektat-(Polygalacturonsäure)-, Homogalacturonan-, Rhamnogalacturonan- oder Pflanzenzellwandumfassenden Zusammensetzung, das die folgenden Schritte umfasst:
- (a) Bereitstellen des Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
- 55 (b) Bereitstellen einer Zusammensetzung, die ein Pektin, ein Pektat, Homogalacturonan, Rhamnogalacturonan oder eine Pflanzenzellwand umfasst; und
- (c) Inkontaktbringen des Polypeptids aus Schritt (a) mit der Zusammensetzung aus Schritt (b) unter Bedingun-

gen, bei denen das Polypeptid die Zusammensetzung hydrolysiert, aufbricht oder disruptiert.

10. Verfahren zum Verflüssigen oder Entfernen eines Pektins, eines Pektats (Polygalacturonsäure), eines Homogalacturonans oder eines Rhamnogalacturonans aus einer Zusammensetzung, das die folgenden Schritte umfasst:

(a) Bereitstellen des Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
 (b) Bereitstellen einer Zusammensetzung, umfassend ein Pektin, ein Pektat (Polygalacturonsäure), ein Homogalacturonan oder ein Rhamnogalacturonan; und
 (c) Inkontaktbringen des Polypeptids aus Schritt (a) mit der Zusammensetzung aus Schritt (b) unter Bedingungen, bei denen das Polypeptid das Pektin, das Pektat (Polygalacturonsäure), das Homogalacturonan oder ein Rhamnogalacturonan entfernt oder verflüssigt.

11. Formulierung zum Behandeln eines Materials mit einer Pektatlyase, umfassend ein Polypeptid nach Anspruch 6 oder ein Polypeptid, für das die Nucleinsäure nach Anspruch 1 kodiert, wobei die Formulierung eine Dosierung von Pektatlyase von zwischen etwa 0,1 ml bis etwa 1 ml pro kg Material umfasst.

12. Bio-Scouring-Prozess, der die folgenden Schritte umfasst:

(a) Bereitstellen eines Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
 (b) Bereitstellen eines Pektin-, Polygalacturonsäure-(Pektat-), Homogalacturonan-, Rhamnogalacturonan- oder Pflanzenzellwand-umfassenden Materials;
 (c) Inkontaktbringen der Pektatlyase aus (a) mit dem Material aus (b) unter Bedingungen, die die Folgenden umfassen

- i) von einem pH-Wert von etwa 8,5 bis zu einem pH-Wert von etwa 10,0,
 ii) einen Bicarbonatpuffer
 iii) ein nichtionisches Benetzungsmittel mit etwa 1 g/L,
 iv) ein Liquorverhältnis von etwa 10:1 bis 50:1 L Liquor:kg Material,
 v) eine Dosis zwischen etwa 0,1 und etwa 1 ml pro kg Material, oder entsprechend,
 vi) eine Temperatur im Bereich von etwa 70 °C bis etwa 95 °C,
 vii) eine Behandlungszeit von etwa 20 min oder
 viii) eine Kombination von beliebigen von i) bis einschließlich vii);

ferner den Gebrauch eines Chelatliganden umfassend, wobei der Chelatligand aus dem Enzymbad ausgenommen ist und nach etwa 20 Minuten Enzymbehandlung zugegeben wird und etwa 10 Minuten beibehalten wird, bevor das Bad abgelassen wird.

13. Verfahren zum Waschen eines Gegenstands, Folgendes umfassend:

(a) Bereitstellen eines Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
 (b) Bereitstellen eines Gegenstands; und
 (c) Inkontaktbringen des Polypeptids aus Schritt (a) und des Gegenstands aus Schritt (b) unter Bedingungen, wobei die Zusammensetzung den Gegenstand waschen kann.

14. Verfahren zum Herstellen eines Frucht- oder Pflanzensafts, -sirups, -pürees oder -extrakts, das die folgenden Schritte umfasst:

(a) Bereitstellen eines Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
 (b) Bereitstellen einer Zusammensetzung oder einer Flüssigkeit, die ein Frucht- oder Pflanzenmaterial umfasst; und
 (c) Inkontaktbringen des Polypeptids aus Schritt (a) und der Zusammensetzung, so dass der/das Frucht- oder Pflanzensaft, -sirup, -püree oder -extrakt hergestellt wird.

15. Verfahren zum Behandeln eines Papiers, eines Papierprodukts, eines Holzzellstoffs oder eines Zellulosematerials, die folgenden Schritte umfassend:

- (a) Bereitstellen eines Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
- (b) Bereitstellen einer Zusammensetzung, umfassend ein Papier, ein Papierprodukt, einen Holzzellstoff oder ein Zellulosematerial; und
- (c) Inkontaktbringen des Polypeptids aus Schritt (a) und der Zusammensetzung aus Schritt (b) unter Bedingungen, unter denen die Pektatlyase das Papier, das Papierprodukt, den Papierzellstoff, den Holzzellstoff oder das Zellulosematerial behandeln kann.

16. Verfahren zum Verbessern der Extraktion von Öl aus einem ölreichen Pflanzenmaterial, das die folgenden Schritte umfasst:

- (a) Bereitstellen eines Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
- (b) Bereitstellen eines ölreichen Pflanzenmaterials; und
- (c) Inkontaktbringen des Polypeptids aus Schritt (a) und des ölreichen Pflanzenmaterials.

17. Verfahren zum Faser-, Faden-, Textil-, Stoff- oder Zellulosematerial-Scouring (Entfetten), das die folgenden Schritte umfasst:

- (a) Bereitstellen eines Polypeptids nach Anspruch 6 oder eines Polypeptids, für das die Nucleinsäure nach Anspruch 1 kodiert;
- (b) Bereitstellen einer Faser, eines Fadens, einer Textilie, eines Stoffs oder eines Zellulosematerials; und
- (c) Inkontaktbringen des Polypeptids aus Schritt (a) und der Faser, des Fadens, der Textilie, des Stoffs oder des Zellulosematerials aus Schritt (b) unter Bedingungen, unter denen die Pektatlyase die Faser, den Faden, die Textilie, den Stoff oder das Zellulosematerial entfetten kann.

Revendications

1. Acide nucléique isolé, synthétique ou recombinant comprenant

- (a) une séquence d'acide nucléique telle que précisée dans SEQ ID N° 99 ;
- (b) une séquence d'acide nucléique ayant au moins 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % ou plus d'identité de séquence à SEQ ID N° 99, dans lequel l'acide nucléique code au moins un polypeptide ayant une activité de pectate lyase,
- (c) un acide nucléique qui s'hybride dans des conditions d'hybridation stringentes avec un acide nucléique comprenant une séquence telle que précisée dans SEQ ID N° 99, dans lequel les conditions d'hybridation stringentes comprennent une étape de lavage comprenant un lavage dans 0,2X SSC à une température d'environ 65 °C pendant environ 15 minutes, et dans lequel l'acide nucléique code au moins un polypeptide ayant une activité de pectate lyase ;
- (d) un acide nucléique codant un polypeptide ayant une séquence telle que précisée dans SEQ ID N° 100, dans lequel l'acide nucléique code un polypeptide ayant une activité de pectate lyase ;
- (e) la séquence d'acide nucléique de (a), (b), (c) ou (d), mais manquant d'une séquence d'acide nucléique codant une séquence signal ;
- (f) la séquence d'acide nucléique de l'un quelconque de (a) à (e), comprenant en outre une séquence d'acide nucléique codant pour un peptide ou polypeptide hétérologue ; ou
- (g) une séquence d'acide nucléique complètement complémentaire de l'un quelconque de (a) à (f).

2. Cassette d'expression ou vecteur comprenant l'acide nucléique de la revendication 1.

3. Cellule isolée ou transformée comprenant (a) l'acide nucléique de la revendication 1 ou (b) la cassette d'expression ou le vecteur de la revendication 2.

4. Procédé de production d'un polypeptide recombinant comprenant les étapes de : (a) fourniture d'un acide nucléique de la revendication 1 ; et (b) expression de l'acide nucléique de l'étape (a) dans des conditions qui permettent

l'expression du polypeptide, produisant ainsi un polypeptide recombinant.

5. Animal non humain transgénique comprenant (a) l'acide nucléique de la revendication 1 ou (b) la cassette d'expression ou le vecteur de la revendication 2.

6. Polypeptide isolé, synthétique ou recombinant comprenant :

(a) une séquence d'acides aminés telle que précisée dans SEQ ID N° 100 ;

(b) un séquence d'acides aminés ayant au moins environ 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % d'identité de séquence à SEQ ID N° 100, dans lequel le polypeptide a une activité de pectate lyase,

(c) un polypeptide ayant une activité de pectate lyase codé par l'acide nucléique de la revendication 1 ;

(d) une séquence d'acides aminés de (a), (b) ou (c) ayant une activité de pectate lyase et ayant au moins un remplacement de résidu d'acides aminés conservatif qui comprend le remplacement d'un acide aminé par un autre aux caractéristiques similaires, tous les remplacements étant conservatifs et le polypeptide conservant essentiellement une activité de pectate lyase ;

(e) la séquence d'acides aminés de l'un quelconque de (a) à (d), mais manquant d'une séquence signal ;

(f) la séquence d'acides aminés de l'un quelconque de (a) à (e) comprenant en outre un peptide ou polypeptide hétérologue ; ou

(g) le polypeptide de l'un quelconque de (a) à (f), dans lequel le polypeptide comprend au moins un site de glycosylation ou est glycosylé.

7. Composition comprenant un polypeptide tel que précisé dans la revendication 6, dans lequel la composition est choisie parmi : une composition détergente, une nourriture, un aliment, une composition de nourriture, une composition alimentaire, une fibre, un fil, un textile, une étoffe, un papier, un produit de papier, de la pâte à papier, de la pâte de bois et une matière cellulosique.

8. Anticorps isolé ou recombinant qui se lie spécifiquement au polypeptide constitué de la séquence d'acides aminés telle que précisée dans SEQ ID N° 100.

9. Procédé d'hydrolyse, de décomposition ou de rupture d'une composition comprenant de la pectine, du pectate (poly(acide galacturonique)), de l'homogalacturonane, du rhamnogalacturonane ou une paroi cellulaire végétale comprenant les étapes suivantes :

(a) la fourniture du polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;

(b) la fourniture d'une composition comprenant une pectine, un pectate, un homogalacturonane, un rhamnogalacturonane ou une paroi cellulaire végétale ; et

(c) la mise en contact du polypeptide de l'étape (a) avec la composition de l'étape (b) dans des conditions dans lesquelles le polypeptide hydrolyse, décompose ou rompt ladite composition.

10. Procédé de liquéfaction ou d'élimination d'une pectine, d'un pectate (poly(acide galacturonique)), d'un homogalacturonane ou d'un rhamnogalacturonane d'une composition comprenant :

(a) la fourniture du polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;

(b) la fourniture d'une composition comprenant une pectine, un pectate (poly(acide galacturonique)), un homogalacturonane ou un rhamnogalacturonane, et

(c) la mise en contact du polypeptide de l'étape (a) avec la composition de l'étape (b) dans des conditions dans lesquelles le polypeptide élimine ou liquéfie la pectine, le pectate (poly(acide galacturonique)), l'homogalacturonane ou le rhamnogalacturonane.

11. Formulation de traitement d'une matière avec une pectate lyase comprenant un polypeptide de la revendication 6 ou un polypeptide codé par l'acide nucléique de la revendication 1, dans laquelle la formulation comprend un dosage de pectate lyase entre environ 0,1 ml et environ 1 ml par kg de matière.

12. Procédé de biodécapage comprenant les étapes suivantes :

- (a) la fourniture d'un polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;
 (b) la fourniture d'une matière comprenant de la pectine, du poly(acide galacturonique) (pectate), de l'homo-galacturonane, du rhamnogalacturonane ou une paroi cellulaire végétale ;
 (c) la mise en contact de la pectate lyase de (a) avec la matière de (b) dans des conditions comprenant

- i) entre un pH de 8,5 environ et un pH 10,0 environ,
 ii) un tampon de bicarbonate
 iii) un agent mouillant non ionique à environ 1 g/l,
 iv) un rapport de liqueur entre environ 10 : 1 et 50 : 1 l de liqueur : kg de matière,
 v) une dose entre environ 0,1 et environ 1 ml par kg de matière, ou équivalente,
 vi) une plage de températures entre environ 70 °C et environ 95 °C,
 vii) un temps de traitement d'environ 20 min, ou
 viii) une combinaison de l'un quelconque de i) à vii) ;

comprenant en outre l'utilisation d'un chélatant, le chélatant étant exclu du bain d'enzyme et étant ajouté après environ 20 minutes de traitement à l'enzyme et retenu pendant environ 10 minutes avant d'évacuer le bain.

13. Procédé de lavage d'un objet comprenant :

- (a) la fourniture d'un polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;
 (b) la fourniture d'un objet ; et
 (c) la mise en contact du polypeptide de l'étape (a) et de l'objet de l'étape (b) dans des conditions dans lesquelles la composition peut laver l'objet.

14. Procédé de préparation d'un jus, d'un sirop, d'une purée ou d'un extrait de fruit ou de légume comprenant les étapes suivantes :

- (a) la fourniture d'un polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;
 (b) la fourniture d'une composition ou d'un liquide comprenant une matière de fruit ou de légume ; et
 (c) la mise en contact du polypeptide de l'étape (a) et de la composition, préparant ainsi le jus, le sirop, la purée ou l'extrait de fruit ou de légume.

15. Procédé de traitement d'un papier, d'un produit de papier, d'une pâte de bois ou d'une matière cellulosique, comprenant les étapes suivantes :

- (a) la fourniture d'un polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;
 (b) la fourniture d'une composition comprenant un papier, un produit de papier, une pâte de bois ou une matière cellulosique ; et
 (c) la mise en contact du polypeptide de l'étape (a) et de la composition de l'étape (b) dans des conditions dans lesquelles la pectate lyase peut traiter le papier, le produit de papier, la pâte à papier, la pâte de bois ou la matière cellulosique.

16. Procédé d'amélioration de l'extraction d'huile d'une matière végétale riche en huile comprenant les étapes suivantes :

- (a) la fourniture d'un polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;
 (b) la fourniture d'une matière végétale riche en huile ; et
 (c) la mise en contact du polypeptide de l'étape (a) et de la matière végétale riche en huile.

17. Procédé de décapage de fibre, fil, textile, étoffe ou matière cellulosique comprenant les étapes suivantes :

- (a) la fourniture d'un polypeptide de la revendication 6, ou d'un polypeptide codé par l'acide nucléique de la revendication 1 ;
 (b) la fourniture d'une fibre, d'un fil, d'un textile, d'une étoffe ou d'une matière cellulosique ; et

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(c) la mise en contact du polypeptide de l'étape (a) et de la fibre, du fil, du textile, de l'étoffe ou de la matière cellulosique de l'étape (b) dans des conditions dans lesquelles la pectate lyase peut décaper la fibre, le fil, le textile, l'étoffe ou la matière cellulosique.

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FIGURE 1

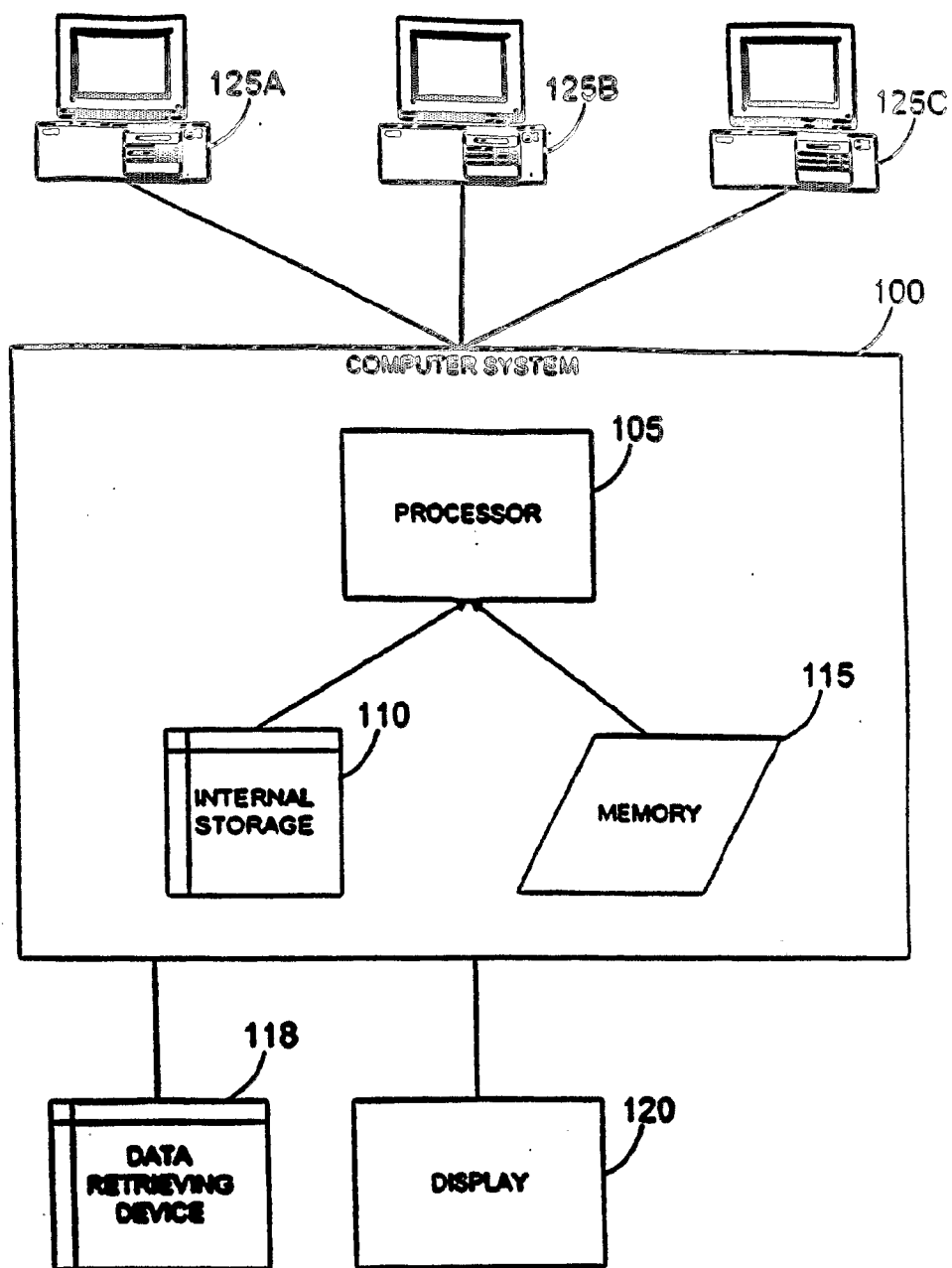


FIGURE 2

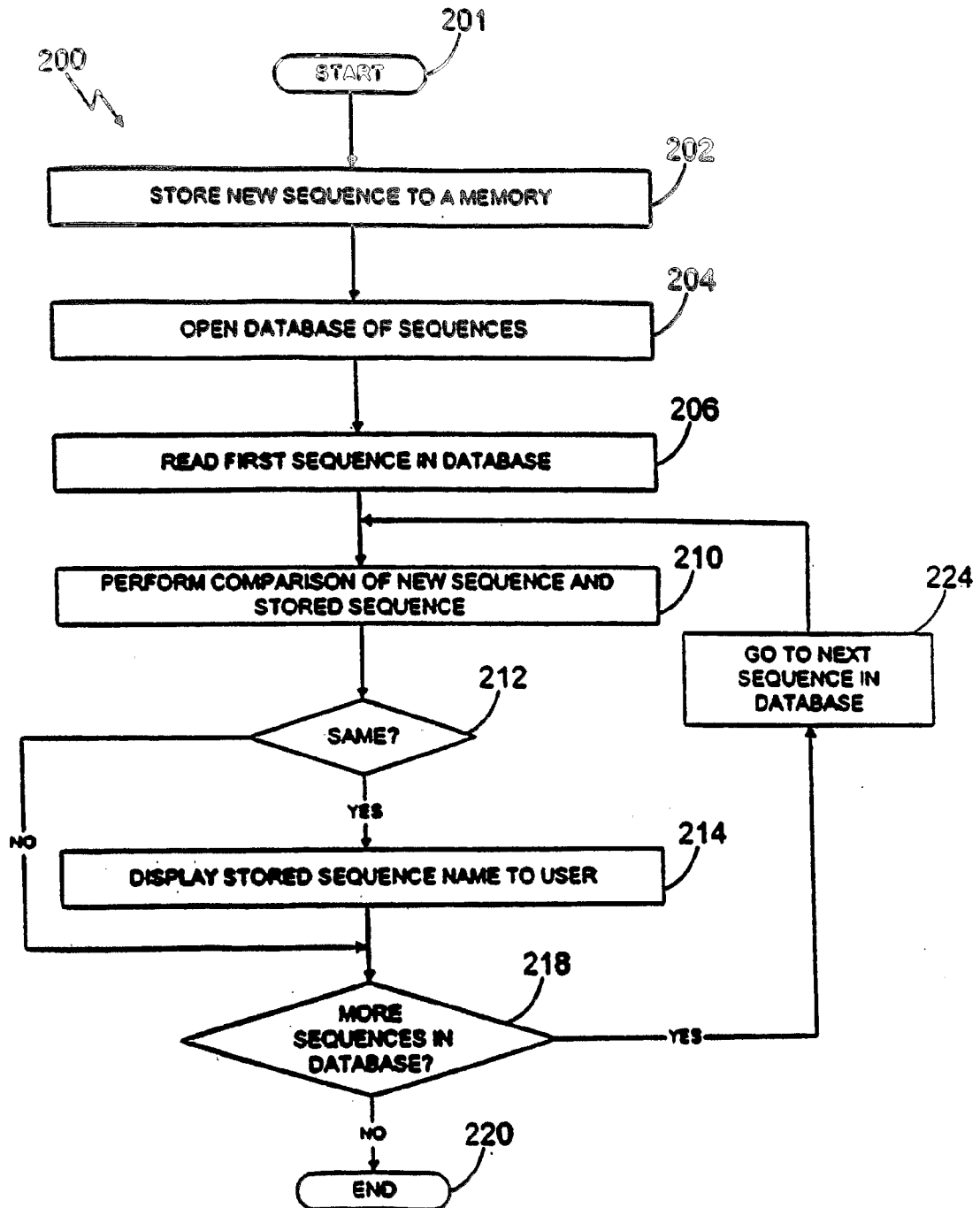


FIGURE 3

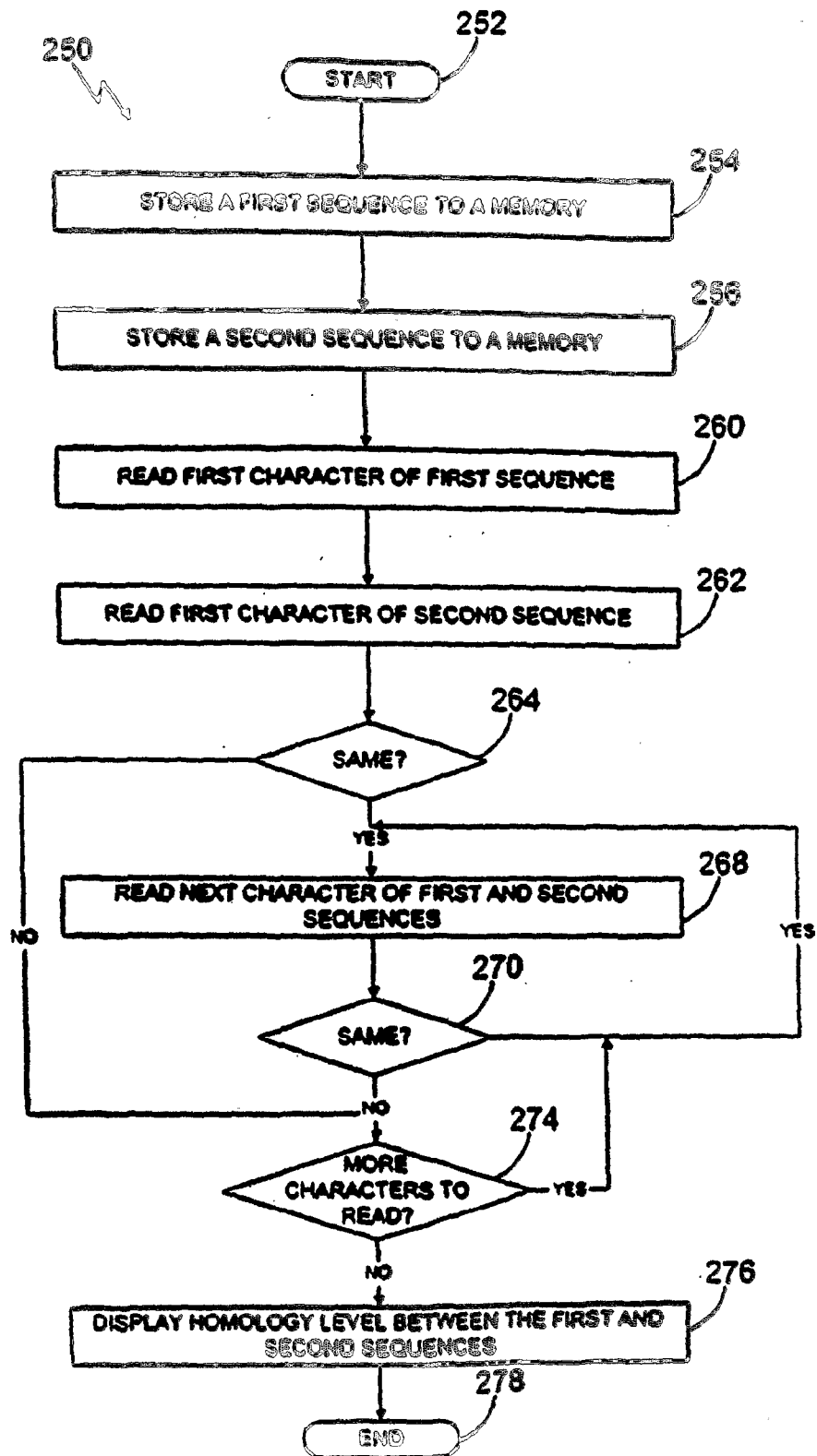


FIGURE 4

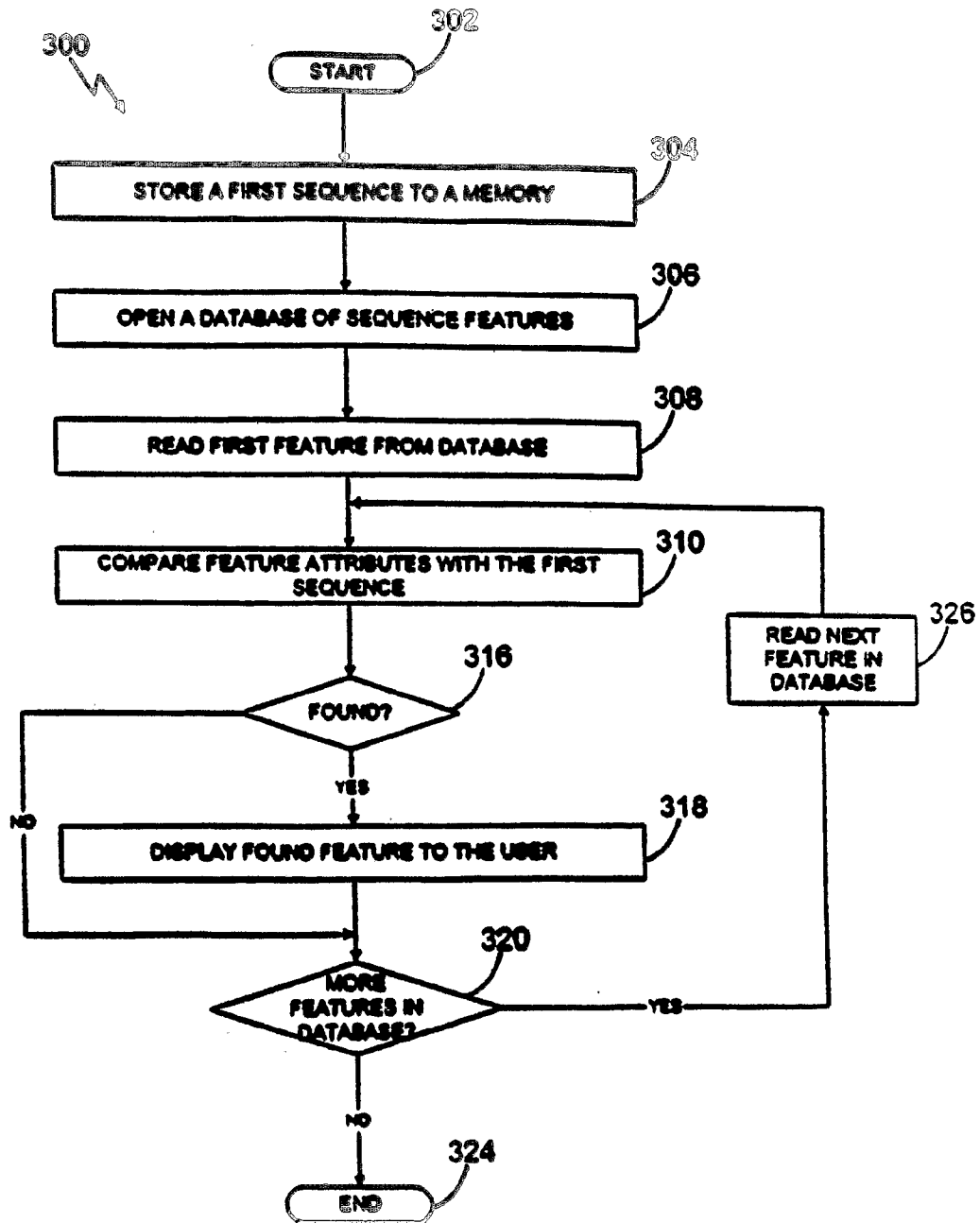


FIGURE 5A

SEQ ID NO: in provisional	Relative Substrate Specificity	Relative Substrate Specificity Value	Characterization Activity Temp	Characterization Activity pH	Enzyme	Characterization Description	Characterization Substrate
101, 102 1, 2			room temp	9	Pectinase	pga assay	Polygalacturonic acid
103, 104					Pectate lyase		
105, 106	Cotton	4.8	40	9	Pectate lyase	Application Bioscoursing	Cotton
107, 108	Cotton	1.5	40	9	Pectate lyase	Application Bioscoursing	Cotton
107, 108			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
109, 110					Pectate lyase		
11, 12					Pectate lyase		
111, 112					Pectinase		
113, 114					Pectate lyase		
115, 116			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
117, 118	Cotton	3	40	9	Pectate lyase	Application Bioscoursing	Cotton
119, 120			room temp	9	Pectate lyase		
121, 122	Cotton	7.8	50	9	Pectate lyase	Application bioscoursing	Cotton
121, 122	Cotton	10.3	40	9	Pectate lyase	Application Bioscoursing	Cotton
123, 124	Cotton	3.6	40	9	Pectate lyase	Application Bioscoursing	Cotton
125, 126	Cotton	2.5	40	9	Pectate lyase	Application Bioscoursing	Cotton
127, 128			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
127, 128			room temp	11	Pectate lyase	pga assay	Polygalacturonic acid
129, 130	Cotton	1.8	40	9	Pectate lyase	Application Bioscoursing	Cotton
13, 14			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
15, 16					Pectate lyase		
17, 18					Pectate lyase		
19, 20					Pectate lyase		
21, 22			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
23, 24					Pectate lyase		
25, 26			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
25, 26			room temp	10	Pectate lyase		
27, 28			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
29, 30			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
3, 4					Pectinase		
					Pectinase		

FIGURE 5B

31, 32	Cotton	4.3	40	9	Pectate lyase	Application Bioscoursing	Cotton
31, 32			room temp	8	Pectate lyase	pga assay	Polygalacturonic acid
33, 34			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
35, 36	Cotton	1.6	40	9	Pectate lyase	Application Bioscoursing	Cotton
35, 36			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
37, 38	Cotton	11.2	50	9	Pectate lyase	Application Bioscoursing	Cotton
39, 40			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
41, 42	Cotton	2	40	9	Pectate lyase	Application Bioscoursing	Cotton
43, 44				9	Pectate lyase		
45, 46			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
47, 48			room temp	10	Pectate lyase	pga assay	Polygalacturonic acid
49, 50					Pectate lyase		
5, 6							
51, 52					Pectate lyase		
53, 54	Cotton	13.4	40	9	Pectate lyase	Application Bioscoursing	Cotton
55, 56					Pectate lyase		
55, 56			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
57, 58			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
59, 60			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
61, 62	Cotton	12.5	40	9	Pectate lyase	Application Bioscoursing	Cotton
61, 62			room temp	10	Pectate lyase	pga assay	Polygalacturonic acid
63, 64					Pectate lyase		
65, 66					Pectate lyase		
67, 68	Cotton	9.8	40	9	Pectate lyase	Application Bioscoursing	Cotton
69, 70			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
7, 8			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
71, 72			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
73, 74	Cotton	0.8	40	9	Pectate lyase	Application Bioscoursing	Cotton
73, 74			room temp	10	Pectate lyase	pga assay	Polygalacturonic acid
75, 76					Pectinase		
77, 78	Cotton	16.2	40	9	Pectate lyase	Application Bioscoursing	Cotton
79, 80					Pectinase		
81, 82	Cotton	1.6	40	9	Pectate lyase	Application Bioscoursing	Cotton
81, 82			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
83, 84	Cotton	3.2	40	9	Pectate lyase	Application Bioscoursing	Cotton

FIGURE 5C

85, 86	Cotton	0.1	40	9	Pectate lyase	Application Bioscoursing	Cotton
85, 86			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
87, 88	Cotton	9.4	40	9	Pectate lyase	Application Bioscoursing	Cotton
89, 90					Pectinase		
9, 10			room temp	10	Pectate lyase	pga assay	Polygalacturonic acid
91, 92					Pectate lyase		
93, 94					Pectate lyase		
95, 96	Cotton	5.4	40	9	Pectate lyase	Application Bioscoursing	Cotton
95, 96			room temp	9	Pectate lyase	pga assay	Polygalacturonic acid
97, 98					Pectate lyase		
99, 100	Cotton	16	50	9	Pectate lyase	Application Bioscoursing	Cotton
99, 100	Cotton	12.2	40	9	Pectate lyase	Application Bioscoursing	Cotton

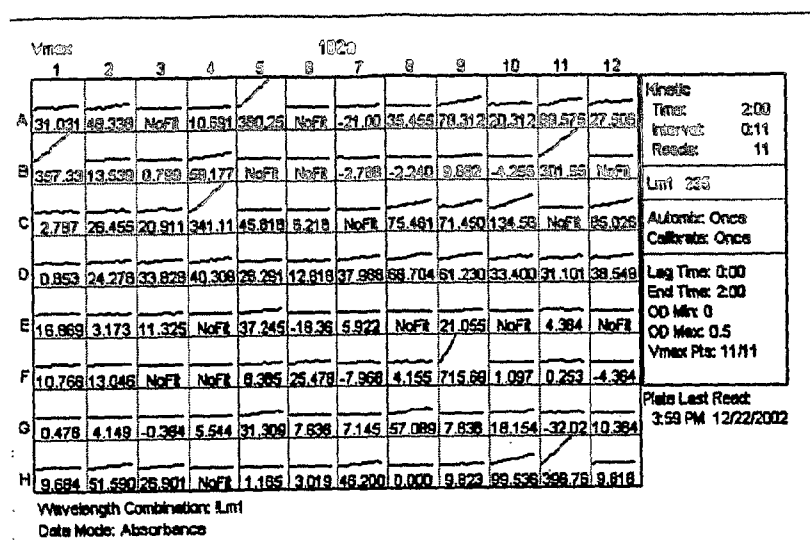
FIGURE 6

Pectate lyase GSSM™ upmutants													
Upmutant	Mutation												
	A118H	A182V	T190L	A197G	S208K	T219M	T223E	S255R	S263K	N275Y	Y309W	S312V	
A	x			x	x	x				x	x	x	
B	x		x		x	x				x	x	x	
C	x	x			x		x		x	x	x		
D	x	x	x		x			x	x	x	x		
E	x				x				x	x	x		
F	x	x			x		x	x		x	x	x	
G	x		x		x		x	x	x	x	x	x	
H	x	x	x	x	x		x	x		x	x	x	
I	x			x	x		x		x		x		
J	x			x	x				x	x	x	x	
K	x		x	x	x	x	x		x		x	x	
L	x	x	x		x		x	x	x	x	x		
M	x	x		x	x		x		x	x	x		
N	x		x	x	x				x	x	x	x	
O	x			x	x				x		x	x	
P	x	x		x	x	x		x		x	x		
Q	x		x		x				x		x	x	
R	x		x	x	x				x	x	x		
S	x		x	x		x	x	x	x	x	x		

FIGURE 7

Upmutant	Melting temperature (°C)	Specific Activity (SA)				
		at 30°C	SA at 40°C	SA at 50°C	SA at 60°C	SA at 70°C
AA	60.6	370.3	582.1	848.2	ND	ND
BB	58.3	366.8	542.4	648.6	ND	ND
CC	57	331.1	439.7	713.6	ND	ND
DD	58	468.6	595.8	714.6	ND	ND
EE	60	102.2	202.5	378.1	ND	ND
FF	58.5	585.5	744.5	955.8	ND	ND
GG	57.2	323.8	590.4	909.3	ND	ND
HH	58.7	267.6	425.5	706.3	ND	ND
II	57.8	357.1	527.3	875.1	ND	ND
JJ	57.5	372.2	537.6	834.9	ND	ND
KK	58.9	444.1	678.9	859.4	ND	ND
LL	58.4	375.2	557.6	1007.1	ND	ND
A	71	ND	ND	311.1	483.3	777.7
B	70.4	ND	ND	317.0	432.4	628.0
C	72.5	ND	ND	377.6	468.6	849.1
D	73.25	ND	ND	323.8	352.2	926.4
E	71	ND	ND	340.4	557.6	641.7
F	71.75	ND	ND	389.3	550.8	438.3
G	73	ND	ND	297.4	545.9	790.4
H	72.25	ND	ND	356.1	480.3	980.2
I	70.4	ND	ND	363.9	701.4	666.2
J	72.8	ND	ND	241.6	622.2	694.6
K	71.5	ND	ND	298.4	701.4	700.4
L	73	ND	ND	245.5	947.0	1003.7
M	73	ND	ND	304.2	547.8	309.1
N	73	ND	ND	685.8	1010.5	1284.5
O	71.3	ND	ND	268.0	517.5	330.7
P	72	ND	ND	398.2	542.9	689.7
Q	70.8	ND	ND	354.1	382.5	526.3
R	71.9	ND	ND	361.0	408.9	722.0
S	70.9	ND	ND	753.3	1186.6	579.1

FIGURE 8



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Pektát-liázok, az azokat kódoló nukleinsavak és eljárások előállításukra és alkalmazásukra

Szabadalmi igénypontok

1. Izolált, szintetikus vagy rekombináns nukleinsav, amely tartalmaz

(a) SEQ ID NO:99 szerinti nukleotid-szekvenciát;

(b) a SEQ ID NO:99 szerintivel legalább 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% vagy nagyobb szekvencia-azonosságú nukleotid-szekvenciát, ahol a nukleinsav legalább egy, pektát-liáz aktivitású polipeptidet kódol,

(c) olyan nukleinsavat, amely sztringens hibridizációs körülmények között hibridizál a SEQ ID NO:99 szerinti szekvenciát tartalmazó nukleinsavhoz, ahol a sztringens hibridizációs körülmények mosási lépést foglalnak magukban 0,2X SSC-vel körülbelül 65°C hőmérsékleten körülbelül 15 percen keresztül, és ahol a nukleinsav legalább egy, pektát-liáz aktivitású polipeptidet kódol;

(d) a SEQ ID NO:100 szerinti szekvenciájú polipeptidet kódoló nukleinsav, ahol a nukleinsav legalább egy, pektát-liáz aktivitású polipeptidet kódol;

(e) az (a), (b), (c) vagy (d) szerinti nukleinsav-szekvencia, de amely nem tartalmaz szignálszekvenciát kódoló nukleinsav-szekvenciát;

(f) az (a) - (e) bármelyike szerinti nukleinsav-szekvencia, amely továbbá heterológ peptidet vagy polipeptidet kódoló nukleinsav-szekvenciát is tartalmaz; vagy

(g) az (a) - (f) bármelyikével teljesen komplementer nukleinsav-szekvencia.

2. Expressziós kazetta vagy vektor, amely 1. igénypont szerinti nukleinsavat tartalmaz.

3. Izolált vagy transzformált sejt, amely (a) 1. igénypont szerinti nukleinsavat vagy (b) 2. igénypont szerinti expressziós kazettát vagy vektort tartalmaz

4. Eljárás rekombináns polipeptidet előállítására, amely magában foglalja a következő lépéseket: (a) 1. igénypont szerinti nukleinsav biztosítása; és (b) az (a) lépésbeli nukleinsav expresszáltatása olyan körülmények között, amelyek lehetővé teszik a polipeptid expresszióját, ezáltal rekombináns polipeptid keletkezik.

5. Transzgenikus nem-humán állat, amely (a) 1. igénypont szerinti nukleinsavat vagy (b) 2. igénypont szerinti expressziós kazettát vagy vektort tartalmaz.

6. Izolált, szintetikus vagy rekombináns polipeptid, amely tartalmaz

(a) SEQ ID NO:100 szerinti aminosav-szekvenciát;

(b) a SEQ ID NO:100 szerintivel legalább körülbelül 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% vagy 99% szekvencia-azonosságú aminosav-szekvenciát, ahol a polipeptidnek pektát-liáz aktivitása van,

(c) az 1. igénypont szerinti nukleinsav által kódolt pektát-liáz aktivitású polipeptidet;

(d) az (a), (b) vagy (c) szerinti aminosav-szekvencia, amelynek pektát-liáz aktivitása van, és amely legalább egy konzervatív aminosav-oldallánc szubsztitúciót tartalmaz, amely egy aminosav hasonló tulajdonságú aminosavval történő szubsztitúcióját tartalmazza, ahol az összes szubsztitúció konzervatív és a polipeptid lényegében megtartja a pektát-liáz aktivitást;

(e) az (a) - (d) bármelyike szerinti aminosav-szekvenciát, de amely nem tartalmaz szignálszekvenciát;

(f) az (a) - (e) bármelyike szerinti aminosav-szekvenciát, amely továbbá heterológ peptidet vagy polipeptidet is tartalmaz; vagy

(g) az (a) - (f) bármelyike szerinti polipeptidet, ahol a polipeptid legalább egy glikozilációs helyet is tartalmaz vagy glikozilált.

7. Készítmény, amely 6. igénypont szerinti polipeptidet tartalmaz, ahol a készítmény a következők által alkotott csoportból van kiválasztva: detergens készítmény, takarmány, élelmiszer, takarmány-készítmény, élelmiszer-készítmény, rost, szál, textil, szövet, papír, papírtermék, papírpép, fapép és cellulóztartalmú anyag.

8. Izolált vagy rekombináns ellenanyag, amely specifikusan kötődik a SEQ ID NO:100 szerinti aminosav-szekvenciából álló polipeptidhez.

9. Eljárás pektin-, pektát (poligalakturonsav)-, homogalakturonán-, ramnogalakturonán- vagy növényi sejtfa-tartalmú készítmény hidrolizálására, elhasítására vagy megtörésére, amely eljárás magában foglalja a következő lépéseket:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) pektint, pektátot, homogalakturonánt, ramnogalakturonánt vagy növényi sejtfa-tartalmú készítmény biztosítása; és

(c) az (a) lépés szerinti polipeptid érintkeztetése a (b) lépés szerinti készítménnyel olyan körülmények között, ahol a polipeptid hidrolizálja, elhasítja vagy megtöri a készítményt.

10. Eljárás pektin-, pektát (poligalakturonsav), homogalakturonán vagy ramnogalakturonán elfolyósítására vagy eltávolítására egy készítményből, amely eljárás magában foglalja a következő lépéseket:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) pektint, pektátot (poligalakturonsavat), homogalakturonánt vagy ramnogalakturonánt tartalmazó készítmény biztosítása; és

(c) az (a) lépés szerinti polipeptid érintkeztetése a (b) lépés szerinti készítménnyel olyan körülmények között, ahol a polipeptid eltávolítja vagy elfolyósítja a pektint, pektátot (poligalakturonsavat), homogalakturonánt vagy ramnogalakturonánt.

11. Anyag pektát-liázzal történő kezelésére szolgáló készítmény, amely 6. igénypont szerinti polipeptidet vagy 1. igénypont szerinti nukleinsav által kódolt polipeptidet tartalmaz, ahol a készítmény pektát-liáz körülbelül 0,1 ml és körülbelül 1 ml per kg közötti mennyiségű dózisát tartalmazza az anyag kg-jára vonatkoztatva.

12. "Biológiai súroló" ("bioscouring") eljárás, amely magában foglalja a következő lépéseket:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) pektin-, poligalakturonsav- (pektát-), homogalakturonán-, ramnogalakturonán- vagy növényi sejtfa-tartalmú készítmény biztosítása;

(c) az (a) lépés szerinti pektát-liáz érintkeztetése a (b) lépés szerinti anyaggal a következőket magában foglaló körülmények között

i) körülbelül pH 8,5 és körülbelül pH 10,0 közötti pH,

ii) bikarbonát puffer

iii) nemionos nedvesítőszer, körülbelül 1 g/l koncentrációban,

iv) körülbelül 10:1 és 50:1 közötti folyadék-liter:anyag-kg folyadékarány,

v) körülbelül 0,1 és körülbelül 1 ml per anyag-kg vagy azzal ekvivalens dózis,

vi) körülbelül 70°C és körülbelül 95°C közötti hőmérséklet-tartomány,

vii) körülbelül 20 perces kezelési idő, vagy

viii) az i) - vii) bármelyikének kombinációja;

és továbbá magában foglalja kelátor alkalmazását, ahol a kelátor ki van zárva az enzím-fürdőből és körülbelül 20 perces enzimes kezelés után van adagolva és körülbelül percig van ott tartva a fürdő leürítése előtt.

13. Eljárás tárgy mosására, amely eljárás magában foglalja:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) tárgy biztosítása; és

(c) az (a) lépés szerinti polipeptid és a (b) lépés szerinti tárgy érintkeztetése olyan körülmények között, ahol a készítmény képes mosni a tárgyat.

14. Eljárás gyümölcs- vagy zöldséglé, -szirup, -püré vagy -extraktum előállítására, amely eljárás magában foglalja a következő lépéseket:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) gyümölcs vagy zöldség anyagot tartalmazó készítmény vagy folyadék biztosítása; és

(c) az (a) lépés szerinti polipeptid és a készítmény érintkeztetése, ezáltal a gyümölcs- vagy zöldséglét, szirupot, pürét vagy extraktumot állítva elő.

15. Eljárás papír, papírtermék, fapép vagy cellulóztartalmú anyag kezelésére, amely eljárás magában foglalja a következő lépéseket:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) papírt, papírterméket, fapépet vagy cellulóztartalmú anyagot tartalmazó készítmény biztosítása; és

(c) az (a) lépés szerinti polipeptid és a (b) lépés szerinti készítmény érintkeztetése olyan körülmények között, ahol a pektát-liáz képes kezelni a papírt, papírterméket, fapépet vagy cellulóztartalmú anyagot.

16. Eljárás olaj extrakciójának javítására olajban gazdag növényből, amely eljárás magában foglalja a következő lépéseket:

(a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid biztosítása;

(b) olajban gazdag növényi anyag biztosítása; és

(c) az (a) lépés szerinti polipeptid és az olajban gazdag növényi anyag érintkeztetése.

17. Eljárás rost, szál, textil, szövet vagy cellulóztartalmú anyag súrolására ("scouring"), amely eljárás magában foglalja a következő lépéseket:

- (a) a 6. igénypont szerinti polipeptid vagy az 1. igénypont szerinti nukleinsav által kódolt polipeptid
- biztosítása;
- (b) rost, szál, textil, szövet vagy cellulóztartalmú anyag biztosítása; és
- (c) az (a) lépés szerinti polipeptid és a rost, szál, textil, szövet vagy cellulóztartalmú anyag érintkeztetése olyan körülmények között, ahol a pektát-liáz képes sűrölni a rostot, szálát, textilt, szövetet vagy cellulóztartalmú anyagot.