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(54) Title: NOVEL PECTINASES AND USES THEREOF

(57) Abstract: The invention relates to newly identified polynucleotide sequences comprising genes that encode novel pectinases isolated from Aspergillus niger. The invention features the full length nucleotide sequence of the novel genes, the cDNA sequences comprising the full length coding sequence of the novel pectinases as well as the amino acid sequence of the full-length functional proteins and functional equivalents thereof. The invention also relates to methods of using these enzymes in industrial processes and methods of diagnosing fungal infections. Also included in the invention are cells transformed with a polynucleotide according to the invention and cells wherein a pectinase according to the invention is genetically modified to enhan e or reduce its activity and/or level of expression.

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NOVEL PECTINASES AND USES THEREOF

Field of the invention

The invention relates to newly identified polynucleotide sequences comprising genes that encode novel pectinases isolated from Aspergillus niger. The invention features the full length nucleotide sequences of the novel genes, the cDNA sequences comprising the full length coding sequences of the novel pectinases as well as the amino acid sequences of the full-length functional proteins and functional equivalents thereof. The invention also relates to methods of using these enzymes in industrial processes and methods of diagnosing fungal infections. Also included in the invention are cells transformed with a polynucleotide according to the invention and cells wherein a pectinase according to the invention is genetically modified to enhance or reduce its activity and/or level of expression.

Background of the invention

Pectin polymers are important constituents of plant primary cell walls. They are composed of chains of 1,4-linked alpha-D-galacturonic acid and methylated derivatives thereof. Enzymes that are able to degrade the above-defined pectin polymers are called pectinases. Degradation in this respect means that at least one sugar residue or estergroup has been removed from the pectin molecule. Pectinases such as polygalacturonase, pectin methylesterase, pectin lyase or pectate lyase are important for the food and feed industry, such as in the art of fruit and vegetable processing such as fruit juice production or wine making, where their ability to catalyse the degradation of the backbone of the pectin polymer is utilised. Another application of pectinases, in particular pectin methyl esterase, is the firming of fruit and vegetables.

The seeds of leguminicaeae like soybeans and other types of peas also contain pectin. This pectin, however, is quite different from pectins described for fruits like apple berries etc. A large part of soybea pectin is water-unsoluble. In addition soybea pectin consists mainly of xylogalacturonan and rhamnogalacturonan and contains only minor amounts of homogalacturonan. The CDTA-soluble pectic substances from soybean meal are composed of

rhamnogalacturonan and xylogalacturonan but not homogalacturonan. Due to these structural differences soybea pectin is much more difficult to degrade by pectinases as compared to fruit pectin. Even the use of technical multi-enzyme preparations was not sufficient to degrade soybea pectin structures (Huisman, MMH; Schols, HA; Voragen, AGJ, **1999**, Enzymatic degradation of cell wall polysaccharides from soybean meal, Carbohydrate Polymers 38, 299-307, and: Ouhida, I; Pérez, JF; Gasa, J; **2002**, Soybean (glycine max) cell wall composition and availability to feed enzymes, J. Agric. Food Chem 50, 1933-1938).

An assortment of different pectin degrading enzymes is known to be present in various microorganisms such as Aspergillus niger.

The following is a non-exhaustive list of pectin-acting enzymes:

Enzyme	E.C. Number
exopolygalacturonase	3.2.1.67
endopolygalacturonase	3.2.1.15
pectin lyase	4.2.2.10
pectate lyase	4.2.2.2
rhamnogalacturonan hydrolase	3.2.1.x
rhamnogalacturonan lyase	4.2.2.x
rhamnogalacturonan acetylesterase	3.1.1.x
RG rhamnohydrolase	3.2.1.x
RG galacturonohydrolase	3.2.1.x
xylogalacturonan hydrolase	3.2.1.x
pectin acetylesterase	3.1.1.x
pectin methylesterase	3.1.1.11
endo-arabinase	3.2.1.99
beta-arabinofuranosidase	3.2.1.55
beta-1,4-galactanase	3.2.1.89
beta-1,3-galactanase	3.2.1.90
beta-galactosidase	3.2.1.23
alpha-galactosidase	3.2.1.22

feruloyl acetyl esterase	3.1.1.x
alpha-fucosidase	3.2.1.51
(beta-fucosidase)	3.2.1.38
beta-apiosidase	3.2.1.x
alpha-rhamnosidase	3.2.1.40
beta-rhamnosidase	3.2.1.43
alpha-arabinopyranosidase	3.2.1.x
beta-glucuronidase	3.2.1.31
(alpha-glucuronidase)	3.2.1.139
beta-xylosidase	3.2.1.37
(alpha-xylosidase)	3.2.1.x

Pectin methylesterase catalyses the removal of methanol from pectin, resulting in the formation of pectic acid (polygalacturonic acid). Pectate lyase cleaves glycosidic bonds in polygalacturonic acid by beta-elimination, pectin lyase cleaves the glycosidic bonds of highly methylated pectins by beta-elimination, and polygalacturonase hydrolyses the glycosidic linkages in the polygalacturonic acid chain.

Commercially available pectinases such as Rapidase Press ® are actually a mixture of enzymes, which, along with other enzymes such as cellulases, are used in the fruit industry to help extract, clarify and modify fruit juices.

It is a disadvantage of these mixtures that they hardly ever contain the optimal mix of enzymes to treat a particular pectin containing composition, such as feed, fruit or juice. Optimal pectin degradation in fruit depends on many factors, including the kind of fruit to be treated, the season in which the fruit is harvested, the ripeness of the fruit, and many more. A well-known example is the release of unwanted methanol and methylated pectin by the action of pectinmethylesterases and/or exo-polygalacturonases when present in too high concentrations.

It is therefore an object of the present invention to provide isolated (recombinant) pectinases that are better able to degrade pectins, for instance in food and feed applications.

Pectinases have already been cloned in a variety of different microorganisms. Molecular cloning of pectinases in fungi has also been described. The DNA and deduced amino acid sequences of several pectinases

from Aspergillus oryzae, Aspergillus kawachii and Emericella nidulans are known.

However, there is still a need for other pectinases with different properties so that feed, fruit and vegetable processing may be optimized.

One of the disadvantages of the currently available enzymes is that they have pH optima above pH 4.0. Many fruit juices are more acidic and a pH optimum below 3.8 would be desirable. Fruit juices may become as acidic as pH 2.0 and it is therefore an object of the present invention to provide pectinases with a more acidic pH optimum. Pectinases with a pH optimum below 3.8 would be especially advantageous.

Fruit processing is also often performed at more extreme temperatures. Use of pectinases with lower temperature optima would improve yield and quality of the juice because more aromas would be released. The currently available enzymes have a very low efficiency at temperatures below 15 °C. Also, at high temperatures the currently available enzymes are easily inactivated. This is a disadvantage for those fruit juices that have to be pasteurized.

Enzymes according to the invention also provide a better temperature stability and may withstand more extreme temperatures in comparison with pectinases according to the prior art.

Also, the specificity of conventional enzymes leaves to be desired. Fruit juice that needs to be filtrated after or during processing tends to clot the filters after some time. This is due to the inability of prior art enzymes to properly digest fruit polysaccharides which causes the fouling of the filters.

The present invention addresses at least one if not all of the above problems.

Object of the invention

It is an object of the invention to provide novel polynucleotides encoding novel pectinases with improved properties. A further object is to provide naturally and recombinantly produced pectinases as well as recombinant strains producing these. Also fusion polypeptides are part of the invention as well as methods of making and using the polynucleotides and polypeptides according to the invention.

Summary of the invention

The invention provides for novel polynucleotides encoding novel pectinases. These pectinases were found to digest pectinases from various

sources in a better way than conventional pectinases. Such better performance was especially evident when the pectinases according to the invention were used to prevent filter fouling in fruit juice applications. Also, pectinases according to the invention may advantageously be employed for improving the nutritional value of soybean extracts. Moreover, the availability of improved, isolated enzymes allows a cocktail of enzymes to be be prepared which is optimized for the digestion of pectin from a particular feed, fruit or juice.

More in particular, the invention provides for polynucleotides having a nucleotide sequence that hybridises preferably under highly stringent conditions to a sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32.

In one embodiment, the invention provides for an isolated polynucleotide comprising a nucleic acid sequence encoding a polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

The sequences revealed herein are shown in the accompanying sequence listing. For clarity reasons, the group consisting of sequences according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19; SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29; SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO:37, SEQ ID NO: 38, SEQ ID NO: 39; SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49; SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59; SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69; SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79; SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89; SEQ ID NO: 90, SEQ ID NO: 91,

SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96 is hereinafter collectively termed the group consisting of SEQ ID NO: 1 - 96

The group consisting of sequences according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19; SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29; SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32 is hereinafter collectively termed the group consisting of SEQ ID NO: 1 - 32

The group consisting of sequences according to SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO:37, SEQ ID NO: 38, SEQ ID NO: 39; SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49; SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59; SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64 is hereinafter collectively termed the group consisting of SEQ ID NO: 33 - 64

The group consisting of sequences according to SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69; SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79; SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89; SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96 is hereinafter collectively termed the group consisting of SEQ ID NO: 65 - 96

The group consisting of sequences according to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19; SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29; SEQ ID NO: 30,

SEQ ID NO: 31 and SEQ ID NO: 32 is hereinafter collectively termed the group consisting of SEQ ID NO: 6 - 32

The group consisting of sequences according to SEQ ID NO: 38, SEQ ID NO: 39; SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49; SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59; SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64 is hereinafter collectively termed the group consisting of SEQ ID NO: 38 – 64.

The group consisting of sequences according to SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79; SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89; SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96 is hereinafter collectively termed the group consisting of SEQ ID NO: 70 – 96.

The group consisting of sequences according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19; SEQ ID NO: 20 and SEQ ID NO: 21 is hereinafter collectively termed the group consisting of SEQ ID NO: 1 - 21

The group consisting of sequences according to SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO:37, SEQ ID NO: 38, SEQ ID NO: 39; SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49; SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52 and SEQ ID NO: 53 is hereinafter collectively termed the group consisting of SEQ ID NO: 33 – 53.

The group consisting of sequences according to SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69; SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79; SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84

and SEQ ID NO: 85 is hereinafter collectively termed the group consisting of SEQ ID NO: 65 – 85.

The invention provides nucleic acids that are more than 40% such as about 60%, preferably 65%, more preferably 70%, even more preferably 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32.

In a more preferred embodiment the invention provides for such an isolated polynucleotide obtainable from a filamentous fungus, in particular A. niger is preferred.

In a further preferred embodiment, the invention provides an isolated polynucleotide encoding at least one functional domain of a polypeptide selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

In a preferred embodiment the invention provides a pectinase gene selected from the group consisting of SEQ ID NO: 33 - 64 . In another aspect the invention provides a polynucleotide, preferably a cDNA encoding an A. niger pectinase selected from the group consisting of SEQ ID NO: 65 - 96 or variants or fragments of that polypeptide. In a preferred embodiment the cDNA has a sequence selected from the group consisting of SEQ ID NO: 1 - 32 or functional equivalents thereof.

In an even further preferred embodiment, the invention provides for a polynucleotide comprising the coding sequence of the polynucleotides according to the invention, preferred is a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1 - 32.

The invention also relates to vectors comprising a polynucleotide sequence according to the invention and primers, probes and fragments that may be used to amplify or detect the DNA according to the invention.

In a further preferred embodiment, a vector is provided wherein the polynucleotide sequence according to the invention is functionally linked with regulatory sequences suitable for expression of the encoded amino acid sequence in a suitable host cell, such as A. niger or A. oryzea. The invention also provides methods for preparing polynucleotides and vectors according to the invention.

The invention also relates to recombinantly produced host cells

that contain heterologous or homologous polynucleotides according to the invention.

In another embodiment, the invention provides recombinant host cells wherein the expression of a pectinase according to the invention is significantly increased or wherein the activity of the pectinase is increased.

In another embodiment, the invention provides recombinant host cells wherein the expression of a pectinase according to the invention is significantly decreased or wherein the activity of the pectinase is decreased. Such a host is especially useful if the polypeptide expresses an unwanted activity in an otherwise useful mixture of enzyme activities.

In another embodiment the invention provides for a recombinantly produced host cell that contains heterologous or homologous DNA according to the invention and wherein the cell is capable of producing a functional pectinase according to the invention, preferably a cell capable of over-expressing the pectinase according to the invention, for example an Aspergillus strain comprising an increased copy number of a gene or cDNA according to the invention.

In yet another aspect of the invention, a purified polypeptide is provided. The polypeptides according to the invention include the polypeptides encoded by the polynucleotides according to the invention. Especially preferred is a polypeptide selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

Fusion proteins comprising a polypeptide according to the invention are also within the scope of the invention. The invention also provides methods of making the polypeptides according to the invention.

The invention also relates to the use of the pectinase according to the invention in any industrial process as described herein

Detailed description of the invention

Polynucleotides

The present invention provides polynucleotides encoding a number of novel pectinases, collectively called PEC 1 – PEC 32, having an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof. The term PEC1 – PEC 32 is hereinafter used to refer to any polypeptide with pectinase activity selected from the group consisiting of SEQ ID NO: 65 – 96. The sequences of the genes encoding PEC 1 - PEC 32

were determined by sequencing genomic clones obtained from Aspergillus niger. The invention provides polynucleotide sequences comprising the genes encoding the PEC 1 - PEC 32 pectinases as well as their complete cDNA sequences. Accordingly, the invention relates to an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 or functional equivalents thereof.

More in particular, the invention relates to an isolated polynucleotide hybridisable under stringent conditions, preferably under highly stringent conditions, to a polynucleotide selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32. Advantageously, such polynucleotides may be obtained from filamentous fungi, in particular from Aspergillus niger. More specifically, the invention relates to an isolated polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32.

The invention also relates to an isolated polynucleotide encoding at least one functional domain of a polypeptide selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which may be isolated from chromosomal DNA, which include an open reading frame encoding a protein, e.g. an A. niger pectinase. A gene may include coding sequences, non-coding sequences, introns and regulatory sequences. Moreover, a gene refers to an isolated nucleic acid molecule as defined herein.

A nucleic acid molecule of the present invention, such as a nucleic acid molecule selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 or a functional equivalent thereof, may be isolated using standard molecular biology techniques and the sequence information provided herein. For example, using all or portion of a nucleic acid selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 as a hybridization probe, nucleic acid molecules according to the invention may be isolated using standard hybridization and cloning techniques (e. g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual.2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion

of a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 may be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence information contained herein.

A nucleic acid according to the invention may be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid thus amplified may be cloned into an appropriate vector and characterized by DNA sequence analysis.

Furthermore, oligonucleotides corresponding to or hybridisable to nucleotide sequences according to the invention may be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence selected from the group consisting of SEQ ID NO: 1 - 32. The sequences of the cDNAs selected from the group consisting of SEQ ID NO: 1 - 32 correspond to the coding region of the A. niger PEC 1 - PEC 32 pectinases. Pectinases PEC 1 - PEC 32 correspond to polypeptides selected from the group consisting of SEQ ID NO: 65 - 96.

In another preferred embodiment, an isolated nucleic acid molecule according to the invention comprises a nucleic acid molecule which is the complement of a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 or a functional equivalent of these nucleotide sequences.

A nucleic acid molecule which is complementary to another nucleotide sequence is one which is sufficiently complementary to the other nucleotide sequence to such an extent that it may hybridize to the other nucleotide sequence thereby forming a stable duplex.

One aspect of the invention pertains to isolated nucleic acid molecules that encode a polypeptide of the invention or a functional equivalent thereof such as a biologically active fragment or domain, as well as nucleic acid molecules sufficient for use as hybridisation probes to identify nucleic acid molecules encoding a polypeptide of the invention and fragments of such nucleic acid molecules suitable for use as PCR primers for the amplification or mutation of nucleic acid molecules.

An "isolated polynucleotide" or "isolated nucleic acid" is a DNA or RNA that is not immediately contiguous with both of the coding sequences with

which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. Thus, in one embodiment, an isolated nucleic acid includes some or all of the 5' non-coding (e.g., promotor) sequences that are immediately contiguous to the coding sequence. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA that is part of a hybrid gene encoding an additional polypeptide that is substantially free of cellular material, viral material, or culture medium (when produced by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Moreover, an "isolated nucleic acid fragment" is a nucleic acid fragment that is not naturally occurring as a fragment and would not be found in the natural state.

As used herein, the terms "polynucleotide" or "nucleic acid molecule" are intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA. The nucleic acid may be synthesized using oligonucleotide analogs or derivatives (e.g., inosine or phosphorothioate nucleotides). Such oligonucleotides may be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a PEC 1 - PEC 32 nucleic acid molecule, e.g., the coding strand of a PEC 1 - PEC 32 nucleic acid molecule. Also included within the scope of the invention are the complement strands of the nucleic acid molecules described herein.

Sequencing errors

The sequence information as provided herein should not be so narrowly construed as to require inclusion of erroneously identified bases. The specific sequences disclosed herein may be readily used to isolate the complete gene from filamentous fungi, in particular A. niger which in turn may easily be subjected to further sequence analyses thereby identifying sequencing errors.

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence may be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

The person skilled in the art is capable of identifying such erroneously identified bases and knows how to correct for such errors.

Nucleic acid fragments, probes and primers

A nucleic acid molecule according to the invention may comprise only a portion or a fragment of the nucleic acid sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32, for example a fragment which may be used as a probe or primer or a fragment encoding a portion of a PEC 1 - PEC 32 protein. The nucleotide sequence determined from the cloning of the PEC 1 - PEC 32 gene and cDNA allows for the generation of probes and primers designed for use in identifying and/or cloning other PEC 1 - PEC 32 family members, as well as PEC 1 - PEC 32 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide which typically comprises a region of nucleotide sequence that hybridizes preferably under highly stringent conditions to at least about 12 or 15, preferably about 18 or 20, preferably about 22 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 or more consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 or a functional

equivalent thereof.

Probes based on the PEC 1 - PEC 32 nucleotide sequences may be used to detect transcripts or genomic PEC 1 - PEC 32 sequences encoding the same or homologous proteins for instance in other organisms. In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group may be a radioisotope, a fluorescent compound, an enzyme, or an enzyme cofactor. Such probes may also be used as part of a diagnostic test kit for identifying cells which express a PEC 1 - PEC 32 protein.

Identity & homology

The terms "homology" or "percent identity" are used interchangeably herein. For the purpose of this invention, it is defined here that in order to determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps may be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions (i.e. overlapping positions) x 100). Preferably, the two sequences are the same length.

The skilled person will be aware of the fact that several different computer programms are available to determine the homology between two sequences. For instance, a comparison of sequences and determination of percent identity between two sequences may be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J. Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. The skilled person will appreciate that all these different parameters will yield slightly different results but that the overall percentage identity of two sequences is not significantly altered

when using different algorithms.

In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity two amino acid or nucleotide sequence is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989) which has been incorporated into the ALIGN program (version 2.0) (available at: http://vega.igh.cnrs.fr/bin/align-guess.cgi) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention may further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches may be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403—10. BLAST nucleotide searches may be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to PEC 1 - PEC 32 nucleic acid molecules of the invention. BLAST protein searches may be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to PEC 1 - PEC 32 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST may be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) may be used. See http://www.ncbi.nlm.nih.gov.

Hybridisation

As used herein, the term "hybridizing" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least about 50%, at least about 60%, at least about 70%, more preferably at least about 80%, even more preferably at least about 85% to 90%, more preferably at least 95% homologous to each other typically remain hybridized to each other.

A preferred, non-limiting example of such hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45 °C, followed by one or more washes in 1 X SSC, 0.1 % SDS at 50 °C, preferably at 55 °C, preferably at 60 °C and even more preferably at 65 °C.

Highly stringent conditions include, for example, hybridizing at 68 ^{0}C in 5x SSC/5x Denhardt's solution / 1.0% SDS and washing in 0.2x SSC/0.1% SDS at room temperature. Alternatively, washing may be performed at 42 ^{0}C.

The skilled artisan will know which conditions to apply for stringent and highly stringent hybridisation conditions. Additional guidance regarding such conditions is readily available in the art, for example, in Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of mRNAs), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to specifically hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-standed cDNA clone).

Obtaining full length DNA from other organisms

In a typical approach, cDNA libraries constructed from other organisms, e.g. filamentous fungi, in particular from the species Aspergillus may be screened.

For example, Aspergillus strains may be screened for homologous PEC 1 - PEC 32 polynucleotides by Northern blot analysis. Upon detection of transcripts homologous to polynucleotides according to the invention, cDNA libraries may be constructed from RNA isolated from the appropriate strain, utilizing standard techniques well known to those of skill in the art. Alternatively, a total genomic DNA library may be screened using a probe hybridisable to a PEC 1 - PEC 32 polynucleotide according to the invention.

Homologous gene sequences may be isolated, for example, by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of nucleotide sequences as taught herein.

The template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from strains known or suspected to express a polynucleotide according to the invention. The PCR product may be subcloned and sequenced to ensure that the amplified sequences represent the sequences

of a new PEC 1 - PEC 32 nucleic acid sequence, or a functional equivalent thereof.

The PCR fragment may then be used to isolate a full length cDNA clone by a variety of known methods. For example, the amplified fragment may be labeled and used to screen a bacteriophage or cosmid cDNA library. Alternatively, the labeled fragment may be used to screen a genomic library.

PCR technology may also be used to isolate full length cDNA sequences from other organisms. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis.

The resulting RNA/DNA hybrid may then be "tailed" (e.g., with guanines) using a standard terminal transferase reaction, the hybrid may be digested with RNase H, and second strand synthesis may then be primed (e.g., with a poly-C primer). Thus, cDNA sequences upstream of the amplified fragment may easily be isolated. For a review of useful cloning strategies, see e.g.,Sambrook et al., supra; and Ausubel et al., supra.

Vectors

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a PEC 1 - PEC 32 protein or a functional equivalent thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. The terms "plasmid" and "vector"

may be used interchangeably herein as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vector includes one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operatively linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signal). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in a certain host cell (e.g. tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention may be introduced into host cells to thereby produce proteins or peptides, encoded by nucleic acids as described herein (e.g. PEC 1 - PEC 32 proteins, mutant forms of PEC 1 - PEC 32 proteins, fragments, variants or functional equivalents thereof, fusion proteins, etc.).

The recombinant expression vectors of the invention may be designed for expression of PEC 1 - PEC 32 proteins in prokaryotic or eukaryotic cells. For example, PEC 1 - PEC 32 proteins may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector may be transcribed and translated *in vitro*, for example using T7 promoter regulatory

sequences and T7 polymerase.

Expression vectors useful in the present invention include chromosomal-, episomal- and virus-derived vectors e.g., vectors derived from bacterial plasmids, bacteriophage, yeast episome, yeast chromosomal elements, viruses such as baculoviruses, papova viruses, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled person. In a specific embodiment, promoters are preferred that are capable of directing a high expression level of pectinases in filamentous fungi. Such promoters are known in the art. The expression constructs may contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

Vector DNA may be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-percipitation, DEAE-dextran-mediated transfection, transduction, infection, lipofection, cationic lipidmediated transfection or electroporation. Suitable methods for transforming or transfecting host cells may be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual, 2nd,ed. Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), Davis et al., *Basic Methods in Molecular Biology* (1986) and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer

resistance to drugs, such as G418, hygromycin and methatrexate. Nucleic acid encoding a selectable marker may be introduced into a host cell on the same vector as that encoding a PEC 1 - PEC 32 protein or may be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid may be identified by drug selection (e.g. cells that have incorporated the selectable marker gene will survive, while the other cells die).

Expression of proteins in prokaryotes is often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, e.g. to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognation sequences, include Factor Xa, thrombin and enterokinase.

As indicated, the expression vectors will preferably contain selectable markers. Such markers include dihydrofolate reductase or neomycin resistance for eukarotic cell culture and tetracyline or ampicilling resistance for culturing in *E. coli* and other bacteria. Representative examples of appropriate host include bacterial cells, such as *E. coli*, Streptomyces and Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS and Bowes melanoma; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria are pQE70, pQE60 and PQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16A, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are PWLNEO, pSV2CAT, pOG44, pZT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Among known bacterial promotors for use in the present

invention include *E. coli* lacI and lacZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR, PL promoters and the trp promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Transcription by higher eukaryotes of a DNA encoding a polypeptide according to the present invention may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretation signal may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification.

Polypeptides according to the invention

The invention provides an isolated polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96, an amino acid sequence obtainable by expressing a polynucleotide selected from the group consisting of SEQ ID NO: 33 - 64 in an appropriate host, as well as an amino acid sequence obtainable by expressing a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1 - 32 in an appropriate host. Also, a peptide or polypeptide comprising a functional equivalent of the above polypeptides is comprised within the present invention. The above polypeptides are collectively comprised in the term "polypeptides according to the invention"

The terms "peptide" and "oligopeptide" are considered synonymous (as is commonly recognized) and each term may be used interchangeably as the context requires to indicate a chain of at least two amino acids coupled by peptidyl linkages. The word "polypeptide" is used herein for chains containing more than seven amino acid residues. All oligopeptide and polypeptide formulas or sequences herein are written from left to right and in the direction from amino terminus to carboxy terminus. The one-letter code of amino acids used herein is commonly known in the art and may be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual, 2nd,ed. Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989)

By "isolated" polypeptide or protein is intended a polypeptide or protein removed from its native environment. For example, recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention as are native or recombinant polypeptides which have been substantially purified by any suitable technique such as, for example, the single-step purification method disclosed in Smith and Johnson, Gene 67:31-40 (1988).

The PEC 1 - PEC 32 pectinase according to the invention may be recovered and purified from recombinant cell cultures by well-known 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. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Protein fragments

The invention also features biologically active fragments of the polypeptides according to the invention.

Biologically active fragments of a polypeptide of the invention include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of a PEC 1 - PEC 32 protein (e.g., an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96), which include fewer amino acids than the full length protein, and exhibit at least one biological activity of the corresponding full-length protein. Typically, biologically active fragments comprise a domain or motif with at least one activity of the PEC 1 - PEC 32 protein. A biologically active fragment of a protein of the invention may be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, may be prepared by recombinant techniques and evaluated for one or more of the biological activities of the native form of a polypeptide of the invention.

The invention also features nucleic acid fragments which encode the above biologically active fragments of the PEC 1 - PEC 32 protein.

Fusion proteins

The proteins of the present invention or functional equivalents thereof, e.g., biologically active portions thereof, may be operatively linked to a non-PEC 1 - PEC 32 polypeptide (e.g., heterologous amino acid sequences) to form fusion proteins. As used herein, a PEC 1 - PEC 32 "chimeric protein" or "fusion protein" comprises a PEC 1 - PEC 32 polypeptide operatively linked to a non-PEC 1 - PEC 32 polypeptide. A "PEC 1 - PEC 32 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to PEC 1 - PEC 32, whereas a "non-PEC 1 - PEC 32 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the PEC 1 - PEC 32 protein, e.g., a protein which is different from the PEC 1 - PEC 32 protein and which is derived from the same or a different organism. Within a PEC 1 - PEC 32 fusion protein the PEC 1 - PEC 32 polypeptide may correspond to all or a portion of a PEC 1 - PEC 32 protein. In a preferred embodiment, a PEC 1 - PEC 32 fusion protein comprises at least one biologically active fragment of a PEC 1 - PEC 32 protein. In another preferred embodiment, a PEC 1 - PEC 32 fusion protein comprises at least two biologically active portions of a PEC 1 - PEC 32 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the PEC 1 - PEC 32 polypeptide and the non-PEC 1 - PEC 32 polypeptide are fused in-frame to each other. The

non-PEC 1 - PEC 32 polypeptide may be fused to the N-terminus or C-terminus of the PEC 1 - PEC 32 polypeptide.

For example, in one embodiment, the fusion protein is a GST-PEC 1 - PEC 32 fusion protein in which the PEC 1 - PEC 32 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins may facilitate the purification of recombinant PEC 1 - PEC 32. In another embodiment, the fusion protein is a PEC 1 - PEC 32 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian and Yeast host cells), expression and/or secretion of PEC 1 - PEC 32 may be increased through use of a hetereologous signal sequence.

In another example, the gp67 secretory sequence of the baculovirus envelope protein may be used as a heterologous signal sequence (*Current Protocols in Molecular Biology*, Ausubel et al., eds., John Wiley & Sons, 1992). Other examples of eukaryotic heterologous signal sequences include the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene; La Jolla, California). In yet another example, useful prokarytic heterologous signal sequences include the phoA secretory signal (Sambrook et al., *supra*) and the protein A secretory signal (Pharmacia Biotech; Piscataway, New Jersey).

A signal sequence may be used to facilitate secretion and isolation of a protein or polypeptide of the invention. Signal sequences are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein may then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence may be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain. Thus, for instance, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available.

As described in *Gentz et al, Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purificaton of the fusion protein. The HA tag is another peptide useful for purification which corresponds to an epitope derived of influenza hemaglutinin protein, which has been described by Wilson *et al., Cell 37*:767 (1984), for instance.

Preferably, a PEC 1 - PEC 32 chimeric or fusion protein according to the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene may be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments may be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which may subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g, a GST polypeptide). A nucleic acid encoding a PEC 1 - PEC 32 polypeptide may be cloned into such an expression vector such that the fusion moiety is linked in-frame to the PEC 1 - PEC 32 polypeptide.

Functional equivalents

The terms "functional equivalents" and "functional variants" are used interchangeably herein. Functional equivalents of PEC 1 - PEC 32 DNA are isolated DNA fragments that encode a polypeptide that exhibits a particular function of a PEC 1 - PEC 32 A. niger pectinase as defined herein. A functional equivalent of a PEC 1 - PEC 32 polypeptide according to the invention is a polypeptide that exhibits at least one function of an A. niger pectinase as defined herein. Functional equivalents therefore also encompass biologically active fragments.

Functional protein or polypeptide equivalents may contain only conservative substitutions of one or more amino acids of a sequence selected from the group consisting of SEQ ID NO: 65 - 96 or substitutions, insertions or

deletions of non-essential amino acids. Accordingly, a non-essential amino acid is a residue that may be altered in a sequence selected from the group consisting of SEQ ID NO: 65 - 96 without substantially altering the biological function. For example, amino acid residues that are conserved within their own family among any of the PEC 1 - PEC 32 proteins of the present invention, are predicted to be particularly unamenable to alteration.

The term "conservative substitution" is intended to mean that a substitution in which the amino acid residue is replaced with an amino acid residue having a similar side chain. These families are known in the art and include amino acids with basic side chains (e.g.lysine, arginine and hystidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagines, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine tryptophan, histidine).

Functional nucleic acid equivalents may typically contain silent mutations or mutations that do not alter the biological function of encoded polypeptide. Accordingly, the invention provides nucleic acid molecules encoding PEC 1 - PEC 32 proteins that contain changes in amino acid residues that are not essential for a particular biological activity. Such PEC 1 - PEC 32 proteins differ in amino acid sequence from a sequence selected from the group consisting of SEQ ID NO: 65 - 96 yet retain at least one biological activity. In one embodiment the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises a substantially homologous amino acid sequence of at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J.U. et al., Science 247:1306-1310 (1990) wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selects or screens to identify sequences that maintain functionality. As the authors state,

these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require non-polar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie et al, supra, and the references cited therein.

An isolated nucleic acid molecule encoding a PEC 1 - PEC 32 protein homologous to the protein selected from the group consisting of SEQ ID NO: 65 - 96 may be created by introducing one or more nucleotide substitutions, additions or deletions into the coding nucleotide sequences selected from the group consisting SEQ ID NO: 65 - 963 - 64 or from the group consisting of SEQ ID NO: 1 - 32 such that one or more amino acid substitutions, deletions or insertions are introduced into the encoded protein. Such mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

The term "functional equivalents" also encompasses orthologues of the A. niger PEC 1 - PEC 32 protein. Orthologues of the A. niger PEC 1 - PEC 32 protein are proteins that may be isolated from other strains or species and possess a similar or identical biological activity. Such orthologues may readily be identified as comprising an amino acid sequence that is substantially homologous to a sequence selected from the group consisting of SEQ ID NO: 65 - 96.

As defined herein, the term "substantially homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., with similar side chain) amino acids or nucleotides to a second amino acid or nucleotide sequence such that the first and the second amino acid or nucleotide sequences have a common domain. For example, amino acid or nucleotide sequences which contain a common domain having about 60%, preferably 65%, more preferably 70%, even more preferably 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity or more are defined herein as sufficiently identical.

Also, nucleic acids encoding other PEC 1 - PEC 32 family members, which thus have a nucleotide sequence that differs from a sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32, are within the scope of the invention. Moreover, nucleic acids encoding PEC 1 - PEC 32 proteins from different species which thus

may have a nucleotide sequence which differs from a sequence selected from the group consisting of SEQ ID NO: 33 - 64.or from the group consisting of SEQ ID NO: 1 - 32 are within the scope of the invention.

Nucleic acid molecules corresponding to variants (e.g. natural allelic variants) and homologues of the PEC 1 - PEC 32 DNA of the invention may be isolated based on their homology to the PEC 1 - PEC 32 nucleic acids disclosed herein using the cDNAs disclosed herein or a suitable fragment thereof, as a hybridisation probe according to standard hybridisation techniques preferably under highly stringent hybridisation conditions.

In addition to naturally occurring allelic variants of the PEC 1 - PEC 32 sequence, the skilled person will recognise that changes may be introduced by mutation into the nucleotide sequences selected from the group consisting of SEQ ID NO: 33 - 64.or from the group consisting of SEQ ID NO: 1 - 32 thereby leading to changes in the amino acid sequence of the PEC 1 - PEC 32 protein without substantially altering the function of the PEC 1 - PEC 32 protein.

In another aspect of the invention, improved PEC 1 - PEC 32 proteins are provided. Improved PEC 1 - PEC 32 proteins are proteins wherein at least one biological activity is improved. Such proteins may be obtained by randomly introducing mutations along all or part of the PEC 1 - PEC 32 coding sequence, such as by saturation mutagenesis, and the resulting mutants may be expressed recombinantly and screened for biological activity. For instance, the art provides for standard assays for measuring the enzymatic activity of pectinases and thus improved proteins may easily be selected.

In a preferred embodiment the PEC 1 - PEC 32 protein has an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96. In another embodiment, the PEC 1 - PEC 32 polypeptide is substantially homologous to the amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96 and retains at least one biological activity of a polypeptide selected from the group consisting of SEQ ID NO: 65 - 96, yet differs in amino acid sequence due to natural variation or mutagenesis as described above.

In a further preferred embodiment, the PEC 1 - PEC 32 protein has an amino acid sequence encoded by an isolated nucleic acid fragment capable of hybridising to a nucleic acid selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32, preferably under highly stringent hybridisation conditions.

Accordingly, the PEC 1 - PEC 32 protein is a protein which

comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous to the amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96 and retains at least one functional activity of a polypeptide selected from the group consisting of SEQ ID NO: 65 - 96.

Functional equivalents of a protein according to the invention may also be identified e.g. by screening combinatorial libraries of mutants, e.g. truncation mutants, of the protein of the invention for pectinase activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level. A variegated library of variants may be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display). There are a variety of methods that may be used to produce libraries of potential variants of the polypeptides of the invention from a degenerate oligonucleotide sequence. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) Tetrahedron 39:3; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; Ike et al. (1983) Nucleic Acid Res. 11:477).

In addition, libraries of fragments of the coding sequence of a polypeptide of the invention may be used to generate a variegated population of polypeptides for screening a subsequent selection of variants. For example, a library of coding sequence fragments may be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which may include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library may be derived which encodes N-terminal and internal fragments of various sizes of the protein of interest.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations of truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for

screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, may be used in combination with the screening assays to identify variants of a protein of the invention (Arkin and Yourvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delgrave et al. (1993) Protein Engineering 6(3):327-331).

In addition to the PEC 1 - PEC 32 gene sequence selected from the group consisting of SEQ ID NO: 33 - 64, it will be apparent for the person skilled in the art that DNA sequence polymorphisms that may lead to changes in the amino acid sequence of the PEC 1 - PEC 32 protein may exist within a given population. Such genetic polymorphisms may exist in cells from different populations or within a population due to natural allelic variation. Allelic variants may also include functional equivalents.

Fragments of a polynucleotide according to the invention may also comprise polynucleotides not encoding functional polypeptides. Such polynucleotides may function as probes or primers for a PCR reaction.

Nucleic acids according to the invention irrespective of whether they encode functional or non-functional polypeptides, may be used as hybridization probes or polymerase chain reaction (PCR) primers. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having a PEC 1 - PEC 32 activity include, inter alia, (1) isolating the gene encoding the PEC 1 - PEC 32 protein, or allelic variants thereof from a cDNA library e.g. from other organisms than A. niger; (2) in situ hybridization (e.g. FISH) to metaphase chromosomal spreads to provide precise chromosomal location of the PEC 1 - PEC 32 gene as described in Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988); (3) Northern blot analysis for detecting expression of PEC 1 - PEC 32 mRNA in specific tissues and/or cells and 4) probes and primers that may be used as a diagnostic tool to analyse the presence of a nucleic acid hybridisable to the PEC 1 - PEC 32 probe in a given biological (e.g. tissue) sample.

Also encompassed by the invention is a method of obtaining a functional equivalent of a PEC 1 - PEC 32 gene or cDNA. Such a method entails obtaining a labelled probe that includes an isolated nucleic acid which encodes all

or a portion of the sequence selected from the group consisting of SEQ ID NO: 65 - 96 or a variant thereof; screening a nucleic acid fragment library with the labelled probe under conditions that allow hybridisation of the probe to nucleic acid fragments in the library, thereby forming nucleic acid duplexes, and preparing a full-length gene sequence from the nucleic acid fragments in any labelled duplex to obtain a gene related to the PEC 1 - PEC 32 gene.

In one embodiment, a PEC 1 - PEC 32 nucleic acid of the invention is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 33 - 64 , from the group consisting of SEQ ID NO: 1 - 32 or the complement thereof.

In another preferred embodiment a PEC 1 - PEC 32 polypeptide according to the invention is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96.

Host cells

In another embodiment, the invention features cells, e.g., transformed host cells or recombinant host cells that contain a nucleic acid encompassed by the invention. A "transformed cell" or "recombinant cell" is a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid according to the invention. Both prokaryotic and eukaryotic cells are included, e.g., bacteria, fungi, yeast, and the like, especially preferred are cells from filamentous fungi, in particular Aspergillus niger.

A host cell may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in a specific, desired fashion. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may facilitate optimal functioning of the protein.

Various host cells have characteristic and specific mechanisms for post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems familiar to those of skill in the art of molecular biology and/or microbiology may be chosen to ensure the desired and correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may

be used. Such host cells are well known in the art.

Host cells also include, but are not limited to, mammalian cell lines such as CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and choroid plexus cell lines.

If desired, the polypeptides according to the invention may be produced by a stably-transfected cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, methods for constructing such cell lines are also publicly known, e.g., in Ausubel et al. (supra).

Antibodies

The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind PEC 1 - PEC 32 proteins according to the invention.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of specifically binding to PEC 1 - PEC 32 protein. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the PEC 1 - PEC 32 protein or an antigenic fragment thereof may be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of PEC 1 - PEC 32 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or PEC 1 - PEC 32 protein binding fragments thereof). Such monoclonal antibodies may be prepared using hybridoma technology (Kohler *et al., Nature 256*:495 (1975); Kohler *et al., Eur. J. Immunol. 6*:511 (1976); Hammerling *et al.,* In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a PEC 1 - PEC 32 protein antigen or, with a PEC 1 - PEC 32 protein expressing cell. The splenocytes of

such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present inventoin; however, it is preferably to employ the parent myeloma cell line (SP_2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al. (Gastro-enterology 80:*225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the PEC 1 - PEC 32 protein antigen. In general, the polypeptides may be coupled to a carrier protein, such as KLH, as described in Ausubel et al., supra, mixed with an adjuvant, and injected into a host mammal.

In particular, various host animals may be immunized by injection of a polypeptide of interest. Examples of suitable host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), adjuvant mineral gels such as aluminum hydroxide, surface actve substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, BCG (bacille Calmette-Guerin) and *Corynebacterium parvum.* Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.

Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridomas producing the mAbs of this invention may be cultivated *in vitro* or *in vivo.*

Once produced, polyclonal or monoclonal antibodies are tested for specific recognition of a PEC 1 - PEC 32 polypeptide or functional equivalent thereof in an immunoassay, such as a Western blot or immunoprecipitation analysis using standard techniques, e.g., as described in Ausubel et al., supra. Antibodies that specifically bind to PEC 1 - PEC 32 proteins or functional equivalents thereof are useful in the invention. For example, such antibodies may be used in an immunoassay to detect PEC 1 - PEC 32 in pathogenic or non-pathogenic strains of *Aspergillus* (e.g., in *Aspergillus* extracts).

Preferably, antibodies of the invention are produced using fragments of the PEC 1 - PEC 32 polypeptides that appear likely to be antigenic, by criteria such as high frequency of charged residues. For example, such fragments may be generated by standard techniques of PCR, and then cloned

into the pGEX expression vector (Ausubel et al., supra). Fusion proteins may then be expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, et al., supra. If desired, several (e.g., two or three) fusions may be generated for each protein, and each fusion may be injected into at least two rabbits. Antisera may be raised by injections in a series, typically including at least three booster injections. Typically, the antisera are checked for their ability to immunoprecipitate a recombinant PEC 1 - PEC 32 polypeptide or functional equivalents thereof whereas unrelated proteins may serve as a control for the specificity of the immune reaction.

Alternatively, techniques decribed for the production of single chain antibodies (U.S. Patent 4,946,778 and 4,704,692) may be adapted to produce single chain antibodies against a PEC 1 - PEC 32 polypeptide or functional equivalents thereof. Kits for generating and screening phage display libraries are commercially available e.g. from Pharmacia.

Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library may be found in, for example, U.S. Patent No. 5,223, 409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 20791; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246;1275-1281; Griffiths et al. (1993) *EMBO J.* 12:725-734.

Polyclonal and monoclonal antibodies that specifically bind PEC 1 - PEC 32 polypeptides of functional equivalents thereof may be used, for example, to detect expression of a PEC 1 - PEC 32 gene or a functional equivalent thereof e.g. in another strain of *Aspergillus*. For example, PEC 1 - PEC 32 polypeptide may be readily detected in conventional immunoassays of *Aspergillus* cells or extracts. Examples of suitable assays include, without limitation, Western blotting, ELISAs, radioimmune assays, and the like.

By "specifically binds" is meant that an antibody recognizes and binds a particular antigen, e.g., a PEC 1 - PEC 32 polypeptide, but does not substantially recognize and bind other unrelated molecules in a sample.

Antibodies may be purified, for example, by affinity chromatography methods in which the polypeptide antigen is immobilized on a

resin.

An antibody directed against a polypeptide of the invention (e.g., monoclonal antibody) may be used to isolate the polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, such an antibody may be used to detect the protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The antibodies may also be used diagnostically to monitor protein levels in cells or tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen or in the diagnosis of Aspergillosis..

Detection may be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive materials include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Preferred epitopes encompassed by the antigenic peptide are regions that are located on the surface of the protein, e.g., hydrophilic regions. Hydrophobicity plots of the proteins of the invention may be used to identify hydrophilic regions.

The antigenic peptide of a protein of the invention comprises at least 7 (preferably 10, 15, 20, or 30) contiguous amino acid residues of the amino acid sequense of a sequence selected from the group consisting of SEQ ID NO: 65 - 96 and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

Preferred epitopes encompassed by the antigenic peptide are regions of PEC 1 - PEC 32 that are located on the surface of the protein, e.g., hydrophilic regions, hydrophobic regions, alpha regions, beta regions, coil regions, turn regions and flexible regions.

Immunoassays

Qualitative or quantitative determination of a polypeptide according to the present invention in a biological sample may occur using any art-known method. Antibody-based techniques provide special advantages for assaying specific polypeptide levels in a biological sample.

In these, the specific recognition is provided by the primary antibody (polyclonal or monoclonal) but the secondary detection system may utilize fluorescent, enzyme, or other conjugated secondary antibodies. As a result, an immunocomplex is obtained.

Accordingly, the invention provides a method for diagnosing whether a certain organism is infected with Aspergillus comprising the steps of:

- Isolating a biological sample from said organism suspected to be infected with Aspergillus,
- reacting said biological sample with an antibody according to the invention,
- determining whether immunecomplexes are formed.

Tissues may also be extracted, e.g., with urea and neutral detergent, for the liberation of protein for Western-blot or dot/slot assay. This technique may also be applied to body fluids.

Other antibody-based methods useful for detecting PEC 1 - PEC 32 gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). For example, PEC 1 - PEC 32-specific monoclonal antibodies may be used both as an immunoabsorbent and as an enzyme-labeled probe to detect and quantify the PEC 1 - PEC 32 protein. The amount of PEC 1 - PEC 32 protein present in the sample may be calculated by reference to the amount present in a standard preparation using a linear regression computer algorithm. In another ELISA assay, two distinct specific monoclonal antibodies may be used to detect PEC 1 - PEC 32 protein in a biological fluid. In this assay, one of the antibodies is used as the immuno-absorbent and the other as the enzyme-labeled probe.

The above techniques may be conducted essentially as a "one-step" or "two-step" assay. The "one-step" assay involves contacting PEC 1 - PEC 32 protein with immobilized antibody and, without washing, contacting the mixture with the labeled antibody. The "two-step" assay involves washing before contacting the mixture with the labeled antibody. Other conventional methods may also be employed as suitable. It is usually desirable to immobilize one component of the assay system on a support, thereby allowing other components of the system to be brought into contact with the component and readily removed from

the sample.

Suitable enzyme labels include, for example, those from the oxidase group, which catalyze the production of hydrogen peroxide by reacting with substrate. Activity of an oxidase label may be assayed by measuring the concentration of hydrogen peroxide formed by the enzyme-labelled antibody/substrate reaction.

Besides enzymes, other suitable labels include radioisotopes, such as iodine (^{125}I, ^{12I}I), carbon (^{14}C), sulphur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Specific binding of a test compound to a PEC 1 - PEC 32 polypeptide may be detected, for example, in vitro by reversibly or irreversibly immobilizing the PEC 1 - PEC 32 polypeptide on a substrate, e.g., the surface of a well of a 96-well polystyrene microtitre plate. Methods for immobilizing polypeptides and other small molecules are well known in the art. For example, the microtitre plates may be coated with a PEC 1 - PEC 32 polypeptide by adding the polypeptide in a solution (typically, at a concentration of 0.05 to 1 mg/ml in a volume of 1-100 ul) to each well, and incubating the plates at room temperature to 37 ^{O}C for 0.1 to 36 hours. Polypeptides that are not bound to the plate may be removed by shaking the excess solution from the plate, and then washing the plate (once or repeatedly) with water or a buffer. Typically, the polypeptide is contained in water or a buffer. The plate is then washed with a buffer that lacks the bound polypeptide. To block the free protein-binding sites on the plates, the plates are blocked with a protein that is unrelated to the bound polypeptide. For example, 300 ul of bovine serum albumin (BSA) at a concentration of 2 mg/ml in Tris-HCl is suitable. Suitable substrates include those substrates that contain a defined cross-linking chemistry (e.g., plastic substrates, such as polystyrene, styrene, or polypropylene substrates from Corning Costar Corp. (Cambridge, MA), for example) . If desired, a beaded particle, e.g., beaded agarose or beaded sepharose, may be used as the substrate.

Binding of the test compound to the polypeptides according to the invention may be detected by any of a variety of artknown methods. For example, a specific antibody may be used in an immunoassay. If desired, the antibody may be labeled (e.g., fluorescently or with a radioisotope) and detected directly (see, e.g., West and McMahon, J. Cell Biol. 74:264, 1977). Alternatively, a second antibody may be used for detection (e.g., a labeled antibody that binds the

Fc portion of an anti-AN97 antibody). In an alternative detection method, the PEC 1 - PEC 32 polypeptide is labeled, and the label is detected (e.g., by labeling aPEC 1 - PEC 32 polypeptide with a radioisotope, fluorophore, chromophore, or the like). In still another method, the PEC 1 - PEC 32 polypeptide is produced as a fusion protein with a protein that may be detected optically, e.g., green fluorescent protein (which may be detected under UV light). In an alternative method, the PEC 1 - PEC 32 polypeptide may be covalently attached to or fused with an enzyme having a detectable enzymatic activity, such as horse radish peroxidase, alkaline phosphatase, a-galactosidase, or glucose oxidase. Genes encoding all of these enzymes have been cloned and are readily available for use by those of skill in the art. If desired, the fusion protein may include an antigen, and such an antigen may be detected and measured with a polyclonal or monoclonal antibody using conventional methods. Suitable antigens include enzymes (e.g., horse radish peroxidase, alkaline phosphatase, and a-galactosidase) and non-enzymatic polypeptides (e.g., serum proteins, such as BSA and globulins, and milk proteins, such as caseins).

Epitopes, antigens and immunogens.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule. On the other hand, a region of a protein molecule to which an antibody may bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen, H. M. et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1984).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody may bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G. et al., Science 219:660-666 (1984). Peptides capable of eliciting

protein-reactive sera are frequently represented in the primary sequence of a protein, may be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer, soluble peptides, especially those containing proline residues, usually are effective. Sutcliffe et al., supra, at 661. For instance, 18 of 20 peptides designed according to these guidelines, containing 8-39 residues covering 75% of the sequence of the influenza virus hemagglutinin HAl polypeptide chain, induced antibodies that reacted with the HA1 protein or intact virus; and 12/12 peptides from the MuLV polymerase and 18/18 from the rabies glycoprotein induced antibodies that precipitated the respective proteins.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. Thus, a high proportion of hybridomas obtained by fusion of spleen cells from donors immunized with an antigen epitope-bearing peptide generally secrete antibody reactive with the native protein. Sutcliffe et al., supra, at 663. The antibodies raised by antigenic epitope bearing peptides or polypeptides are useful to detect the mimicked protein, and antibodies to different peptides may be used for tracking the fate of various regions of a protein precursor which undergoes posttranslation processing. The peptides and anti-peptide antibodies may be used in a variety of qualitative or quantitative assays for the mimicked protein, for instance in competition assays since it has been shown that even short peptides (e.g., about 9 amino acids) may bind and displace the larger peptides in immunoprecipitation assays. See, for instance, Wilson, I.A. et al., Cell 37:767-778 at 777 (1984). The anti-peptide antibodies of the invention also are useful for purification of the mimicked protein, for instance, by adsorption chromatography using methods well known in the art.

Antigenic epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and

including the entire amino acid sequence of a polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); and sequences containing proline residues are particularly preferred.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies.

Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis. For instance, Houghten has described a simple method for synthesis of large numbers of peptides, such as 10-20 mg of 248 different 13 residue peptides representing single amino acid variants of a segment of the HAl polypeptide which were prepared and characterized (by ELISA-type binding studies) in less than four weeks. Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten et al. (1986). In this procedure the individual resins for the solid-phase synthesis of various peptides are contained in separate solvent-permeable packets, enabling the optimal use of the many identical repetitive steps involved in solid-phase methods.

A completely manual procedure allows 500-1000 or more syntheses to be conducted simultaneously. Houghten et al., supra, at 5134.

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F.J. et al., J. Gen. Virol. 66:2347-2354 (1985).

Generally, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemocyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine may be coupled to carrier using

a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde.

Animals such as rabbits, rats and mice are immunized with either free or carriercoupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 ug peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which may be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. For instance, Geysen et al., 1984, supra, discloses a procedure for rapid concurrent synthesis on solid supports of hundreds of peptides of sufficient purity to react in an enzyme-linked immunosorbent assay. Interaction of synthesized peptides with antibodies is then easily detected without removing them from the support. In this manner a peptide bearing an immunogenic epitope of a desired protein may be identified routinely by one of ordinary skill in the art. For instance, the immunologically important epitope in the coat protein of foot-and-mouth disease virus was located by Geysen et al. with a resolution of seven amino acids by synthesis of an overlapping set of all 208 possible hexapeptides covering the entire 213 amino acid sequence of the protein. Then, a complete replacement set of peptides in which all 20 amino acids were substituted in turn at every position within the epitope were synthesized, and the particular amino acids conferring specificity for the reaction with antibody were determined. Thus, peptide analogs of the epitope-bearing peptides of the invention may be made routinely by this method. U.S. Patent No. 4,708,781 to Geysen (1987) further describes this method of identifying a peptide bearing an immunogenic epitope of a desired protein.

Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of

the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to
Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also may be made routinely by these methods.

Use of PEC 1 - PEC 32 pectinases in industrial processes

Pectins and pectinases play an important role in the food industry. Pectinases are used in the production of fruit and vegetable juices and purees, and in the extraction of useful components, like arome compounds, or even pharmaceuticals, from plant materials.

One of the main applications of pectinases is in the production of clear fruit and vegetable juice. The production of juices from fruit and vegetables typically involves, after an optional washing step, grinding, crushing or otherwise destroying the integrity of the fruit or vegetables thus obtaining a fruit or vegetable pulp. Subsequently, the pulp may be treated with enzymes to decrease soluble pectin, a process generally referred to as maceration. After maceration, the pulp is ready for pressing, leading to a juice fraction and a residue fraction, the latter being referred to as the pomace. The juice obtained after pressing is usually pasteurised, optionally with recovery of the aroma, which may then be added back at the end of the process. In order to obtain a clear concentrate, the pasteurised juice is enzymatically depectinized with the aid of pectinases, optionally pre-concentrated, filtrated, optionally ultra-filtrated and concentrated to obtain a clear concentrate or juice which is ready for shipping and/or blending to obtain a clear apple juice for the consumer market

PEC 1 - PEC 32 pectinases are useful in all production processes in which the integrity of the cell wall should be destroyed in order to release materials from the inside of the cells, or from the cell walls.

Pectinases may also be used to destroy the connection between cells without affecting the integrity of the separate cells. When cells should remain intact, e.g. in the preparation of potato puree, PEC 1 - PEC 32 pectinases may advantageously be used to release the structural connection between the cells.

PEC 1 - PEC 32 pectinases are also able to decrease the water-binding and hence the viscosity of pectin-containing slurries, e.g. fruit or vegetable pulps, which improves the pressability/processability of the pulp.

PEC 1 - PEC 32 pectinases are able to clarify cloudy juices. Attacking the outer pectin layer of the cloud particles changes the charge of the particles, thus promoting the aggregation of the particles.

PEC 1 - PEC 32 pectinases may also be used in the retting of flax (to release the cellulose fibres from the plant tissue) and the scouring of cotton (to increase the water absorbance and dye uptake of the cotton fibres).

Plant and pectin-containing materials include plant pulp, parts of plants and plant extracts. In the context of this invention an extract from a plant material is any substance which may be derived from plant material by extraction (mechanical and/or chemical), processing or by other separation techniques. The extract may be juice, nectar, base, or concentrates made thereof. The plant material may comprise or be derived from vegetables, e.g., carrots, celery, onions, legumes or leguminous plants (soy, soybean, peas) or fruit, e.g., pome or seed fruit (apples, pears, quince etc.), grapes, tomatoes, citrus (orange, lemon, lime, mandarin), melons, prunes, cherries, black currants, redcurrants, raspberries, strawberries, cranberries, pineapple and other tropical fruits, trees and parts thereof (e.g. pollen, from pine trees).

Fruit juice that needs to be filtrated after or during processing tends to clot the filters after some time. This is due to the inability of prior art enzymes to properly digest fruit polysaccharides which causes the fouling of the filters. Use of the enzymes according to the invention prevents fouling of filters because of the unique properties of the PEC 1 - PEC 32 pectinase.

PEC 1 - PEC 32 pectinases may conviently be produced in microorganisms. Microbial pectinases are available from a variety of sources; Bacillus spec. are a common source of bacterial enzymes, whereas fungal enzymes are commonly produced in Aspergillus spec.

In the above processes, it is advantageous to use pectinases that are obtained by recombinant DNA techniques. Such recombinant enzymes have a number of advantages over their traditionally purified counterparts.

Recombinant enzymes may be produced at a low cost price, high yield, free from contaminating agents like bacteria or viruses but also free from bacterial toxins or contaminating other enzyme activities.

The polypeptides of the invention may be used to treat plant material including plant pulp and plant extracts. For example, they may be used to treat apple pulp and/or raw juice during the production of apple juice. They may also be used to treat liquid or solid foodstuffs or edible foodstuff ingredients. Conveniently the polypeptide of the invention is combined with suitable (solid or liquid) carriers or diluents including buffers to produce a composition or enzyme preparation. The polypeptide is typically stably formulated either in liquid or dry form. Typically, the product is made as a composition which will optionally include, for example, a stabilising buffer and/or preservative. The compositions may also include other enzymes capable of digesting plant material or pectin, for example other pectinases such as an endo-arabinanase, rhamnogalacturonases, and/or polygalacturonase. For certain applications, immobilization of the enzyme on a solid matrix or incorporation on or into solid carrier particles may be preferred. The composition may also include a variety of other plant material-degrading enzymes, for example cellulases and other pectinases.

The polypeptides and compositions of the invention may therefore be used in a method of processing plant material to degrade or modify the pectin constituents of the cell walls of the plant material

Typically, the polypeptides of the invention are used as a composition/ enzyme preparation as described above. The composition will generally be added to plant pulp obtainable by, for example mechanical processing such as crushing or milling plant material. Incubation of the composition with the plant material will typically be carried out for a time of from 10 minutes to 5 hours, such as 30 minutes to 2 hours, preferably for about 1 hour. The processing temperature is preferably 10-55°C, e.g. from 15 to 25°C, optimally about 20°C and one may use 10-300g, preferably 30-70g, optimally about 50g of enzyme per ton of material to be treated. All the enzyme(s) or their compositions used may be added sequentially or at the same time to the plant pulp. Depending on the composition of the enzyme preparation the plant material may first be macerated (e.g. to a purée) or liquefied. Using the polypeptides of the invention processing parameters such as the yield of the extraction, viscosity of the extract and/or quality of the extract may be improved.

Alternatively, or in addition to the above, a polypeptide of the

invention may be added to raw juice obtained from pressing or liquefying the plant pulp. Treatment of raw juice may be carried out in a similar manner to the plant pulp in respect of dosage, temperature and holding time. Again, other enzymes than pectinases may be included.

A composition containing a polypeptide of the invention may also be used during the preparation of fruit or vegetable purees. The end product of these processes is typically heat–treated at 85°C for a time of from 1 minute to 1 hour, under conditions to partially or fully inactivate the polypeptides of the invention.

Due to the highly specific action on pectins the polypeptides of the invention may also be used to prepare pectins with modified chara teristics, e.g. modified gelation capacities for specific applications.

The polypeptides of the invention may be added to animal feeds rich in pectin, e.g. soy-containing food, to improve the breakdown of the plant cell wall leading to improved utilisation of the plant nutrients by the animal. The polypeptides of the invention may be added to the feed or silage if pre-soaking or wet diets are preferred. Advantageously, the polypeptides of the invention may continue to degrade pectins in the feed in vivo. Polypeptides according to the invention have low pH optima and are capable of releasing important nutrients in such acidic environments as the stomach of an animal. The invention thus also provides (e.g. animal) feeds and/or foodstuffs comprising one or more polypeptides of the inv ntion, as well as feeds and/or foodstuffs obtainable by incubation and/or digestion with at least one enzyme according to the invention.

PEC 1 - PEC 32 pectinases may also be advantageously used during the production of milk substitutes (or replacers) from soy bean. These milk substitutes may be consumed by both humans and animals. A typical problem during the preparation of these milk substitutes is the high viscosity of the soy bean slurry, resulting in the need for an undesirable dilution of the slurry to a concentration of dry solids of 10 to 15%. An enzyme preparation containing a polypeptide according to the invention may be added to the slurry, either before or during the processing, enabling processing at a higher concentration (typically 40 to 50%) dry solids. Addition of pectinases according to the invention to a soy suspension as described in WO 95/29598 results in a decrease in waterbinding and a concommitant decrease in viscosity of the slurry.

The enzyme may also be used in the preparation of savoury product(s), e.g. from soy bean.

The invention also relates to the use of PEC 1 - PEC 32 pectinases in a selected number of industrial and pharmaceutical processes. Despite the long term experience obtained with such processes, the pectinase according to the invention features a number of significant advantages over the prior art enzymes. Depending on the specific application, these advantages may include aspects like lower production costs, higher specificity towards the substrate, less antigenic, less undesirable side activities, higher yields when produced in a suitable microorganism, more suitable pH and temperature ranges, better tastes of the final product as well as food grade and kosher aspects.

An important aspect of the pectinases according to the invention is that they cover a whole range of pH and temperature optima which are ideally suited for a variety of applications. For example many large scale processes benefit from relatively high processing temperatures of 50 degrees C or higher, e.g. to control the risks of microbial infections. Several pectinases according to the invention comply with this demand but at the same time they are not that heat stable that they resist attempts to inactivate the enzyme by an additional heat treatment. The latter feature allows production routes that yield final products free of residual enzyme activity. Similarly many feed and food products have slightly acidic pH values so that pectinases with acidic or near neutral pH optima are preferred for their processing. A PEC 1 - PEC 32 pectinase complies with this requirement as well.

The polypeptides of the invention may also be added to animal feeds rich in pectin or xylogalacturonan, to reduce anti-nutritional effects of plant pectins and consequently improve production performances and welfare of the animals, as well as reducing environmental pollution.

It has been shown that plant pectins increase digesta viscosity, fermentation and colonisation of bacterial proliferation in the gastro intestinal tract (Langhout, D.J. (1998). The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks. PhD thesis, Agricultural University Wageningen, The Netherlands). As a consequence, several digestive physiological parameters are altered (Johnson, I.T. and Gee, J.M. (1981). Effect of gel-forming gums on the intes Mu . R. (1984). Effect of dietary

supplements of guar gumcor ellulose on intestinal cell proliferation, enzyme levels

as present in the expression vector pGBTOPFYT1 as and glucose transport in the rat. British Journal of Nutrition 52:477-487) resulting in reduced dietary nutrient, mineral and energy utilization (Langhout, D.J. and Schutte, J.B. (1996). Nutritional implications of pectins in chicks in relation to esterification and origin of pectins. Poultry Science 75:1236-1242, Smits, C.M.H., Veldman, A., Verkade, H.J. and Beynen, A.C. (1998). The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. Poultry Science 77:1534-1539). The magnitude efficiency of dietary nutrient utilization is reduced, the effectivity in production performances (e.g. efficiency of producing milk, eggs, wool and meat) declines. Moreover, at reduced dietary nutrient utilization by the animal, excretion of and environmental pollution with e.g. nitrogenous compounds and heavy metals is increased.

Advantagous is that these fungal derived polypeptides of the invention have generally lower pH optima and their activities are therefore (more) capable of hydrolysis in such acidic environments as the stomach of an animal. With that, anti-nutritional properties of the plant cell wall fractions are already reduced early on in the digestive tract of the animal.

The improved characteristics of pectinases according to the invention were established as described in the examples below. PEC 1 – PEC 32 were expressed in A. niger by cloning of cDNAs acco ding to SEQ ID NO: 1 – 32. The resulting enzyme preparations were subsequently purified by ultrafiltration and used in food and feed conversion as described in the examples. It was found that PEC 1 – PEC 32, when added to animal feed, were able to increase the nutritional value of the feed, as measured by an improved body weight gain in the range of 1 to 5% and in an improvement of the feed conversion ration of 1.5 to 6%. In particular, PEC 1 – PEC 21 corresponding to polypeptides with SEQ ID NO: 65 – 85 were found especially useful.

EXAMPLES

Example 1.

DNA fragments having a sequence according to SEQ ID NO: 1 – 32 were cloned under the control of the glucoamylase promoter. To this end, the *Aspergillus niger* phytase (*phyA)* gene
described in international patent application WO 98/46772 was replaced with the

pectinase genes described above, yielding the vectors pPECT1 - 32 respectively. The expression vectors pPECT1 - 32 were introduced into *Aspergillus niger* CBS 646.97 (described in WO 98/46772). Using PCR, transformants containing pPECT1 - 32 were selected. 10^7 spores of selected transformants were inoculated into shake flasks, containing 20 ml of liquid preculture medium containing per liter: 30 g maltose.H_2O; 5 g yeast extract; 10 g hydrolyzed casein; 1 g KH_2PO_4; 0.5 g $MgSO_4.7H_2O$; 0.03 g $ZnCl_2$; 0.02 g $CaCl_2$; 0.01 g $MnSO_4$. $4H_2O$; 0.3 g $FeSO_4$. $7H_20$; 3 g Tween 80; 10 ml penicillin (5000 IU/ml)/Streptomycin (5000 UG/ml); pH 5.5. These cultures were grown at 34°C for 20-24 hours. 10 ml of this culture was inoculated into 100 ml of *A. niger* fermentation medium containing per liter: 70 g maltodextrines; 25 g hydrolyzed casein; 12.5 g yeast extract; 1 g KH_2PO_4; 2 g K_2SO_4; 0.5 g $MgSO_4.7H_2O$; 0.03 g $ZnCl_2$; 0.02 g $CaCl_2$; 0.01 g $MnSO_4.4H_2O$; 0.3 g $FeSO_4.7H_2O$; 10 ml penicillin (5000 IU/ml)/Streptomycin (5000 UG/ml); adjusted to pH 5.6 with 4 N H_2SO_4. These cultures were grown at 34°C for about 6 days. Samples taken from the fermentation broth were centrifuged (10', 5.000 rpm in a swinging bucket centrifuge) and supernatants collected. The culture supernatants were concentrated by ultrafiltration and used as enzyme source.

An experime t was designed to establish whether PEC 1 – PEC 32 pectinases, expressed as described above, could improve the nutritional value of soybean-based feed. The experiment was performed using male broilers (Ross 308) housed in floor pens. Directly after arrival from the hatchery, the animals were randomly distributed over 40 pens, each pen containing 20 broilers. Eight pens were allocated to each treatment. The pens were set up in an artificially heated, illuminated and ventilated broiler house, applying conditions similar to those found in practice. Animals were vaccinated according to the normal vaccination program. The experiment was performed till day 35 of age.

Five treatments were included in this trial. To the basal diet (table 1), no enzyme was added (control). To diet 2 a multi-enzyme (pectinase) mixture (Rapidase Press ® DSM) was added in a concentration of 100 ppm. Diets 3, 4 and 5 additionally contained one single pectinase selected from the group consisting of SEQ ID NO: 65 - 95, in three different doses 1, 5 and 25 ppm, respectively. The feeds were pelleted without the addition of steam in the process. Feed and water were offered *ad libitum* to the animals.

Table 1: Feed composition and contents of main nutrients of diet 1.

Ingredient	Contents (%)
Wheat	60
Soybean meal	29
Soy oil	1
Blended animal fat	6
Minerals, vitamins, amino acids	4
ME (MJ/kg)	12.8
Crude protein (%)	21
Crude fat (%)	9

The diet was not supplemented with an antibiotic growth promoter or a coccidiostat. The results of this trial are presented in table 2.

Table 2. Average Body Weight Gain and Feed Conversion Ratio of the broilers (day 1-35).

Treatment	BWG (g/bird)	FCR (g/g)
1. Control	1957	1.613
2. Control + Rapidase Press 100 ppm	2019	1.613-1.601
3. Control + Rapidase Press + PEC 1 – PEC 32, 1 ppm	2019-2044	1.610-1.589
4. Control + Rapidase Press + PEC 1 – PEC 32, 5 ppm	2037 -2051	1.594-1.578
5. Control + Rapidase Press + PEC 1 – PEC 32, 25 ppm	2035-2055	1.589- 1.505

Rapidase Press improved performance (treatment 2 compared to treatment 1), especially the growth of the animals. Additional supplementation with any enzyme chosen from PEC 1 – PEC 32, (treatments 3 – 5) further improved the performance of the animals, in particular supplementation with an enzyme chosen from PEC 1 – PEC 21 (corresponding to SEQ ID NO: 65 – 85) lead to a 1 - 4% gain in body weight and improved the feed conversion ratio with 1.5 - 6%.

Example 2.

In this experiment the apparent metabolisable energy (AME) content of the experimental diets was determined, as well as the apparent faecal digestibility of fat and protein. Male broilers (Ross 308) of seven days of age were randomly distributed over 18 cages, each cage housing 8 broilers. Six cages were allocated to each treatment. The cages were set up in an artificially heated, illuminated and ventilated broiler house, using a three-tier cage system. Tiers were blocked over the treatments. The room was illuminated 24 hours/day, but the light intensity was gradually decreased during the trial. Also the temperature was decreased gradually during the experiment, according to a practical schedule. The humidity during the trial was kept at approximately 60%. Animals were vaccinated according to the normal vaccination program against Infectious Bronchitis and New Castle Disease. The experiment was performed till day 28 of age. Excreta were collected from day 24 to day 28. To the diets 0.25% chromic oxide was added as an indigestible marker, at the expense of wheat.

Three treatments were included in this trial: treatments 1, 2 and 5 from example 1. The treatments were: 1. basal diet (table 1 [example 1]), without enzyme was added (control), 2. addition of Rapidase Press, and 5. as diet 2, but with the addition of 25 ppm of additional pectinases PEC 1 – PEC 32.

The feeds were pelleted without the addition of steam in the process. Feed and water were offered *ad libitum* o the animals. Analytical procedures applied were similar to those typically applied in animal experimentation institutes, usually AOAC-methods.

The results of this trial are presented in table 3.

Table 3. AME (MJ/kg) content and apparent faecal digestibility coefficients (%) of fat and protein of the three experimental diets. Excreta of the male broilers were collected from day 24 - 28.

Treatment	AME (MJ/kg)	Faecal digestibility (%)	
		Fat	Protein
1. Control	12.57	78.4	79.4

2. Control + Rapidase Press 100 ppm	12.67	79.6	82.3
5. Control + Rapidase Press + PEC 1 – PEC 32, 25 ppm	12.77-13.51	80.0-81.7	81.0-83.4

It may be concluded that Rapidase Press improved digestibility, especially of protein. Addition of PEC 1 – PEC 32 had a large effect on energy metabolisability and on faecal digestibility of fat and protein, in particular PEC 1 – PEC 21 showed significant improvements in the order of 2 to 5%.

Example 3.

A next experiment was performed using mixed-sex broilers (Ross) fed a maize-soybean meal-based diet. The animals were housed in floor pens. Directly after arrival from the hatchery, the animals were randomly distributed over 48 pens, each pen containing 24 broilers; eight pens (four pens of males and four pens of females) were allocated to each treatment.
The pens were set up in an artificially heated, illuminated and ventilated broiler house, applying conditions similar to those found in practice. Animals were vaccinated according to the normal vaccination program against Infectious Bronchitis and New Castle Disease. The experiment was performed up to day 42 of age.

The following treatments were included in this trial:

1. Basal diet (table 4) without addition of enzyme (control)
2. Control + Rapidase Press 100 ppm
3. Diet 1 + PEC 1 – PEC 32, 25 ppm
4. Diet 2 + PEC 1 – PEC 32, 1 ppm
5. Diet 2 + PEC 1 – PEC 32, 5 ppm
6. Diet 2 + PEC 1 – PEC 32, 25 ppm

The feeds were pelleted without the addition of steam in the process. Feed and water were offered *ad libitum* t the animals.

Table 4: Feed composition and contents of main nutrients of diet 2.

Ingredient	Contents (%)
Maize	50.5
Soybean meal	31.7
Tapioca	6.0
Fish meal	1.0
Meat meal	2.1
Soy oil	2.1
Blended animal fat	3.0
Mono calcium phosphate	1.2
Limestone	0.45
Stenerol (coccidiostat)	0.05
Other minerals, vitamins, amino acids	1.9
ME (MJ/kg)	11.9
Crude protein (%)	21.5
Crude fat (%)	8.0
Digestible lysine (%)	1.06
Digestible methionine + cystine	0.80

The diet was not supplemented with an antibiotic growth promoter. The results of this trial are presented (as averages over the period 1-42 days and over the sexes) in table 5.

Table 5. Average BWG and FCR of the broilers (day 1-42).

Treatment	BWG (g/bird)	FCR (g/g)
1	2166	1.811
2	2204	1.820
3	2170 - 2210	1.776 – 1.803
4	2190 - 2206	1.781 - 1.806
5	2195 - 2222	1.771 - 1.823
6	2215 - 2241	1.752 – 1.795

Rapidase Press improved growth by 1.8% (treatment 2 compared to treatment 1). A single enzyme selected from PEC 1 – 32 (treatment 3) showed a similar effect, but improved FCR as well (average 1.4%). Supple entation with both enzymes, Rapidase Press and an enzyme selected from PEC 1 – PEC 32 in different doses (treatments 4 – 6) showed the best results. In particular PEC 1 – PEC 21 were found to be useful enzymes in this respect.

Example 4.

Also the diet tested in example 3 was submitted to a digestibility experiment. In this experiment the apparent metabolisable energy (AME) content of the experimental diets was determined, as well as the apparent faecal digestibility of fat and protein. Also the apparent ileal digestibility of amino acids was determined. Male broilers (Ross) of nine days of age were used in this experiment. The animals were randomly distributed over 24 cages, each cage housing 8 broilers. Six cages were allocated to each treatment. The cages were three-tier cage system. Tiers were blocked over the treatments. The room was illuminated 24 hours/day, but the light intensity was gradually decreased during the trial. Also the temperature was decreased gradually during the experiment, according to a practical schedule. The humidity during the trial was kept at approximately 60%. Animals were vaccinated according to the normal vaccination program against Infectious Bronchitis and New Castle Disease. The experiment was performed till day 29 of age. Excreta were collected from day 25 to day 29. At day 29, the animals were euthanized using T61, and the contents of the last 30

cm of the small intestine were collected. The digesta were pooled per cage, to form six samples per treatment. The diets were identical to those mentioned in table 4, but 0.25% chromic oxide was added as an indigestible marker, at the expense of tapioca.

Four treatments were included in this trial: treatments 1, 2, 3 and 6 from example 3. The treatments were:

1. Basal diet (table 4) without addition of enzyme (control)
2. Control + Rapidase Press 100 ppm
3. Diet 1 + PEC 1 – PEC 32, 25 ppm
4. Diet 2 + PEC 1 – PEC 32, 25 ppm

The feeds were pelleted without the addition of steam in the process. Feed and water were offered *ad libitum* to the animals. Analytical procedures applied were similar to those typically applied in animal experimentation institutes, usually AOAC-methods. Methionine was determined after prior oxidation with performic acid. The results of this trial are presented in tables 6 (AME and faecal digestibility) and in table 7 (ileal amino acid digestibility).

Table 6. AME (MJ/kg) content and apparent faecal digestibility coefficients (%) of fat and protein of the three experimental diets. Excreta of the male broilers were collected from day 24 - 28.

Treatment	AME (MJ/kg)	Faecal digestibility (%)	
		Fat	Protein
1	12.2	80.5	81.3
2.	12.4	80.6	82.4
3	12.2 – 12.6	80.4 – 80.9	81.5 – 82.9
6	12.3 – 12.7	80.9 - 82.4	81.2 - 83.3

Table 7. Apparent ileal digestibility coefficients (%) for lysine, methionine, and threonine. Digesta collected at day 29.

Treatment	Lysine	Methionine	Threonine
1	83.9	86.5	78.6
2	85.1	88.1	79.8
3	83.9 - 85.3	86.5 – 88.5	78.1 - 80.1
6	83.8 - 86.9	86.8 - 89.6	78.4 - 81.2

The enzymes according to the invention improved AME and digestibility of the different nutrients. In particular PEC 1 – PEC 21 were found to be suitable in the above application. Combination of PEC 1 – PEC 21 with a commercial pectinase preparartion was found to be the better treatment in this experiment, and seemed especially to have a synergistic effect on AME and fat digestibility.

EXAMPLE 5

Substrate preparation: Preparation of MHR-S from apples

Modified hairy regions (MHR) from apples were isolated as a filter retentate after treatment of apples with Rapidase Press, a commercially available pectinase preparation, and subsequently the MHR was saponified resulting in MHR-S.

EXAMPLE 6

Improvement of filtration rate by PEC 1 – PEC 32 and combinations thereof

Experiments were done to see if the pectinases according to the invention could improve filterfouling during filtration. Apple MHR-S prepared as described in Example 2.1 was used as a substrate. A solution of 0.5% in 50 mM acetate buffer pH 4.0 was incubated with each individual enzyme chosen from PEC 1 – PEC 32 for 17 hours at 30□C. The solutions were filtrated using an Amicon device equipped with a 30 kD filter at a pressure of 2 bars. The increase in weight of the filtrate was followed over time. In particular PEC 6 – PEC 32 showed significant improvements in filter fouling. Further improvements were obtained when enzymes with different specificities within this group were combined.

CLAIMS

1) An isolated polynucleotide hybridisable to a polynucleotide selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32

2) An isolated polynucleotide according to claim 1 hybridisable under high stringency conditions to a polynucleotide selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32

3) An isolated polynucleotide according to claims 1 or 2 obtainable from a filamentous fungus.

4) An isolated polynucleotide according to claim 3 obtainable from A. niger.

5) An isolated polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

6) An isolated polynucleotide encoding at least one functional domain of a polypeptide selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

7) An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32 or functional equivalents thereof

8) An isolated polynucleotide selected from the group consisting of SEQ ID NO: 33 - 64 or from the group consisting of SEQ ID NO: 1 - 32.

9) A vector comprising a polynucleotide sequence according to claims 1 to 8.

10) A vector according to claim 9 wherein said polynucleotide sequence according to claims 1 to 8 is operatively linked with regulatory sequences suitable for expression of said polynucleotide sequence in a suitable host cell.

11) A vector according to claim 10 wherein said suitable host cell is a filamentous fungus

12) A method for manufacturing a polynucleotide according to claims 1 – 8 or a vector according to claims 9 to 11 comprising the steps of culturing a host cell transformed with said polynucleotide or said vector and isolating said polynucleotide or said vector from said host cell.

13) An isolated polypeptide selected from the group consisting of SEQ ID NO: 65 - 96 or functional equivalents thereof.

14) An isolated polypeptide according to claim 13 obtainable from Aspergillus niger

15) An isolated polypeptide obtainable by expressing a polynucleotide according to claims 1 to 8 or a vector according to claims 9 to 11 in an appropriate host cell, e.g. Aspergillus niger.

16) Recombinant pectinase comprising a functional domain of a PEC 1 - PEC 32 polypeptide.

17) A method for manufacturing a polypeptide according to claims 13 to 16 comprising the steps of transforming a suitable host cell with an isolated polynucleotide according to claims 1 to 8 or a vector according to claims 9 to 11, culturing said cell under conditions allowing expression of said polynucleotide and optionally purifying the encoded polypeptide from said cell or culture medium.

18) A recombinant host cell comprising a polynucleotide according to claims 1 to 8 or a vector according to claims 9 to 11.

19) A recombinant host cell expressing a polypeptide according to claims 13 to 16.

20) Purified antibodies reactive with a polypeptide according to claims 13 to 16.

21) Fusion protein comprising a polypeptide sequence according to claims 13 to 16.

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Lys Ser Pro Gly Asn Phe Lys Pro Ala Ser Gly Arg Gln Asn Ser Thr
35 40 45

Ser Asn Val Cys Glu Val Lys Pro Asn Gln Thr Asp Ala Ala Pro Gly
50 55 60

Ile Leu Ala Ala Ala His Thr Cys Asn Asn Gly Gly Thr Val Phe Leu
65 70 75 80

Pro Pro Gly Asp Phe Val Val Ala Thr Ala Leu Asp Leu Thr Phe Leu
85 90 95

Asn Asn Ile Asp Phe Ala Ile Trp Gly Asn Ile Thr Phe Lys Lys Asp
100 105 110

Ile Asp Leu Trp Thr Thr Gln Ala Phe Gln Tyr Thr Phe Gln Thr Ala
115 120 125

Ser Leu Phe Trp Arg Phe Gly Gly Asn Asn Val Asn Ile Tyr Gly Asp

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Gly Lys Gly Val Ile Asp Gly Ala Gly Gln Tyr Trp Trp Ser Ala Met
145 150 155 160

Ala Glu Asp Ser Ser Val Met Arg Pro Cys Leu Leu Gly Thr Asp Gly
165 170 175

Leu His His Ala Thr Ile Ser Gly Leu Thr Met Leu Asn Ser Pro Asn
180 185 190

Trp Phe Asn Leu Ile Ala Asn Ser Thr Asp Ile Leu Ile Ser Asn Met
195 200 205

Thr Met Leu Val Glu Ser Glu Ile Ser Asp Ala Pro Ala Lys Asn Thr
210 215 220

Asp Gly Trp Asp Ile Tyr Arg Ser Ser Asn Ile Val Ile Gln Asp Ser
225 230 235 240

Arg Ile Val Asn Thr Asp Asp Cys Val Ser Phe Lys Pro Asn Ser Thr
245 250 255

Gln Ile Val Ile Gln Asn Leu Asp Cys Thr Gly Ser His Gly Ile Ser
260 265 270

Val Gly Ser Leu Gly Gln Tyr Gln Gly Glu Thr Asp Ile Val Glu Asp
275 280 285

Leu Tyr Ile Tyr Asn Ile Ser Met Thr Asp Ala Ser Asp Val Ala Arg
290 295 300

Ile Lys Val Trp Pro Gly Val Pro Ala Asp Thr Ser Gly Ser Thr Ser
305 310 315 320

Gly Gly Gly Leu Gly Arg Val Arg Asn Val Thr Tyr Glu His Met Gln
325 330 335

Ser Glu Asn Asn Asp His Ile Ile Ser Val Ser Gln Cys Tyr Glu Ser

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340 345 350

Lys Asn Gln Thr Met Cys Asp Ser Tyr Pro Ser Lys Leu Val Ile Glu
355 360 365

Asp Val Leu Phe Lys Asp Phe Lys Gly Thr Thr Ser Lys Lys Tyr Asp
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Pro Glu Ile Gly Glu Leu Thr Cys Ser Ser Pro Asp Val Cys His Asn
385 390 395 400

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Gln Ala Met Ala Pro Ile Ala Leu Lys Ile Leu Leu Phe Thr Ser Leu
35 40 45

Ile Val Pro Ser Ile Ser Leu Ser Asp Gln Ala Arg Asn Gly His Ala
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Arg Thr Ile Cys Glu Val Lys Pro Gly Gly Ser Ser Glu Ile Asp Asp
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Val Pro Ala Ile Val Asp Ala Leu Thr Thr Cys Gly Ser Gly Gly Arg
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Val Ile Phe Ser Asn Asn Thr Tyr His Ile Asn Ser Val Met Asn Thr
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Thr Trp Leu Asp Asp Val Glu Ile Asp Leu Gln Gly Thr Leu Leu Trp
        115                 120                 125

Ser Thr Asn Ile Ser Tyr Trp Leu Asn His Ser Leu Pro Val Gly Tyr
    130                 135                 140

Gln Asn Gln Ser Thr Ala Trp Ile Leu Gly Gly Lys Asp Ile Val Phe
145                 150                 155                 160

Glu Gly His Gly Tyr Gly Thr Phe Asn Gly Ser Gly Gln Thr Trp Tyr
                165                 170                 175

Arg Tyr Val Gly Ser Thr Ser Asn Tyr Pro Arg Arg Pro Asn Gln Leu
            180                 185                 190

Thr Val Ser Gly Ala Met Gly Ala Val Phe Lys Gly Leu Arg Phe Val
        195                 200                 205

Gln Ser Gln Met Trp Thr Met Ser Ile Ile His Thr Ser Asn Ser Trp
    210                 215                 220

Phe Asp Ser Ile Tyr Val Asn Asn Leu Tyr Asp Asp Gly Gly Ser Ala
225                 230                 235                 240

Gln Asn Thr Asp Gly Ala Asn Thr Ile Tyr Ser Lys Asn Ile Thr Leu
                245                 250                 255

Thr Asn Trp Glu Val Val Asn Gly Asp Asp Ser Ile Ser Thr Lys Ala
            260                 265                 270

Asn Ser Thr Asp Ile Thr Ile Ala Asn Cys Thr Phe Thr Ser Gly Leu
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Val Glu Arg Leu Lys Ile Ser Asn Ile Thr Tyr Glu Lys Thr Thr His
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Ala Val Tyr Phe Lys Thr Trp Thr Gly Asp Gln Val Gly Tyr Pro Pro
                325                 330                 335

Asn Gly Gly Gly Gly Gly Leu Gly Tyr Ala Ser Asp Ile Val Ala Thr
            340                 345                 350

Asn Leu Lys Thr Asn Asn Leu Lys Gly Ala Pro Phe Thr Ile Ser Gln
        355                 360                 365

Cys Thr Thr Phe Ser Gly Ala Ser Gly Asn Cys Thr Asn Ser Lys Phe
    370                 375                 380

Gln Ile Arg Asp Leu Val Phe Thr Asp Ile Ser Gly Thr Thr Asp Ser
385                 390                 395                 400

Ser Asp Val Ala Ser Phe Gln Cys Ser Ala Val Ala Pro Cys Glu Asp
                405                 410                 415

Ile Thr Ile Glu Asn Val Ser Leu Arg Ile Ala Gly Asn Thr Thr His
            420                 425                 430

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Arg Ala Asn Gly Gly His Gln Asp Asp Val Pro Asn Ile Met Ala Ala
        35                  40                  45

Phe Lys Glu Cys Gly Asn Gly Gly Thr Ile Ile Phe Pro Glu Asp Gln
    50                  55                  60

Ser Tyr Trp Ile Ala Thr Arg Leu His Pro Thr Leu Lys Asp Val Ala
65                  70                  75                  80

Ile Glu Trp Arg Gly Lys Trp Thr Phe Ser Asp Asn Leu Thr Tyr Trp
                85                  90                  95

Arg Asn Asn Ser Tyr Pro Ile Ala Phe Gln Asn His His Ala Gly Phe
            100                 105                 110

Ile Ile Ser Gly Asp Asn Ile Thr Ile Asn Gly Tyr Gly Thr Gly Gly
        115                 120                 125

Ile Asp Gly Asn Gly Asn Thr Trp Tyr Thr Ala Glu Lys Gly Asp Thr
    130                 135                 140

Gln Pro Gly Arg Pro Met Pro Phe Val Phe Trp Asn Val Ser Glu Val
145                 150                 155                 160

Ile Val Asp Ser Phe Tyr Val Lys Asp Pro Pro Leu Trp Ser Val Asn
                165                 170                 175

Ile Met Asn Gly Thr Asn Met Arg Phe Asn Asn Ile Tyr Cys Asn Ala
            180                 185                 190

Thr Ala Val Asp Ala Pro Trp Gly Asp Asn Trp Val Gln Asn Thr Asp
        195                 200                 205
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pectinases.ST251

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Gly Phe Asp Thr Met Asp Ala Thr Asn Ile Gln Leu Thr Asn Phe Val
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Tyr Gln Gly Gly Asp Asp Cys Ile Ala Ile Lys Pro Arg Ser Tyr Asn
225                 230                 235                 240

Ile Asp Ile Gln Asn Val Thr Cys Arg Gly Gly Asn Gly Ile Ala Ile
                245                 250                 255

Gly Ser Leu Gly Gln Tyr Leu Glu Asp Ser Ser Val Ala Asn Ile Arg
            260                 265                 270

Val Asp Lys Val Asn Ile Ile Arg Tyr Asn Glu Asp Met His Asn Ser
        275                 280                 285

Ala Tyr Leu Lys Thr Trp Val Gly Ala Leu Val Pro Gln Ser Ser Tyr
    290                 295                 300

Glu Ser Ala Gly Val Pro Arg Gly Asp Gly Trp Gly Ser Ile Arg Asn
305                 310                 315                 320

Val Leu Phe Ser Asn Phe Asn Val Gln Gly Ala Ser Ala Gly Pro Ser
                325                 330                 335

Ile Ser Gln Asp Ser Gly Asp Asn Gly Ser Tyr Ala Gly Thr Ser Lys
            340                 345                 350

Met Ser Ile Ser Asn Val Ala Phe Val Asn Phe Thr Gly Trp Val Asp
        355                 360                 365

Thr Glu Lys Ser Val Val Ser Thr Val Ser Cys Ser Glu Val His Pro
    370                 375                 380

Cys Tyr Asn Ile Asp Tyr Asp Asn Val Val Leu Tyr Pro Gly Lys Asn
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Ala Thr Thr Ala Gly Thr Gly Ser Cys Lys Tyr Thr Ala Asp Gly Gly
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Ile Pro Leu Gly Asn Gly Gln Asp Asp Val Pro Asn Ile Leu Ser Ala
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Val Asp Glu Cys Gly Gln Thr Ser Gly Gly Arg Val Val Leu Pro Ala
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Pro Tyr Thr Tyr Arg Ile Asn Gln Arg Met Thr Thr His Leu Thr Asp
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Ser Arg Leu Glu Ile Gly Gly Thr Leu Leu Phe Ser Asp Asp Ile Asp
85 90 95

Tyr Trp Val Asn Asn Ser Tyr Arg Val Asp Phe Gln Asn Gln Ser Ser
100 105 110

Ala Trp Arg Ile Thr Gly His Asp Tyr Val Val Asp Gly Gly Pro Arg
115 120 125

Gln Gly Gly Val Asp Gly Asn Gly Gln Leu Trp Tyr Thr Trp Ala Lys
130 135 140

Gly Gly Ser Asn Val Phe Gly Arg Pro Met Pro Val His Val Phe Glu
145 150 155 160

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Ser Thr Arg Ala Thr Leu Arg Asn Leu Ala Ile Arg Gln Pro Gln Phe
                165                 170                 175

Trp Ala Val Leu Val Asp Ser Ser Ser His Ile Asn Leu Asp Asn Phe
            180                 185                 190

Tyr Val Asn Ala Thr Asn His Asp Ser Ser Val Ser Pro Glu Gly Glu
        195                 200                 205

Trp Val Gln Asn Thr Asp Gly Ile Asp Thr Tyr Arg Ser Asp His Ile
    210                 215                 220

Thr Val Thr Asn Trp Val Tyr Gln Gly Gly Asp Asp Ala Val Ala Phe
225                 230                 235                 240

Lys Gly Asn Ser Thr Asn Ile His Val Glu Asn Val Thr Val Tyr Gly
                245                 250                 255

Gly Pro Gly Ile Ala Phe Gly Ser Leu Gly Gln Tyr Pro Asp Arg Thr
            260                 265                 270

Asp Ile Val Glu Asn Val Thr Val Arg Asn Val Arg Val Gln Pro Ser
        275                 280                 285

Phe Gln Arg Ala Met Asn Ser Gly Val Tyr Phe Lys Ser Trp Ile Gly
    290                 295                 300

Val Asn Tyr Gly Val Pro Pro Asn Gly Gly Gly Gly Gly His Gly Tyr
305                 310                 315                 320

Val Arg Asn Val Ser Val Glu Asn Leu Arg Leu Lys Asp Val Gln Leu
                325                 330                 335

Pro Val Tyr Ile Asp Thr Trp
            340
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<210> 69
<211> 438
<212> PRT
<213> Aspergillus niger

pectinases.ST251

<400> 69

Met Tyr Leu Leu Pro Leu Thr Leu Phe Leu Thr Ala Ala Phe Gly Val
1 5 10 15

Ser Ile Pro Arg Ser Pro Leu Ile Pro Gly Ala Gln Ile Val Pro Ala
20 25 30

Ser Ser Thr Ala Asp Leu Arg Ala Ile Gly Ala Gln His His Lys Tyr
35 40 45

Pro Asp Arg Glu Thr Val Thr Ile Arg Ala Ser Arg Asn Ala Leu Asp
50 55 60

Asp Val Ser Ser Asp Phe Leu Trp Gly Leu Lys Gln Ala Asn His Gly
65 70 75 80

Gly Arg Leu Leu Leu Lys Gln Gly Glu Thr Tyr Val Ile Gly Lys Lys
85 90 95

Leu Asp Leu Thr Phe Leu Asp Asn Ile Glu Val Gln Leu Glu Gly Glu
100 105 110

Ile Gln Phe Thr Asn Asn Ile Thr Tyr Trp Gln Ala Asn Asn Phe Tyr
115 120 125

Tyr Asp Phe Gln Lys Ser Ile Thr Phe Trp Arg Trp Gly Gly Gln Asp
130 135 140

Ile Lys Ile Phe Gly Ser Gly Val Leu Asn Gly Asn Gly Gln Lys Trp
145 150 155 160

Tyr Asp Glu Phe Ala Gly Lys Gln Ile Leu Val Tyr Asn Thr Phe Tyr
165 170 175

Arg Pro Ile Leu Phe Leu Thr Asp Asn Ala Thr Arg Ile Ser Val Glu
180 185 190

Gly Ile Thr Gln Leu Asn Ser Pro Cys Trp Thr Asn Phe Phe Val Arg

pectinases.ST251

195 200 205

Thr Asn Asp Val Ser Phe Asp Asn Val Tyr Ile His Ala Phe Ser Thr
210 215 220

Asn Ala Ser Ser Asp Pro Ala Asn Thr Asp Gly Met Asp Ser Leu Asp
225 230 235 240

Val Asp Gly Val Ser Phe Thr Asn Met Arg Ile Asp Val Gly Asp Asp
245 250 255

Cys Phe Ser Pro Lys Pro Asn Thr Thr Asn Ile Phe Val Gln Asn Met
260 265 270

Trp Cys Asn Asn Thr His Gly Val Ser Met Gly Ser Ile Gly Gln Tyr
275 280 285

Ala Gly Glu Met Asp Ile Ile Glu Asn Val Tyr Ile Glu Asn Val Thr
290 295 300

Leu Leu Asn Gly Gln Asn Gly Ala Arg Leu Lys Ala Trp Ala Gly Gln
305 310 315 320

Asp Val Gly Tyr Gly Arg Ile Asn Asn Val Thr Tyr Lys Asn Ile Gln
325 330 335

Ile Gln Asn Thr Asp Ala Pro Ile Val Leu Asp Gln Cys Tyr Phe Asp
340 345 350

Ile Asn Ala Thr Glu Cys Ala Lys Tyr Pro Ser Ala Val Asn Ile Thr
355 360 365

Asn Ile Leu Phe Glu Asn Ile Trp Gly Ser Ser Ser Gly Lys Asp Gly
370 375 380

Lys Ile Val Ala Asp Leu Val Cys Ser Pro Asp Ala Val Cys Thr Asn
385 390 395 400

Ile Thr Leu Ser Asn Val Asn Leu Thr Ser Pro Lys Gly Thr Ala Glu

pectinases.ST251

405 410 415

Ile Val Cys Asp Asp Ile Gln Gly Gly Ile Gly Val Asp Cys Val Ser
420 425 430

Asp Glu Ser Val Thr Arg
435

<210> 70
<211> 397
<212> PRT
<213> Aspergillus niger

<400> 70

Met Leu Leu His Gly Leu Leu Leu Ala Leu Gln Ala Ile Leu Ala Ser
1 5 10 15

Ser Ala Ala Ile Thr Ser Pro Ser Ser Asn His Leu Ser Thr Ala Ala
20 25 30

Arg Glu Lys Cys Gln Thr Thr Leu Gln Cys Pro Pro Gly Thr Leu Ile
35 40 45

Val Ser Asn Thr His Pro Gln Leu Ser Asn Phe Thr Thr Leu Gln Ala
50 55 60

Ala Ile Asn Ala Leu Pro Asn Asp Asn Ser Ser Gln Thr Ile Leu Leu
65 70 75 80

Leu Ser Gly Ser Tyr Asn Glu Gln Val Asn Ile Thr Arg Ser Gly Pro
85 90 95

Ile Thr Leu Leu Gly Gln Gln Pro Asp Arg Ala Ala Leu Thr Asp Pro
100 105 110

Ala Arg Asn Thr Val Asn Leu Thr Phe Ala Gly Ala Asn Ser Asp Ser
115 120 125

Thr Gly Asp Ile Asp Asn Val Trp Phe Ser Val Met Val Val Ala Pro
130 135 140

pectinases.ST251

Thr Leu Asp Ala Ser Leu Thr Gly Ser Gly Thr Thr Gly Tyr Pro Val
145 150 155 160

Pro Ala Asp Thr Pro Phe Gly Asn Thr Asp Phe Arg Val Tyr Asn Ile
165 170 175

Asp Phe Arg Asn Thr Tyr Ala Pro Tyr Ser Ala Gly Pro Ala His Ala
180 185 190

Ile Ser Phe Ser Arg Ser Asn Gly Gly Phe Tyr Tyr Cys Gly Phe Tyr
195 200 205

Ser Tyr Gln Asp Thr Ile Tyr Ile Gly Lys Leu Gly Ser Ala Tyr Met
210 215 220

Tyr Lys Ser Ile Leu Ala Gly Gln Thr Asp Phe Leu Tyr Gly Phe Gly
225 230 235 240

Thr Leu Phe Ile Gln Ser Ser Gln Ile Val Leu Arg Ser Cys Gly Gly
245 250 255

Gly Ile Thr Ala Trp Lys Gly Thr Asn Thr Thr Val Arg Asn Asn Tyr
260 265 270

Gly Val Tyr Ile His Asp Ser Thr Val Asn Ala Ala Asn Thr Ser Ile
275 280 285

Ala Glu Gln Ile Lys Gly Ser Cys Ala Leu Gly Arg Pro Trp Asn Ser
290 295 300

Leu His Arg Ser Ile Phe Ala Asn Thr Tyr Glu Asp Gly Ser Ile Glu
305 310 315 320

Pro Ser Gly Tyr Ile Asn Trp Glu Asp Arg Trp Ser Lys Asn Glu Thr
325 330 335

Leu Met Ala Glu Tyr Lys Ala Tyr Gly Pro Gly Phe Asn Leu Thr Gly
340 345 350

pectinases.ST251

```
Arg Arg Glu Ser Glu Val Ser Val Leu Leu Ser Ser Ser Glu Glu Glu
        355                 360                 365

Arg Tyr Arg Asp Pro Ser Arg Leu Phe Leu Phe Glu Asp Gly Arg Glu
    370                 375                 380

Gly Asn Val Ala Trp Ile Asp Trp Asp Val Val Ser Ser
385                 390                 395
```

<210> 71
<211> 327
<212> PRT
<213> Aspergillus niger

<400> 71

```
Met His Thr Pro Tyr Leu Leu Gly Ala Leu Ala Ala Leu Ala Ala Thr
1               5                   10                  15

Ala Val Gly Ala Pro Ala Glu His Ile Lys Lys Arg Glu Ser Arg Thr
            20                  25                  30

Ser Ala Pro Ser Gly Cys Leu Thr Val Gly Ser Asp Gly Thr Tyr Ser
        35                  40                  45

Thr Ile Gly Asp Ala Leu Asp Ala Leu Gly Ser Ser Thr Ser Ser Ala
    50                  55                  60

Cys Ile Tyr Val Ala Ser Gly Thr Tyr Glu Glu Gln Leu Thr Ile Asp
65                  70                  75                  80

Tyr Ala Gly Asn Leu Thr Leu Tyr Gly Glu Thr Thr Asp Thr Ser Thr
                85                  90                  95

Tyr Lys Asp Asn Val Val Thr Ile Thr His Thr Ile Ser Ser Ser Asp
            100                 105                 110

Ala Gly Ser Leu Asp Lys Ser Ala Thr Val Asn Val Val Ser Asp Gly
        115                 120                 125
```

pectinases.ST251

```
Phe Ser Met Tyr Asn Ile Asn Val Glu Asn Gly Tyr Gly Glu Gly Ala
    130                 135                 140

Gln Ala Val Ala Leu Val Gly Asn Ala Asp Gln Leu Gly Phe Tyr Gly
145                 150                 155                 160

Cys Gln Phe Ser Gly Tyr Gln Asp Thr Leu Tyr Val Lys Ala Gly Thr
                165                 170                 175

Gln Tyr Tyr Ser Asn Cys Met Ile Glu Gly Ala Val Asp Tyr Ile Phe
            180                 185                 190

Gly Asp Ala Ser Val Trp Phe Gly Glu Cys Asp Ile Val Ser Asn Gly
        195                 200                 205

Ala Gly Ala Ile Thr Ala Ser Ser Arg Glu Thr Ser Ser Asp Ser Gly
    210                 215                 220

Trp Tyr Ala Ile Asp Asn Cys Asn Ile Lys Ala Ala Ser Gly Val Ser
225                 230                 235                 240

Leu Thr Glu Glu Val Tyr Leu Gly Arg Pro Trp Arg Val Leu Ala Arg
                245                 250                 255

Val Ile Tyr Gln Asn Ser Val Leu Ser Asp Ile Ile Asn Pro Lys Gly
            260                 265                 270

Trp Thr Thr Met Ala Asp Gly Ala Thr Pro Leu Tyr Tyr Glu Tyr Asn
        275                 280                 285

Asn Ser Gly Ala Gly Ser Asp Thr Ser Asp Arg Glu Tyr Glu Thr Ser
    290                 295                 300

Ile Ser Ala Ala Val Asp Lys Thr Thr Val Leu Gly Glu Thr Trp Gly
305                 310                 315                 320

Asp Trp Ile Asp Arg Ser Tyr
                325
```

pectinases.ST251

<210> 72
<211> 459
<212> PRT
<213> Aspergillus niger

<400> 72

Met Arg Ala Ser Ile Leu Pro Leu Thr Leu Phe Leu Ala Thr Leu Ala
1 5 10 15

Gly Ala Gln Leu Ser Gly Pro Val Gly Pro Leu Val Asp Tyr Ser Thr
20 25 30

Lys Ala Arg Asn Gln Thr Cys Asn Ile Ile Asp Tyr Gly Ala Val Ala
35 40 45

Asp Gly Lys Thr Asp Ile Ser Gln Ala Leu Leu Asp Ala Trp Gly Asn
50 55 60

Cys Ser Val Gly Gly Leu Val Tyr Ile Pro Pro Gly Asn Tyr Ser Leu
65 70 75 80

Ala Glu Asp Ile Glu Leu Lys His Gly Gln Ser Ser Ala Ile Gln Leu
85 90 95

Asp Gly Val Val Met Arg Gly His Arg Gly Ser Tyr Gln Met Ile Leu
100 105 110

Ile Arg Asp Cys Asn Asp Phe Glu Phe Phe Ser Gly Asn Ser Arg Gly
115 120 125

Ala Ile Gln Gly Phe Gly Tyr Glu Tyr Leu Gln Asn Asp Thr Tyr Gly
130 135 140

Glu Arg Leu Leu Arg Ile Gln Glu Val Asn Asn Phe Ser Val His Gly
145 150 155 160

Phe Ala Leu Ile Asp Ser Pro Ser Tyr Tyr Ile Val Phe Asp Thr Val
165 170 175

pectinases.ST251

Thr Ser Gly Glu Val Tyr Asn Ile Leu Ile Arg Gly Val Thr Ser Val
180 185 190

Gly Ala Thr Asp Ala Ile Asp Val Trp Gly Glu Asn Met Trp Phe His
195 200 205

Asp Ile Glu Val Ser Asn Gly Asp Glu Cys Val Thr Val Lys Ser Pro
210 215 220

Ala His Asn Tyr Leu Ile Glu Asn Ile Tyr Cys Asn Leu Ser Gly Gly
225 230 235 240

Thr Ala Ile Gly Ser Leu Gly Thr Gly Thr Asn Ile Ser Asp Ile His
245 250 255

Tyr Arg Asn Leu Tyr Met Asn Gln Ala Asp Ala Cys Phe Leu Lys Ser
260 265 270

Asn Asn Gly Asp Gly Ile Val Lys Asn Ile Ile Trp Glu Asn Val Ile
275 280 285

Val His Gly Gly Pro Tyr Pro Leu Ala Ile Asp Glu Ala Trp Gly Asp
290 295 300

Asp Arg Gly Ser Val Gly Val Gln Val Ser Asn Leu Thr Phe Arg Asn
305 310 315 320

Trp His Gly Glu Ser Val Ser Ala Ser Arg Pro Val Ile Arg Leu Gln
325 330 335

Cys Asp Ser Asp Val Pro Cys Tyr Asp Ile Thr Ile Glu Asn Val Asn
340 345 350

Leu Trp Ala Asn Asp Ser Asn Tyr Val Val Trp Gln Cys Glu Asn Ala
355 360 365

Tyr Gly Asp Gly Ala Cys Leu Ser Ser Ala Glu Gly Thr Lys Asp Leu
370 375 380

pectinases.ST251

Glu Thr Phe Thr Ser Lys Gln Thr Ile Thr Ala Thr Pro Ser Tyr Ala
385 390 395 400

Ala Pro Thr Met Ala Ala Asp Phe Thr Phe Asn Leu Pro Ser Thr Ser
405 410 415

Pro Phe Thr Ile Pro Pro Met Pro Thr Ser Phe Tyr Pro Gly Ala Thr
420 425 430

Pro Ile Ser Thr Leu Leu His Leu His Gly Ala Gly Gly Leu Pro Ser
435 440 445

Ala Ser Pro Ile Ser His His Arg Arg His Gln
450 455

<210> 73
<211> 474
<212> PRT
<213> Aspergillus niger

<400> 73

Met Arg Ser Ile Leu Phe Ile Leu Phe Ser Val Phe Ala Gly Leu Ala
1 5 10 15

Ala Gly Gln Leu Ile Gly Pro Val Gly Pro Thr Thr Gln Leu Glu Asp
20 25 30

Lys Asp Ile Glu Cys Asn Ile Leu Asp Tyr Gly Gly Val Ala Asp Asn
35 40 45

Glu Thr Asp Val Ala Thr Ser Ile Glu Thr Thr Phe Thr Glu Cys Val
50 55 60

Leu Asn Asn Pro Lys Ser Arg Leu Val Ile Pro Glu Gly Asp Tyr Leu
65 70 75 80

Ile Lys Arg Ser Val Val Leu Ser Asn Gly Thr Asn Trp Ala Phe Gln
85 90 95

Leu Asp Gly Leu Ile Thr Ala Ala Tyr Gly Gly Asn Trp Thr Val Asp

pectinases.ST251

100 105 110

Pro Val Asp Phe Glu Phe Tyr Ser Ser Asn Gly Leu Gly Ala Phe Gln
115 120 125

Gly Gln Gly Tyr Ile Tyr Arg Asn Leu Ala Asn Thr Asp Arg Pro Arg
130 135 140

Leu Val Arg Leu Ile Ser Pro Thr Asn Ala Ser Val His Asp Leu Ile
145 150 155 160

Leu Val Asp Ser Pro Lys Phe His Ile Val Phe Asp Phe Ala Val Asn
165 170 175

Leu Glu Ala Tyr His Leu Thr Ile Arg Gly Ala Asn Leu Gly Ser Tyr
180 185 190

Asp Gly Ile Asp Ala Ile Gly Thr Asn Tyr Tyr Ile His Asp Asn Glu
195 200 205

Ala Ile Thr Asn Arg Asp Glu Cys Val Ser Val Lys Ser Pro Ser His
210 215 220

His Ala Leu Val Glu Asn Leu Val Cys Asn Gln Ala Gly Ser Gly Val
225 230 235 240

Ser Ile Gly Ser Leu Asn Val Ser Ala Glu Ile Ser Asn Ile Glu Ala
245 250 255

Arg Asn Ile Ser Ile Ile Gln Gly Asn Asn Ile Ala Phe Ile Lys Thr
260 265 270

Tyr Pro Gly Gly Ser Gly Tyr Val Lys Asp Val Thr Phe Glu Asn Phe
275 280 285

Arg Ser Leu Asn Ser Leu Tyr Gly Leu Asp Ile Asn Gln Tyr Trp Gln
290 295 300

Asn Thr Trp Glu Pro Asp Thr Gly Ser Val Thr Leu Ser Asn Leu Val

pectinases.ST251

305 310 315 320

Phe Lys Asn Phe Ser Gly Ser Val Ala Asp Gly Ala Leu Arg Pro Pro
325 330 335

Leu Tyr Leu Phe Ala Ser Asp Leu Thr Phe Ala Thr Asn Val Thr Val
340 345 350

Glu Glu Phe Ser Val Trp Thr Glu Thr Gly Thr Thr Val Val Asn Lys
355 360 365

Ile Ser Asn Ile Phe Gly Thr Gly Asp Asp Ser Tyr Gly Glu Asn Asp
370 375 380

Gly Ile Glu Ser Leu Gln Ser Gly Glu Ser Pro Tyr Thr Tyr Thr Ser
385 390 395 400

Thr Tyr Thr Ile Thr Ala Ser Pro Thr Asn Trp Gln Ala Pro Ser Thr
405 410 415

Pro Thr Trp Ala Leu Pro Ser Thr Gly Tyr Gly Ser Lys Phe Asn Glu
420 425 430

Asn Cys Ser Gly Tyr Phe Tyr Ile Ala Asn Leu Ser Thr Ala Ala Ser
435 440 445

Pro Ile Pro Val Tyr Thr Pro Ala Pro Leu Trp Arg Pro Gly Gly Ile
450 455 460

Asp Tyr Asn Leu His Tyr Trp Gly Ser Phe
465 470

<210> 74
<211> 443
<212> PRT
<213> Aspergillus niger

<400> 74

Met Leu Val Thr Ser Leu Ile Ala Leu Leu Pro Ala Ile Ala Ala Ala
1 5 10 15

pectinases.ST251

```
Gln Val Ser Gly Thr Val Gly Pro Arg Thr Ser Ala Ser Ala Lys Ala
            20                  25                  30

Ala Glu Lys Val Cys Asn Val Leu Asp Tyr Gly Ala Ser Ala Asn Ser
        35                  40                  45

Thr Ile Asp Ile Gly Pro Pro Leu Lys Glu Ala Phe Gln Asp Cys Gln
    50                  55                  60

Thr Gly Gly Leu Val Tyr Ile Pro Glu Gly Asp Tyr Leu Leu Ser Ser
65                  70                  75                  80

Trp Val Ser Leu Val Tyr Gly Ser Gly Trp Ala Leu Gln Leu Asp Gly
                85                  90                  95

Ile Ile Tyr Arg Asp Lys Asn Val Thr Asp Gly Gly Asn Met Ile Phe
            100                 105                 110

Ile Glu His Thr Ser Asp Ile Glu Ile Phe Ser Asn Asn Ser Ala Gly
        115                 120                 125

Ala Ile Gln Gly Tyr Gly Tyr Leu Phe His Glu Gln Asp Glu Tyr Gly
    130                 135                 140

Pro Arg Ile Leu Arg Leu Asn Asn Val Thr Asp Phe Ser Val His Asp
145                 150                 155                 160

Leu Ile Leu Val Asp Ser Pro Ala Tyr Phe Leu Asn Leu Val Glu Ser
                165                 170                 175

Tyr Asn Gly Glu Val Tyr Asn Met Val Ile Arg Gly Ala Ser Met Gly
            180                 185                 190

Gly Leu Asp Gly Ile Asp Ile Ser Gly Ala Asn Tyr Trp Ile His Asp
        195                 200                 205

Val Glu Val Thr Asn Gly Asp Glu Cys Val Thr Val Lys Ser Pro Ser
    210                 215                 220
```

pectinases.ST251

```
Ala Asn Val Arg Val Glu Asn Val Phe Cys Asn His Ser Gly Gly Cys
225                 230                 235                 240

Ala Met Gly Ser Leu Gly Thr Asp Thr Asn Ile Ser Asn Ile Glu Phe
                245                 250                 255

Glu Asn Ile Tyr Thr Tyr Asn Ser Thr Gln Met Tyr Met Ile Lys Ser
            260                 265                 270

Asn Gly Gly Asn Gly Thr Val Thr Asn Cys Ser Phe Lys Asn Phe Ile
        275                 280                 285

Gly Tyr Ser Asn Ala Tyr Met Leu Asp Leu Asp Thr Tyr Trp Gly Asp
    290                 295                 300

Glu Ser Asp Gly Asp Gly Ile Lys Tyr Glu Asn Ile Gly Phe Glu Asn
305                 310                 315                 320

Trp Lys Gly Thr Ser Ser Asn Gly Ile Gln Arg Ser Pro Ile Arg Ile
                325                 330                 335

Leu Cys Pro Asp Ala Asn Pro Cys Thr Asn Ile Thr Leu Thr Ala Val
            340                 345                 350

Glu Leu Trp Thr Asp Thr Gly Asp Tyr Val Lys Gln Glu Cys Ser Ser
        355                 360                 365

Ala Tyr Gly Glu Gly Glu Cys Leu Arg Gln Gln Asn Gly Thr Leu Ala
    370                 375                 380

Ser Tyr Ser Phe Thr Thr Thr Ile Thr Ser Val Pro Val Thr Ala Tyr
385                 390                 395                 400

Ser Pro Thr Thr Thr Met Pro Gly Leu Ile Ser Thr Ser Met Asp Thr
                405                 410                 415

Thr Thr Ser Ile Pro Ile Pro Thr Ile Pro Thr Ser Phe Phe Pro Gly
            420                 425                 430
```

pectinases.ST251

Ala Ser Ala Tyr Ser Thr Leu Met Ala Asn Met
435 440

<210> 75
<211> 447
<212> PRT
<213> Aspergillus niger

<400> 75

Met Thr Trp Ser Thr Ser Phe Leu Val Ala Thr Ser Leu Leu Ser Ile
1 5 10 15

Ile Asn Ser Val His Ala Gln Leu Thr Gly Ser Val Gly Pro Leu Thr
20 25 30

Ser Val Ile Asp Lys Ala Ala Val Lys Thr Cys Asn Val Cys Asp Tyr
35 40 45

Gly Ala Ser Ser Asp Asn Thr Thr Gly Val Gly Gln Pro Ile Ile Asp
50 55 60

Ala Phe Thr Asp Cys Gly Ser Gly Gly Leu Ile His Val Pro Glu Gly
65 70 75 80

Asp Tyr Leu Leu Lys Asp Trp Val Ser Ser Glu Asn Gly Ser Ala Trp
85 90 95

Ser Ile Gln Leu Asp Gly Val Leu His Trp Asp Ser Ser Pro Ser Ala
100 105 110

Gln Ser Tyr Ile Phe Ala Ile Thr Gly Gly Ser Asp Ser Glu Leu Ser
115 120 125

Ser Ser Asn Ala Thr Gly Ala Ile Gln Gly Ser Gly Tyr Leu Tyr His
130 135 140

Arg His Asn Thr Tyr Thr Ser Pro Arg Met Leu Tyr Ile Ser Gly Cys
145 150 155 160

pectinases.ST251

Arg Ile Gly Pro Phe Met Thr Trp Tyr Trp Ser Thr Arg Leu Cys Pro
165 170 175

Thr Leu Ser Leu Thr Ala Val Thr Met Glu Lys Arg Thr Ile Trp Leu
180 185 190

Ser Val Val Val Ile Thr Val Val Trp Met Ala Leu Ile Leu Pro Leu
195 200 205

Leu Leu Asn Val His Ser Ile Ser Gly Asn Ala Asn Ala Thr Ala Ala
210 215 220

Arg Pro Asn Ser His Asn Phe Leu Ile Glu Asn Ile Tyr Cys Asn Pro
225 230 235 240

Ser Gly Gly Cys Ala Ile Gly Ser Leu Gly Ser Ser Val Asn Val Thr
245 250 255

Asn Ile Leu Tyr Arg Asn Val Tyr Thr Trp Asp Ser Asn Gln Met Met
260 265 270

Met Ile Lys Thr Asn Gly Gly Leu Gly Asn Val Ser Asn Ile Val Phe
275 280 285

Glu Asn Phe Ile Gly His Gly Asn Val Asn Ser Leu Asp Leu Asp Ser
290 295 300

Tyr Trp Ser Ser Met Asn Ala Ile Asp Gly Val Gly Ile Tyr Tyr His
305 310 315 320

Asn Ile Thr Ile Tyr Asn Trp Thr Gly Thr Ala Ile Asp Gly Glu Thr
325 330 335

Arg Pro Pro Ile Arg Val Ile Cys Pro Glu Asp Met Pro Cys Thr Glu
340 345 350

Ile Thr Leu Val Gln Ile Asp Leu Leu Val Glu Glu Gly Arg Tyr Asp
355 360 365

pectinases.ST251

```
Glu Tyr Tyr Cys Ala Ile Ala Cys Gly Gly Tyr Cys Leu Asp Ser Ala
    370                 375                 380

Thr Ser Thr Leu Thr Thr Tyr Thr Thr Thr Thr Tyr Gly Asn Ser Ala
385                 390                 395                 400

Ser Thr Gly Tyr Glu Ala Pro Thr Met Ala Asp Asp Leu Ala Thr Ala
                405                 410                 415

Phe Gly Thr Thr Ala Ser Ile Pro Thr Pro Thr Thr Pro Ala Ser Phe
            420                 425                 430

Phe Phe Pro Gly Val Ala Pro Val Ser Ala Val Ala Gly Ser Ser
        435                 440                 445
```

<210> 76
<211> 533
<212> PRT
<213> Aspergillus niger

<400> 76

```
Met Leu Ser Lys Thr Ser Leu Leu Ser Leu Leu Ser Leu Ala Ala Gly
1               5                   10                  15

Val Val Asn Ala Asp Phe Gly Ile Thr Thr Asn Asp Asp Ser Tyr Val
            20                  25                  30

Ile Asn Ala Asn Ser Pro Asn Ser Leu Val Phe Thr Val Asp Arg Gly
        35                  40                  45

Ser Cys Asp Ile Thr Ser Ile Val His Tyr Gly Thr Glu Leu Gln Tyr
    50                  55                  60

Ser Gly Lys Gly Ser His Ile Gly Ser Gly Leu Gly Thr Ala Thr Val
65                  70                  75                  80

Ser Ala Thr Lys Ser Gly Ala Gly Asp Tyr Ile Lys Val Thr Cys Glu
                85                  90                  95
```

pectinases.ST251

```
Thr Asp Thr Leu Thr Gln Tyr Met Val Val His Asp Gly Asp Pro Ile
            100                 105                 110

Ile His Met Ala Thr Tyr Ile Thr Glu Glu Pro Ser Ile Gly Glu Leu
        115                 120                 125

Arg Phe Ile Ala Arg Leu Asn Ser Asp Val Leu Pro Asn Glu Glu Pro
    130                 135                 140

Phe Gly Asp Val Ser Asn Thr Ala Asp Gly Glu Ala Ile Glu Gly Ser
145                 150                 155                 160

Asp Val Phe Leu Val Asp Gly Glu Thr Arg Ser Lys Phe Tyr Ser Ser
                165                 170                 175

Gln Arg Phe Ile Asp Asp Gln Arg His Cys Ile Ala Gly Asp Glu His
            180                 185                 190

Arg Val Cys Met Ile Leu Asn Gln Tyr Glu Thr Ser Ser Gly Gly Pro
        195                 200                 205

Phe His Arg Asp Ile Asn Ser Asn Asn Gly Gly Asp Tyr Asn Ser Leu
    210                 215                 220

Tyr Trp Tyr Met Asn Ser Gly His Val Gln Leu Glu Ser Tyr Arg Met
225                 230                 235                 240

Gly Leu His Gly Pro Tyr Ser Met Tyr Phe Ser Arg Ser Gly Thr Pro
                245                 250                 255

Ser Thr Asp Ile Asp Thr Ser Phe Phe Ala Asp Leu Asp Ile Glu Gly
            260                 265                 270

Tyr Val Ala Glu Ser Gly Arg Gly Thr Val Ser Gly Thr Ala Ser Gly
        275                 280                 285

Ala Asp Ser Ser Phe Asp Trp Val Val His Trp Tyr Asn Asp Asp Ala
    290                 295                 300
```

pectinases.ST251

```
Gln Tyr Trp Thr Tyr Thr Ser Ser Ser Gly Ser Phe Thr Ser Pro Ala
305                 310                 315                 320

Met Lys Pro Gly Thr Tyr Thr Met Val Tyr Tyr Gln Gly Glu Tyr Val
                325                 330                 335

Val Ala Thr Ser Glu Val Thr Val Ser Ala Gly Ser Ser Thr Ser Lys
            340                 345                 350

Asp Ile Ser Gly Ser Val Glu Thr Gly Thr Thr Ile Phe Lys Ile Gly
        355                 360                 365

Asp Trp Asp Gly Gln Pro Thr Gly Phe Arg Asn Ala Glu Asn Gln Leu
    370                 375                 380

Arg Met His Pro Ser Asp Ser Arg Met Ser Asp Trp Gly Pro Leu Thr
385                 390                 395                 400

Tyr Thr Val Gly Ser Ser Ser Leu Thr Asp Phe Pro Met Ala Ile Phe
                405                 410                 415

Lys Ser Val Asn Ser Pro Val Thr Ile Lys Phe Thr Ala Thr Ser Asp
            420                 425                 430

Gln Thr Gly Ala Ala Thr Leu Arg Ile Gly Thr Thr Leu Ser Phe Ala
        435                 440                 445

Gly Gly Arg Pro Gln Ala Thr Ile Asn Asp Tyr Glu Gly Ser Ala Pro
    450                 455                 460

Ser Ala Pro Thr Asn Leu Asp Ser Arg Gly Val Thr Arg Gly Ala Tyr
465                 470                 475                 480

Arg Gly Tyr Gly Asp Val Tyr Asp Val Ser Val Pro Glu Gly Thr Ile
                485                 490                 495

Val Glu Gly Glu Asn Thr Ile Thr Ile Ser Val Ile Ser Gly Ser Ser
            500                 505                 510
```

pectinases.ST251

Gly Asp Asp Phe Leu Ser Pro Asn Phe Leu Asp Ala Val Phe Ile Ile
515 520 525

Ala Leu Val Asp Asn
530

<210> 77
<211> 706
<212> PRT
<213> Aspergillus niger

<400> 77

Met Arg Leu Leu His Pro Leu Ile Pro Ala Ser Leu Leu Leu Thr Leu
1 5 10 15

Thr Ser Ala Thr Leu His Thr Ser Gln Thr Asn Thr Thr Ile Thr Leu
20 25 30

Thr Asn Asn Arg Leu Thr Ala Asn Phe Ser Lys Ser Gln Gly Arg Ile
35 40 45

Thr Asp Leu Tyr Leu Asp Asn Gln Asp Leu Leu Gly Pro Gln Ser Gly
50 55 60

Asp Thr Gly Val Gly Pro Tyr Leu Asp Cys Tyr Cys Ile Pro Ser Gly
65 70 75 80

Phe Tyr Thr Pro Gly Ser Thr Ser Pro Thr Leu Gln Leu Phe Thr Gly
85 90 95

Thr Asp Lys Ser Gly Thr Ser Tyr Ala Gly Val Leu Met Asp Glu Thr
100 105 110

Tyr Pro Pro Thr Gly Gln His Phe Gln Gln Tyr Trp Phe Leu Arg Asp
115 120 125

Gly Glu Thr Gly Leu His Thr Phe Ser Arg Leu Ala Tyr Tyr Asn Glu
130 135 140

Thr Thr Pro Tyr Leu Arg Asn Leu Gln Glu Phe Arg Thr Leu Phe Arg

pectinases.ST251

```
145                 150                 155                 160

Pro Asn Thr Glu Leu Trp Thr His Leu Ser Ser Ser Glu Val Gln Thr
                165                 170                 175

Ala Pro Leu Pro Ser Lys Lys Ala Val Glu Glu Glu Val Val Val Gln
            180                 185                 190

Asp Ala Thr Trp Thr Phe Asn Asn Thr Pro Thr Asp Glu Tyr Tyr Val
        195                 200                 205

Gln Phe Ala Asp Tyr Phe Thr Lys Tyr Thr Phe Ser Asn Ala Trp Arg
    210                 215                 220

Asp Asn Ser Val His Gly Met Tyr Ala Asp Gly Ser Thr Ser Asn Gly
225                 230                 235                 240

Ser Thr Phe Gly Ala Trp Leu Val Met Asn Thr Lys Asp Thr Tyr Tyr
                245                 250                 255

Gly Gly Pro Leu His Ser Asp Leu Thr Val Asp Gly Ile Val Tyr Asn
            260                 265                 270

Tyr Leu Val Ser Asn His His Gly Glu Gly Thr Pro Asn Ile Thr Tyr
        275                 280                 285

Gly Phe Asp Arg Thr Phe Gly Pro Gln Tyr Tyr His Phe Asn Gly Gly
    290                 295                 300

Lys Gly Ser Thr Ala Ser Leu Gln Glu Leu Lys Ser Asp Ala Glu Thr
305                 310                 315                 320

Leu Ala Asp Pro Ser Trp Asn Val Asp Phe Tyr Asp Ser Ile Ala Lys
                325                 330                 335

His Val Val Gly Tyr Thr Pro Ser Ser Gln Arg Gly Ser Val Gln Gly
            340                 345                 350

Lys Ile Lys Leu Pro Lys Gly Ala Thr Arg Pro Ile Ala Val Leu Thr
```

pectinases.ST251

355 360 365

Val Asp Gly Gln Tyr Phe Gln Asp Asn Ser Val Asn Ser Ser Ser Tyr
370 375 380

Gln Tyr Trp Ala Glu Ile Asp Asp Ser Gly His Phe Ser Val Asp His
385 390 395 400

Val Lys Glu Gly Pro Tyr Arg Leu Thr Val Tyr Ala Asp Gly Ile Phe
405 410 415

Gly Asp Phe Val Arg Asp Gly Val Gln Val Lys Ala Gly Lys Lys Thr
420 425 430

Thr Ile Gln Glu Thr Trp Glu Ala Glu Ser Ala Gly Thr Glu Ile Trp
435 440 445

Arg Leu Gly Thr Pro Asp Lys Ser Ser Gly Glu Phe Arg His Gly Val
450 455 460

Ala Arg Asp Pro Thr His Pro Leu His Pro Pro Glu Tyr Leu Ile Tyr
465 470 475 480

Trp Gly Ala Tyr Asp Trp Gln Ser Asp Phe Pro Asp Gly Ile Asn Tyr
485 490 495

Thr Ile Gly Thr Ser Asp Pro Ala Thr Asp Leu Asn Thr Val His Trp
500 505 510

Ser Val Phe Gly Pro Thr Pro Asn Asp Pro Arg Val Glu Tyr Asp Thr
515 520 525

Thr His Asp Trp Thr Ile Asn Phe Pro Leu Ser Glu Asp Asp Leu Ala
530 535 540

Glu Arg Ser Lys Ala Thr Leu Thr Ile Gln Leu Ala Gly Ala Lys Ala
545 550 555 560

Ala Ser Gly Asn Thr Asp Val Tyr Asn Ala Ser Glu Pro Tyr Thr Asn

pectinases.ST251

565 570 575

Leu Ala Leu Glu Ser Tyr Ile Asn Asp Gln Ala Glu Pro Leu Thr Leu
580 585 590

Leu Ile Gly Phe Asn Gln Ser Ser Ser Cys Ile Val Arg Ser Ala Val
595 600 605

Ser Cys Tyr Gln Val Arg Ser Arg Met Glu Phe Pro Ala Asp Trp Leu
610 615 620

Lys Val Gly Asn Asn Val Leu Thr Leu His Leu Pro Tyr Asn Ala Thr
625 630 635 640

Asp Thr Glu Thr Ala Ile Leu Pro Ala Thr Val Thr Gly Arg Leu Ile
645 650 655

Leu Pro Pro Gln Pro Ile Tyr Gly Gln Thr Pro Val Ile Leu Ser Val
660 665 670

Ile Gly Ser Glu Lys Leu Glu Pro Leu Pro Ala Ala Ser Ile Leu Ile
675 680 685

Phe Glu Ile Ile Arg His Gln Ser Glu Pro Leu Trp Ser Val Pro Arg
690 695 700

Ser Ala
705

<210> 78
<211> 278
<212> PRT
<213> Aspergillus niger

<400> 78

Met Lys Leu Ser Leu Leu Ser Leu Phe Phe Thr Pro Ile Phe Ala Leu
1 5 10 15

Pro Ser His Leu Thr Pro Arg Asn Asp Ile Pro Pro Phe Phe Leu Leu
20 25 30

pectinases.ST251

```
Ala Gly Asp Ser Thr Thr Ala Val Gln Ser Ser Gly Gly Gly Gly Trp
        35                  40                  45

Gly Asp Gly Phe Ile Asn Thr Thr Leu His Lys Gly Ala Lys Gly Ile
    50                  55                  60

Asn Tyr Gly His Asp Gly Ala Thr Thr Val Ser Phe Arg Ser Gly Gly
65                  70                  75                  80

Asp Trp Ala Thr Val Leu Ser Lys Val Ala Glu Tyr Lys Ser Asp Tyr
                85                  90                  95

Arg Ala Phe Val Thr Ile Gln Phe Gly His Asn Asp Gln Lys Pro Ala
            100                 105                 110

Ala Asn Ile Ser Leu Ala Glu Tyr Thr Ser Asn Leu Glu Gln Phe Ala
        115                 120                 125

Lys Asp Val Lys Asn Ala Gly Gly Thr Pro Ile Leu Val Thr Pro Leu
    130                 135                 140

Ser Arg Arg Asn Tyr Asp Asn Ser Thr Gly Thr Pro Leu Val Ile Glu
145                 150                 155                 160

Asn Leu Ala Asp Gln Arg Ala Ala Thr Ile Asp Ala Ala Lys Asn Thr
                165                 170                 175

Asp Thr Ser Tyr Ile Asp Leu Asn Lys Ala Ser Thr Asp Tyr Leu Asn
            180                 185                 190

Ser Ile Gly Pro Ala Asp Ala Tyr Thr Tyr Asn Leu Ala Ser Asp Asp
        195                 200                 205

Tyr Thr His Leu Asn Gly Glu Gly Ser Ile Val Phe Gly Gly Met Val
    210                 215                 220

Ala Ser Leu Ile Asp Gln Asp Phe Thr Glu Leu Lys Ser Asp Gly Val
225                 230                 235                 240
```

pectinases.ST251

```
Phe Ile His Asp Gln Trp Leu Val Asp Gly Ile Tyr Gln Ala Glu Asn
                245                 250                 255

Asp Ser Cys Tyr Ile Ser Ile Leu Glu Tyr Leu Ser Met Ala Tyr Asp
            260                 265                 270

Leu Ala Asn Pro Met Leu
        275
```

<210> 79
<211> 410
<212> PRT
<213> Aspergillus niger

<400> 79

```
Met Ser Val Phe Lys Ala Ser Phe Leu Phe Leu Leu Ser Ser Ser Leu
1               5                   10                  15

Val His Gly Val Pro His Ser Ser Arg Ala Ser Arg Ser Gln Gln Cys
            20                  25                  30

Val Val Pro Pro Leu His Asn Ala Arg Leu Thr Arg Val Ile Ile Phe
        35                  40                  45

Glu Glu Gly Val Asn Tyr Asn Ile Phe Gln Pro Ile Thr Ala Thr Asn
    50                  55                  60

Leu Ser Asn Val Glu Ile Arg Met His Gly Asn Leu His Leu Pro Gln
65                  70                  75                  80

Asn Ile Thr Ala Val Gln Asn Ile Val Ser Asp Gly Thr Ser Thr Trp
                85                  90                  95

Phe Thr Leu Glu Gly Pro Lys Val Asp Trp Ile Gly Pro Glu Asp Val
            100                 105                 110

Asn Asn Gly Trp Ile Asp Ser Tyr Gly Gln Pro Trp Trp Asp Ala Asn
        115                 120                 125
```

pectinases.ST251

```
Pro Ala Gly Ser Ser Gly Ile Asp Asn Arg Pro His Leu Met Ser Phe
    130                 135                 140

Lys Ser Ser Gln Ala Thr Met Lys Tyr Phe Arg Ser Arg Lys Pro Ile
145                 150                 155                 160

Ala Trp Asn Val Lys Leu His Gly Gln Asp Ile Thr Val Ser His Ala
                165                 170                 175

Ile Ile Asp Ala Thr Ser Thr Gly Ser Phe Pro Phe Asn Thr Asp Gly
            180                 185                 190

Phe Asp Val Glu Gly Thr Asn Ile Gln Ile Thr Asp Ser Ile Met Tyr
        195                 200                 205

Asn Gly Asp Asp Ala Ile Ala Lys His His Arg Leu Pro Asp Ser Arg
    210                 215                 220

His Glu His Gly Ser Leu Gly Lys Asp Pro Thr Asp Phe Ala Asn Ile
225                 230                 235                 240

Ser Asn Ile Arg Phe Asp Asp Val Thr Val Val Asp Gly Leu Tyr Ala
                245                 250                 255

Ala Arg Phe Lys Ser Trp Ser Gly Gly Thr Gly Leu Val Lys Asn Val
            260                 265                 270

Thr Trp Asn Asn Ile Arg Val Phe Asn Val Thr Phe Pro Ile Phe Val
        275                 280                 285

Thr Gln Ser Tyr Ser Asp Gln Gly Ala Ser Arg Ser Gly Thr Val Asn
    290                 295                 300

Ala Ser Ser Ala Val Met Met Glu Asp Phe Thr Trp Ser Asp Phe Ala
305                 310                 315                 320

Gly Ser Ile Asn Thr Tyr Gln Pro Gly Asp Gly Ser Cys Val Ser Asp
                325                 330                 335
```

pectinases.ST251

```
Pro Cys Trp Tyr Asn Val Gly Leu Pro Asn Leu Lys His Thr Glu Ala
            340                 345                 350

Leu Ile Ile Glu Cys His Thr Ala Gln Ser Cys Lys Asn Phe Val Thr
        355                 360                 365

Asp Asn Ile Gln Leu Tyr Pro Gln Val Leu Glu Pro Ala Ser Val Ile
    370                 375                 380

Cys Met Asn Ala Thr Ala Ala Leu Asn Pro Asp Leu Gly Phe Thr Cys
385                 390                 395                 400

Lys Asn Gly Thr Tyr Ser Pro Leu Ser Asn
                405                 410
```

<210> 80
<211> 319
<212> PRT
<213> Aspergillus niger

<400> 80

```
Met Pro Phe Phe Arg Ala Lys Ser Ser Lys Arg Gln Ile Met Phe Val
1               5                   10                  15

Lys Trp His Phe Leu Val Leu Gly Ala Ile Pro Met Ile Pro Ala Tyr
            20                  25                  30

Pro Ser Gly Ala Ala Tyr Lys Gly Gly Phe Glu Trp Asp Ser Thr Lys
        35                  40                  45

Tyr Leu Phe Val Leu Ala Leu Ile Asp Gly Arg Ile Trp Arg Ser Glu
    50                  55                  60

Gly Gly Pro Asn Trp Val Glu Tyr Leu Thr Gly Cys Gly Leu Glu Glu
65                  70                  75                  80

Gly Leu Thr Ser Pro Phe Asp Cys Asp Gln Gln Leu Trp Asp Phe Ala
                85                  90                  95
```

pectinases.ST251

```
Phe Ala Gly Ser Asp Ile Ser Val Glu Tyr Thr Pro Leu His His Asn
            100                 105                 110

Phe Thr Val Ser Leu Val Asn Gln Val Lys Gln Phe Asn Thr Tyr Ala
        115                 120                 125

Gln Pro Val Leu Lys Lys Thr Val Asp Gln Ser His Ala Leu Val Ala
    130                 135                 140

Ile Trp Ile Gly Ile Asn Asp Ile Gly Asp Ser Ser Lys Tyr Asp Val
145                 150                 155                 160

Asp Phe Pro Thr Phe Tyr Asn Glu Leu Met Asn Thr Leu Phe Ser Ser
                165                 170                 175

Val Gln Thr Ile Tyr Ser Gln Gly Tyr Arg Ser Tyr Leu Phe Met Asn
            180                 185                 190

Leu Pro Pro Leu Asp Arg Arg Pro Gly Asn Leu Gly Ser Ala Asp Pro
        195                 200                 205

Ser Pro Asn Ala Thr Gln Ile Thr Trp Tyr Asn Asp Ala Leu Ala Gln
    210                 215                 220

His Ala Ser Ala Phe His Asp Arg Tyr Ala Asp Thr Asn Val Met Leu
225                 230                 235                 240

Phe Asp Ala His Ser Glu Leu Ser Tyr Ile Leu Asp Asn Pro Gly Asp
                245                 250                 255

Phe Gly Ile Val Asn Ile Thr Asn Phe Cys Ala Gly Tyr Asp Gln Pro
            260                 265                 270

Asp Ile Ala Trp Asn Tyr Gln Ala Tyr Gly Cys Pro Thr Pro Leu Asp
        275                 280                 285

Thr Tyr Phe Trp Tyr Asn Ser Gly His Met Thr Ser His Val His Glu
    290                 295                 300
```

pectinases.ST251

Ile Leu Ala Gly Ala Val Glu Arg Lys Leu Glu Glu Trp Ser Asp
305 310 315

<210> 81
<211> 317
<212> PRT
<213> Aspergillus niger

<400> 81

Met Thr Gly Ile Pro Thr Val Thr Ala Arg Pro Trp Thr Gln Arg Pro
1 5 10 15

Arg Ala Glu Asn Ser Thr Thr Asn Pro Thr Tyr Phe Phe Thr Leu Cys
20 25 30

Ala Gln Pro Phe Pro Ser Thr Pro Pro Thr His Ile Thr Asn Asn Ile
35 40 45

Asp Asp Glu Gly Ile Gly Thr Thr Thr Asn Gly Pro Asn Trp Ile Gly
50 55 60

Tyr Leu Thr Thr Thr Glu Asn Ala Ser Leu Val Leu Ser Tyr Asn Leu
65 70 75 80

Ala Ala Gly Gly Ala Thr Ile Asp Asn Ala Leu Val Pro Ala Tyr Pro
85 90 95

Gly Asp Leu Ala Ser Gln Phe Arg Leu Phe Glu Asp Val Tyr Ala Asp
100 105 110

Lys Pro Ala Ser Ala Pro Trp Ser Ala Glu Asp Ala Val Phe Gly Val
115 120 125

Trp Ile Gly Ile Asn Glu Tyr Ile Leu Pro Pro Pro Pro Pro Pro Pro
130 135 140

Pro Pro Pro Pro Pro Leu Leu Leu His Ser Pro Asp Cys Pro Tyr Thr
145 150 155 160

Gly Gly Gly Cys Tyr Ile Gly Asn Ala Tyr Tyr Ser Thr Asp Ala Glu

pectinases.ST251

165 170 175

Thr Tyr Thr Pro Lys Leu Ile Ser Arg Leu Glu Ser Leu Val Glu Glu
180 185 190

Val Tyr Lys Asn Gly Gly Arg Lys Phe Leu Phe Leu Asn Val Pro Pro
195 200 205

Thr Ser Arg Ser Pro Leu Phe Leu Glu Gln Gly Glu Glu Val Val Lys
210 215 220

Gln His Ala Glu Tyr Leu Ser Val Tyr Asn Glu Asn Leu Glu Gly Met
225 230 235 240

Val Asp Asp Phe Thr Lys Lys Lys Gly Asp Val Thr Thr Val Leu Tyr
245 250 255

Asp Ser Trp Ser Phe Met Thr Lys Ile Leu Asp Asp Pro Thr Ala Tyr
260 265 270

Gly Phe Pro Asp Ala Thr Cys Ile Asn Asp Asp Gly Thr Ser Cys Ile
275 280 285

Trp Trp Asn Asn Tyr His Pro Gly Met Lys Tyr His Leu Leu Gln Ala
290 295 300

Glu Asp Met Lys Pro Lys Leu Arg Lys Leu Gly Gly Trp
305 310 315

<210> 82
<211> 536
<212> PRT
<213> Aspergillus niger

<400> 82

Met Asp Phe Pro Arg Leu Leu Leu Ala Leu Cys Phe Leu Leu Thr Phe
1 5 10 15

Ser Leu Thr Ser Ala Tyr Asp Ala Pro Leu Val Thr Leu Asp Tyr Gly
20 25 30

pectinases.ST251

```
Thr Phe Gln Gly Ser Tyr Asp Ala Thr Tyr Asn Leu Ser Tyr Phe Arg
        35                  40                  45

Lys Ile Pro Phe Ala Ala Pro Ala Thr Gly Glu Asn Arg Phe Arg Ala
    50                  55                  60

Pro Gln Pro Pro Leu Asn Ile Thr Asn Gly Thr Tyr Asp Thr Asp Gln
65                  70                  75                  80

Ser Phe Asp Met Cys Pro Gln Arg Thr Val Asn Gly Ser Glu Asp Cys
                85                  90                  95

Leu Tyr Leu Gly Leu Tyr Ser Arg Pro Trp Asp Thr Ser Ser Ser Thr
            100                 105                 110

Thr Ser Arg Pro Val Leu Val Val Phe Tyr Gly Gly Gly Phe Ile Glu
        115                 120                 125

Gly Asp Ala Leu Phe Gly Met Pro Pro Asn Ala Tyr Pro Val Leu Asn
    130                 135                 140

Val Ser Thr Leu Asn Asp Tyr Ile Val Val Tyr Thr Asn Tyr Arg Val
145                 150                 155                 160

Asn Ala Phe Gly Phe Leu Pro Gly Gln Ala Ile Lys Asp Ser Pro Thr
                165                 170                 175

Ser Asp Leu Asn Pro Gly Leu Leu Asp Gln Gln Tyr Ala Leu Lys Trp
            180                 185                 190

Val Lys Ser His Ile His Arg Phe Gly Gly Asn Pro Asn Asn Val Thr
        195                 200                 205

Ile Trp Gly Gln Ser Ala Gly Gly Gly Ser Val Val Ala Gln Ile Leu
    210                 215                 220

Ala Asn Gly Arg Gly Ser Asn Pro Lys Leu Phe Ser Lys Ala Leu Ala
225                 230                 235                 240
```

pectinases.ST251

```
Ser Ser Pro Phe Trp Pro Lys Thr Tyr Ala Tyr Asn Ala Pro Gln Ala
                245                 250                 255

Glu Ala Ile Tyr Thr Gln Leu Val Asn Leu Thr Asn Cys Thr Thr Ala
            260                 265                 270

Ser Asp Thr Leu Lys Cys Leu Lys Glu Val Asp Val Gln Ser Ile Arg
        275                 280                 285

Asp Ala Ser Leu Ile Ile Asp Ala Asp Asn Thr Tyr Thr Thr Ser Ser
    290                 295                 300

Tyr Thr Trp Ala Pro Val Ile Asp Gly Thr Phe Leu Ile Glu Pro Leu
305                 310                 315                 320

Thr Ser Ala Thr Ala Ser Asn Thr Leu Lys Thr Asp Leu Ile Trp Gly
                325                 330                 335

Met Tyr Asn Ala His Glu Gly Glu Asn Phe Ile Pro Pro Gly Leu Glu
            340                 345                 350

Asp Thr Thr Thr Thr Asn Gly Phe Asn Ser Ser Leu Ala Ser Phe His
        355                 360                 365

Asn Trp Leu Thr Gly Phe Leu Pro Gly Leu Asp Thr Ser Asp Ile Asn
    370                 375                 380

Leu Ile Glu Ser Lys Tyr Tyr Pro Val Ser Gly Thr Ala Glu Thr Leu
385                 390                 395                 400

Ser Tyr Asn Thr Thr Phe Val Arg Ala Gly Leu Val Tyr Arg Asp Val
                405                 410                 415

Val Leu Ala Cys Pro Ala Tyr Trp Val Ala Ser Ala Ala Gly Glu Lys
            420                 425                 430

Gly Tyr Val Gly Glu Tyr Thr Ile Pro Pro Ala Arg His Gly Ser Asp
        435                 440                 445
```

pectinases.ST251

```
Thr Glu Trp Trp Asp Thr Val Ser Thr Val Gln Gln Thr Asp Pro Leu
    450                 455                 460

Ile Tyr Asp Gly Tyr Ala Gly Ala Phe Ala Ser Phe Phe Gln Thr Gly
465                 470                 475                 480

Asp Pro Asn Ala His Lys Leu Thr Asn Gly Ser Glu Pro Gly Val Pro
                485                 490                 495

Glu Val Gln Gln Thr Ala Glu Glu Phe Val Ile Ala Thr Glu Gly Phe
            500                 505                 510

Glu Asn Val Gly Leu Gly Glu Leu Glu Asp Arg Cys Ala Phe Trp Lys
        515                 520                 525

Ser Val Gly Lys Lys Ile Pro Ile
    530                 535
```

<210> 83
<211> 378
<212> PRT
<213> Aspergillus niger

<400> 83

```
Met Val Thr Ser Ser Ser Val Ile Val Leu Thr Leu Trp Ala Ala Leu
1               5                   10                  15

Val Ser Ala Ser Pro Val Ala Asp Pro Leu Val Thr Pro Ala Pro Lys
            20                  25                  30

Leu Glu Asp Leu Glu Lys Arg Ala Thr Ser Cys Thr Phe Ser Gly Ser
        35                  40                  45

Glu Gly Ala Ser Ser Ala Ser Lys Ser Lys Thr Ser Cys Ser Thr Ile
    50                  55                  60

Val Leu Ser Asp Val Ala Val Pro Ser Gly Thr Thr Leu Asp Leu Thr
65                  70                  75                  80
```

pectinases.ST251

```
Asp Leu Asn Asp Gly Thr His Val Ile Phe Glu Gly Glu Thr Thr Phe
                85                  90                  95

Gly Tyr Glu Glu Trp Ser Gly Pro Leu Val Ser Val Ser Gly Thr Asp
            100                 105                 110

Ile Thr Val Thr Gly Ala Asp Gly Ala Tyr Leu Asn Gly Asp Gly Ser
        115                 120                 125

Arg Trp Trp Asp Gly Glu Gly Ser Asn Gly Gly Lys Thr Lys Pro Lys
    130                 135                 140

Phe Phe Tyr Ala His Asp Leu Thr Ser Ser Thr Ile Ser Gly Ile Tyr
145                 150                 155                 160

Ile Gln Asn Ser Pro Val Gln Val Phe Ser Ile Asp Gly Ser Thr Tyr
                165                 170                 175

Leu Thr Met Glu Asp Ile Thr Ile Asp Asn Thr Asp Gly Asp Asp Gly
            180                 185                 190

Glu Ala Ala Asn Thr Asp Gly Phe Asp Ile Gly Asp Ser Thr Tyr Ile
        195                 200                 205

Thr Ile Thr Gly Ala Asn Val Tyr Asn Gln Asp Asp Cys Val Ala Val
    210                 215                 220

Asn Ser Gly Glu Asn Ile Tyr Phe Ser Gly Gly Val Cys Ser Gly Gly
225                 230                 235                 240

His Gly Leu Ser Ile Gly Ser Val Gly Gly Arg Ser Asp Asn Thr Val
                245                 250                 255

Lys Asn Val Thr Phe Tyr Asp Ser Glu Ile Lys Ser Ser Gln Asn Gly
            260                 265                 270

Val Arg Ile Lys Thr Ile Tyr Gly Asp Thr Gly Ser Val Ser Glu Val
        275                 280                 285
```

pectinases.ST251

```
Thr Tyr Lys Glu Ile Thr Leu Ser Asp Ile Thr Asp Tyr Gly Ile Val
    290                 295                 300

Val Glu Gln Asn Tyr Asp Asp Thr Ser Lys Ser Pro Thr Asp Gly Ile
305                 310                 315                 320

Thr Ile Glu Asp Phe Val Leu Asp Asn Val Gln Gly Ser Val Glu Ser
                325                 330                 335

Ser Gly Thr Asn Ile Tyr Ile Val Cys Gly Ser Asp Ser Cys Thr Asp
            340                 345                 350

Trp Thr Trp Thr Asp Val Asp Val Ser Gly Gly Lys Thr Ser Ser Asp
        355                 360                 365

Cys Glu Asn Val Pro Asp Asp Ile Ser Cys
    370                 375
```

<210> 84
<211> 558
<212> PRT
<213> Aspergillus niger

<400> 84

```
Met Leu Leu Asp Lys Leu Ser Val Leu Ser Phe Leu Gly Leu Ala Pro
1               5                   10                  15

Ile Phe Ala Ala Ala Gln Leu Ser Gly Ser Val Gly Pro Leu Thr Ser
            20                  25                  30

Ala Ser Thr Lys Ala Ala Thr Lys Thr Cys Asn Val Leu Asp Tyr Gly
        35                  40                  45

Ala Lys Ala Asp Lys Ser Thr Asp Leu Gly Ala Pro Leu Ala Ser Ala
    50                  55                  60

Phe Ala Asp Cys Lys Ser Gly Gly Leu Val Tyr Val Pro Ser Gly Asp
65                  70                  75                  80
```

pectinases.ST251

Tyr Ala Leu Ser Thr Trp Ala Arg Leu Ser Gly Gly Glu Ala Trp Ala
85 90 95

Leu Gln Ile Asp Gly Ile Ile Tyr Arg Thr Gly Thr Asp Gly Gly Asn
100 105 110

Met Ile Tyr Ile Glu His Ser Ser Asp Phe Glu Leu Phe Ser Ser Thr
115 120 125

Ser Glu Gly Ala Met Gln Gly Leu Gly Tyr Glu Phe His Ala Asp Asp
130 135 140

Asn Trp Ser Gly Pro Arg Leu Leu Arg Leu Tyr Glu Val Thr Asp Phe
145 150 155 160

Ser Val His Asp Phe Ile Leu Val Asp Ser Pro Ser Phe His Phe Ser
165 170 175

Leu Asp Thr Cys Thr Asn Gly Glu Ile Tyr Asn Met Ala Ile Arg Gly
180 185 190

Gly Asn His Gly Gly Leu Asp Gly Ile Asp Val Trp Ser Asn Asn Ile
195 200 205

Trp Val His Asp Val Glu Val Thr Asn Lys Asp Glu Cys Val Thr Val
210 215 220

Lys Ser Pro Ser Lys Asn Ile Leu Ile Glu Ser Ile Tyr Cys Asn Trp
225 230 235 240

Ser Gly Gly Cys Gly Met Gly Ser Phe Gly Ser Asp Thr Asn Val Ser
245 250 255

Asp Ile Thr Tyr Arg Asn Ile Tyr Thr Trp Ser Ser Asn Asn Met Met
260 265 270

Leu Ile Lys Ser Asn Gly Gly Ser Gly Phe Val Glu Asn Val Leu Leu
275 280 285

pectinases.ST251

```
Glu Asn Phe Ile Gly His Gly Asn Ala Tyr Ser Leu Asp Ile Asp Ser
    290                 295                 300

Tyr Trp Ala Ser Met Ser Ala Val Asp Gly Asp Gly Val Gln Leu Ser
305                 310                 315                 320

Asn Ile Thr Val Lys Asn Trp Lys Gly Thr Glu Ala Tyr Gly Ala Glu
                325                 330                 335

Arg Gly Pro Val Lys Val Val Cys Ala Asp Gly Ala Pro Cys Tyr Asp
            340                 345                 350

Ile Thr Ile Glu Asp Phe Ala Met Trp Thr Glu Glu Gly Asp Ser Gln
        355                 360                 365

Trp Tyr Ser Cys Glu Ser Ala Tyr Gly Ser Gly Tyr Cys Leu Gln Asp
    370                 375                 380

Ser Asp Asp His Val Ser Tyr Ser Val Thr Thr Ser Thr Val Ser Ser
385                 390                 395                 400

Ala Pro Ser Gly Tyr Ser Ala Thr Ser Met Ala Ala Asp Leu Thr Thr
                405                 410                 415

Asp Phe Gly Ser Thr Val Ser Ile Pro Ile Pro Thr Ile Pro Thr Ser
            420                 425                 430

Phe Tyr Pro Gly Ala Thr Pro Tyr Ser Ala Leu Met Ala Asn Ser Ala
        435                 440                 445

Ser Thr Ala Ala Ala Ser Ser Ile Ala Ser His Ala Thr Val His Ser
    450                 455                 460

Ser Ser Ala Ser Val Ala Ala Ser Val Pro Ser Ala Val Ala Pro Ser
465                 470                 475                 480

Glu Ser Ile Pro Ala Ala Thr Ser Ala Val Val Ser Ser Ala Ala Ala
                485                 490                 495
```

pectinases.ST251

Ile Ala Pro Ser Pro Ala Val Gly Ala Gln Glu Gly Ser Thr Thr Ser
500 505 510

Ala Pro Ser Phe Ala Ala Pro Ser Gly Ala Gly Asn Ser Pro Gln Gly
515 520 525

Pro Thr Gly Ala Ser Gly Phe Gly Glu Lys Gly Gln Gln Gly Glu Gln
530 535 540

Gly Glu Gln Gly Glu Gln Gly Glu Gln Gly Val Cys Tyr Val
545 550 555

<210> 85
<211> 350
<212> PRT
<213> Aspergillus niger

<400> 85

Met Ile Tyr Ser Leu Leu Leu Ser Ala Leu Pro Leu Leu Ser Ser Ala
1 5 10 15

Ala Leu Thr Tyr Arg Gly Ala Asp Ile Ser Ser Leu Leu Ile Glu Glu
20 25 30

Asp Ala Gly Ile Ser Tyr Lys Asn Leu Asn Gly Glu Thr Gln Ala Leu
35 40 45

Glu Asp Ile Leu Val Asn Asn Gly Val Asn Ser Ile Arg Gln Arg Val
50 55 60

Trp Val Asp Pro Ser Asp Gly Ser Tyr Asp Leu Asp Tyr Asn Leu Lys
65 70 75 80

Leu Ala Lys Arg Val Gln Ala Ala Gly Met Ser Ile Tyr Leu Asp Leu
85 90 95

His Leu Ser Asp Thr Trp Ala Asp Pro Ser Asp Gln Thr Thr Pro Thr
100 105 110

Gly Trp Ser Thr Thr Asp Ile Asp Thr Leu Thr Trp Gln Leu Tyr Asn

pectinases.ST251

115 120 125

Tyr Thr Leu Asp Val Cys Asn Thr Phe Ala Glu Asn Asp Ile Asp Ile
130 135 140

Glu Ile Val Ser Ile Gly Asn Glu Ile Ser Ser Gly Leu Leu Trp Pro
145 150 155 160

Leu Gly Lys Thr Ser Asn Tyr Asp Asn Ile Ala Lys Leu Leu His Ser
165 170 175

Gly Ala Trp Gly Val Lys Asp Ser Asn Gln Ala Thr Thr Pro Lys Ile
180 185 190

Met Ile His Leu Asp Asn Gly Trp Asp Trp Glu Glu Gln Glu Tyr Phe
195 200 205

Tyr Lys Thr Val Leu Ala Thr Gly Ser Leu Leu Ser Thr Asp Phe Asp
210 215 220

Leu Met Gly Val Ser Tyr Tyr Pro Phe Tyr Ser Ser Glu Ala Thr Leu
225 230 235 240

Ser Ala Leu Gln Thr Ser Leu Thr Asn Met Gln Ser Asn Tyr Asp Lys
245 250 255

Ser Val Val Val Val Glu Thr Asn Trp Pro Val Ser Cys Pro Asp Pro
260 265 270

Glu Tyr Ser Phe Pro Ser Asp Leu Ser Ser Ile Pro Phe Ser Ala Ala
275 280 285

Gly Gln Glu Glu Phe Leu Glu Lys Leu Ala Glu Val Val Glu Gly Val
290 295 300

Thr Asp Gly Leu Gly Ile Tyr Tyr Trp Glu Pro Ala Trp Val Asp Asn
305 310 315 320

Ala Ala Leu Gly Ser Ser Cys Ala Asp Asn Leu Met Val Asp Ile Asp

pectinases.ST251

325 330 335

Thr Asp Glu Val Leu Glu Ser Val Thr Val Phe Glu Asp Leu
340 345 350

<210> 86
<211> 476
<212> PRT
<213> Aspergillus niger

<400> 86

Met Thr Leu Leu Arg His Leu Leu Thr Ala Thr Ala Leu Leu Gly Ala
1 5 10 15

Ser Val Gln Ala Ala Gln Gly Val Thr Gly Ser Pro Phe Gly Phe Ala
20 25 30

Ser Gly Thr Thr Gly Gly Gly Asp Ala Thr Pro Ala Ala Pro Ser Asp
35 40 45

Ile Ser Gln Leu Lys Thr Trp Leu Ser Asp Ser Thr Pro Arg Val Ile
50 55 60

Leu Ile Asp Lys Glu Phe Asn Phe Leu Gly Ser Glu Gly Lys Cys Thr
65 70 75 80

Asn Cys Glu Cys Cys Lys Pro Ala Ser Asn Thr Cys Gly Ser Ser Gly
85 90 95

Gln Asn Ala Val Lys Gln Asn Gly Ser Asp Trp Cys Gly Ser Tyr Pro
100 105 110

Thr Leu Thr Cys Thr Tyr Asp Asn Ala Gly Ile Glu Gly Leu Glu Val
115 120 125

Ala Ser Asn Lys Ser Ile Val Gly Val Gly Ser Ser Gly Val Leu Arg
130 135 140

Gly Lys Gly Leu Arg Leu Val Asn Gly Val Ser Asn Ile Ile Ile Gln
145 150 155 160

pectinases.ST251

```
Asn Ile His Ile Thr Glu Leu Asn Pro Glu Phe Ile Trp Gly Gly Asp
                165                 170                 175

Ala Ile Thr Leu Asp Gly Thr Asn Asn Val Trp Ile Asp His Val Lys
            180                 185                 190

Ile Asn Leu Ile Gly Arg Gln Met Phe Val Ala Gly Tyr Glu Ala Ser
        195                 200                 205

His Ser Val Thr Ile Ser Asn Ser Glu Phe Asp Gly Glu Thr Ser Trp
    210                 215                 220

Ser Ala Thr Cys Asp Gly His His Tyr Trp Thr Val Leu Gly Tyr Gly
225                 230                 235                 240

His Asn Asp Lys Ile Thr Phe Ala Asn Asn Tyr Ile His His Thr Ser
                245                 250                 255

Gly Arg Ser Pro Lys Leu Glu Phe Asn Ser Phe Trp His Ala Tyr Asn
            260                 265                 270

Asn Tyr Trp Tyr Asn Asn Thr Gly His Ala Phe Asp Val Gly Lys Asn
        275                 280                 285

Thr Arg Ala Leu Ile Glu Gly Asn Val Met Val Gln Val Asp Thr Pro
    290                 295                 300

Leu Leu Ala Asp Ser Asn Pro Gly Ala Val Phe Ala Val Asn Thr Ser
305                 310                 315                 320

Asp Val Ser Thr Cys Thr Ser Thr Leu Gly Arg Thr Cys Val Pro Asn
                325                 330                 335

Thr Leu Ile Ser Ser Gly Thr Leu Ser Gly Ser Asp Ser Ser Val Ile
            340                 345                 350

Ser Ser Trp Pro Ser Gly Glu Ser Asp Val Thr Val Met Ala Ala Ser
        355                 360                 365
```

pectinases.ST251

```
Lys Val Ala Ser Tyr Val Lys Ala Asn Ala Gly Ile Gly Lys Leu Gly
    370                 375                 380

Asn Gly Ser Gly Ser Ser Ser Thr Val Gly Ala Ala Ala Thr Ser Ala
385                 390                 395                 400

Val Ala Lys Arg Ala Asp Ser Asp Asp Ala Pro Phe Val Pro Ala Tyr
                405                 410                 415

Ser Glu Ala Gly Pro Gly Ala Ser Ala Val Pro Thr Gln Pro Ser Trp
            420                 425                 430

Ser Trp Arg Thr Val Thr Asn Gly Pro Ala Pro Thr Gly Ala Pro Ser
        435                 440                 445

Asp Ser Pro Ser Ala Pro Gln Gly Leu Gly Ala Pro Val Gln Ala Ser
    450                 455                 460

Asn Lys His His His Gln Gly His Gly Arg Gly Tyr
465                 470                 475
```

<210> 87
<211> 437
<212> PRT
<213> Aspergillus niger

<400> 87

```
Met Thr Pro Asn Trp Ser Lys Leu Trp Thr Phe Ile Ala Asn Pro Lys
1               5                   10                  15

Asp Pro Ser Ser Ser Ser Pro Ser Pro Tyr Thr Leu Arg Arg Ile Ile
            20                  25                  30

Lys Ser Leu Ser Leu Leu Thr Val Phe Ser Ile Phe Leu Tyr Ala Leu
        35                  40                  45

Tyr Ile His Phe Gln Pro Ser Ile Ile Pro Gln Thr Pro Asp Leu Pro
    50                  55                  60
```

pectinases.ST251

```
Asp Pro Asp Leu Pro Pro Ser Pro Glu Asp Ser Tyr Lys Ser Ile Tyr
65                  70                  75                  80

Gly Tyr Pro Pro Thr Asn Pro Thr Ile Pro Pro Leu His Ile His Asp
                85                  90                  95

Pro Ser Ile Leu Tyr Asp Leu Pro Thr Asn Thr Tyr Tyr Ala Tyr Gly
            100                 105                 110

Ser Gly Pro His Ile Pro Ile His Ser Ala Pro Thr Leu Gln Gly Pro
        115                 120                 125

Trp Thr Lys Val Gly Thr Val Leu Asp Ala Asp Ser Ile Leu Pro Lys
    130                 135                 140

Gly Asp Arg Lys Ala Pro Trp Ala Pro Thr Ala Leu Val His Asp Gly
145                 150                 155                 160

Thr Phe Tyr Val Phe Tyr Ala Thr Ser His Ser Gly Cys Arg Asp Ser
                165                 170                 175

Ala Ile Gly Val Ala Thr Ser Thr Ser Pro Gly Pro Gly Gly Trp Glu
            180                 185                 190

Asp His Gly Ala Ile Ala Ile Ser Gly Arg Gly Glu Arg Gly Lys Glu
        195                 200                 205

Tyr Pro Phe Asp Arg Ala Asn Ala Ile Asp Val Ser Val Val Val Asp
    210                 215                 220

Tyr Thr Asp Thr Gln Thr Gln Thr Glu Pro Ser Glu Gly Glu Ile Ser
225                 230                 235                 240

Leu Glu Glu Gly Lys Lys Gly Lys Gly Arg Gly Tyr Met Thr Phe Gly
                245                 250                 255

Ser Phe Trp Thr Gly Ile Trp Gln Val Pro Leu Lys Pro Asn Leu Leu
            260                 265                 270
```

pectinases.ST251

```
His Met Asp Lys Gln Gly Glu Glu Glu Lys Arg Val Lys His Leu Ala
        275                 280                 285

His Glu Pro Ala Ala Ile His Pro Pro Thr Lys Lys Ala Asp Gly Leu
    290                 295                 300

Cys Gly Asp Thr Thr Gly Met His Pro Ile Glu Gly Ala Phe Ile Ser
305                 310                 315                 320

Tyr His Glu Pro Trp Trp Tyr Leu Trp Phe Ser Trp Gly Lys Cys Cys
                325                 330                 335

His Phe Asp Pro Glu Lys Leu Pro Arg Ala Gly Leu Asp Ile Arg Val
            340                 345                 350

Gly Arg Ser Ser Ser Pro Gln Gly Pro Phe Val Asp Lys Glu Gly Lys
        355                 360                 365

Asp Leu Val Asp Gly Gly Gly Glu Ile Val Tyr Gly Ser Asn Gly Asp
    370                 375                 380

Val Tyr Ala Pro Gly Gly Gln Gly Val Leu Ser Gly Glu Val Glu Gly
385                 390                 395                 400

Asp Val Leu Tyr Tyr His Tyr Leu Asn Ile Ser Val Gly Tyr Glu Phe
                405                 410                 415

Lys Glu Ala Arg Leu Gly Tyr Asn Tyr Leu Lys Tyr Val Asp Gly Trp
            420                 425                 430

Pro Val Pro Leu Ser
        435
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<210> 88
<211> 366
<212> PRT
<213> Aspergillus niger

<400> 88

pectinases.ST251

```
Met Leu Pro Ser Leu Leu Ser Val Leu Phe Phe Leu Leu Tyr Ala Val
1               5                  10                  15

Asn Ala Val Pro Leu Ala Pro Arg Ala Ser Ala Leu Ala Gly Ile Asp
            20                  25                  30

Thr Lys Ser Phe Ser Lys Thr Lys Asp Tyr Pro Leu Pro Asn Leu Gly
        35                  40                  45

Asn Ile Val Ala His Asp Pro Asn Val Ile Gln His Asp Gly Tyr Phe
    50                  55                  60

Tyr Leu Tyr Lys Gly Gly Val His Ile Pro Ile His Arg Ala Arg Ser
65                  70                  75                  80

Leu Ser Gly Pro Trp Glu Gln Val Gly Thr Val Leu Asp Asp Ser Ser
                85                  90                  95

Val Ile Pro Lys Gln Asn Arg Ser Arg Pro Trp Ala Pro Thr Thr Ile
            100                 105                 110

Gln His Asp Asn Arg Phe Tyr Cys Phe Tyr Ala Ile Ser Glu Asn Gly
        115                 120                 125

Ser Arg Asp Ser Ala Ile Gly Val Ala Ser Ser Asp Thr Pro Val Gly
    130                 135                 140

Gly Asn Trp Thr Asp His Gly Ala Val Val Asn Thr Gly Lys Gly Asp
145                 150                 155                 160

Leu Ser Asp Ile Tyr Pro Tyr Ser Val Ser Asn Ala Ile Asp Gly Ala
                165                 170                 175

Phe Ile Thr Asp Gln Gln Thr Gly Gln Ser His Leu Leu Tyr Gly Ser
            180                 185                 190

Tyr Trp His Gly Ile Phe Ser Val Pro Leu Ala Asp Asp Leu Leu Ser
        195                 200                 205
```

pectinases.ST251

Val Lys Thr Pro Lys Thr Pro Asn Ala Thr Asn Leu Ala Tyr Ile Pro
210 215 220

Asp Ala Lys Ser Lys Pro Ile Glu Gly Ser Phe Met Thr Tyr Lys Ala
225 230 235 240

Pro Tyr Tyr Tyr Leu Trp Phe Ser His Gly Lys Cys Cys His Phe Asp
245 250 255

Ile His Ala Phe Pro Pro Met Gly Asp Glu Tyr Asn Ile Arg Val Gly
260 265 270

Arg Ser Lys Ser Ala Thr Gly Pro Phe Val Asp Lys Asp Gly His Asp
275 280 285

Thr Leu Lys Gly Gly Gly Thr Ile Val Tyr Gly Ser Asn His Gly Ile
290 295 300

Val Tyr Ala Pro Gly Gly Val Gly Val Leu Ile Asn Asn Gly Ser Glu
305 310 315 320

Ala Asp Val Leu Tyr Tyr His Tyr Leu Asn Thr Thr Ser Gly Phe Ala
325 330 335

Gln Gly Asp Ala His Leu Gly Trp Asn Tyr Leu His Tyr Val Asn Gly
340 345 350

Trp Pro Val Ala Val Glu Gly Tyr Val Asn Ala Asn Gly Lys
355 360 365

<210> 89
<211> 318
<212> PRT
<213> Aspergillus niger

<400> 89

Met Leu Ser Phe Val Leu Leu Leu Cys Val Ala Leu Val Asn Ala Tyr
1 5 10 15

Ser Asp Pro Gly Ala Cys Ser Gly Thr Cys Trp Ala His Asp Pro Asn

pectinases.ST251

20 25 30

Val Ile Arg Arg Val Ser Asp Gly Thr Tyr Phe Arg Phe Ser Thr Gly
35 40 45

Gly Gly Val His Ile Ser Ser Ala Ser Ala Ile Thr Gly Pro Trp Thr
50 55 60

Asp Leu Gly Tyr Ala Leu Pro Asn Gly Ser Ile Val Thr Val Gly Asn
65 70 75 80

Ala Ser Asn Leu Trp Ala Pro Asp Val His Tyr Val Asp Gly Thr Tyr
85 90 95

Tyr Met Tyr Tyr Ala Ser Ser Thr Leu Gly Ser Arg Asp Ser Thr Ile
100 105 110

Gly Val Ala Thr Ser Thr Thr Leu Glu Ala Asp Ser Trp Thr Asp His
115 120 125

Gly Glu Ile Gly Val Thr Ser Ser Ser Ser Thr Pro Tyr Asn Ala Ile
130 135 140

Asp Pro Asn Trp Ile Thr Ile Gly Ser Thr Pro Tyr Leu Gln Phe Gly
145 150 155 160

Ser Tyr Trp Gln Gly Leu Tyr Gln Val Glu Met Thr Asp Ser Leu Ser
165 170 175

Ala Ser Ser Ser Thr Pro Thr Asn Leu Ala Tyr Asn Ala Ser Gly Asn
180 185 190

His Ala Ile Glu Ala Ser Tyr Leu Tyr Glu Tyr Gly Gly Tyr Tyr Tyr
195 200 205

Leu Thr Phe Ser Ser Gly Lys Ala Gln Gly Tyr Thr Thr Ser Leu Pro
210 215 220

Ala Gln Gly Asp Glu Tyr Arg Ile Val Val Cys Arg Ser Lys Thr Gly

pectinases.ST251

225 230 235 240

Thr Gly Asn Phe Val Asp Lys Asp Gly Val Ser Cys Leu Asn Ser Gly
245 250 255

Gly Thr Thr Val Leu Ala Ser His Asp Tyr Val Tyr Gly Pro Gly Gly
260 265 270

Gln Gly Ile Ile Asn Thr Thr Ser His Gly Ile Val Val Tyr Tyr His
275 280 285

Tyr Ala Asn Lys Asn Ile Gly Leu Ala Val Asp Asp Tyr Gln Phe Gly
290 295 300

Trp Asn Thr Leu Thr Trp Thr Asp Gly Trp Pro Val Val Ala
305 310 315

<210> 90
<211> 494
<212> PRT
<213> Aspergillus niger

<400> 90

Met Leu Thr Ser Gln Pro Leu Ser Pro Asn Cys Pro His Arg Arg Ala
1 5 10 15

Tyr His His Leu Pro Ser Leu Leu Glu Val Leu Ile His Ser Pro His
20 25 30

Glu Pro Leu Ser Leu Thr Leu Lys Thr Leu Tyr Leu Phe Ser Thr Asn
35 40 45

Gly Ser Pro His Trp Leu Ser Tyr Leu Thr Pro Gln Pro Ser Pro Leu
50 55 60

Thr Thr Lys His Arg Val Val Asn Asp Thr Leu Gln Ser Glu Asp Tyr
65 70 75 80

Ser Leu Val Arg Ala Arg Gly Trp Gln His Pro Arg Lys Tyr Ile Gln
85 90 95

pectinases.ST251

```
Val Asp Thr Met Lys Gly Leu Pro Ser Leu Leu Asn Ser Ser Ser Thr
            100                 105                 110

Lys Lys Trp Thr Gln Ser Thr Pro Ser Thr Gly Glu Gly Glu Asn Glu
        115                 120                 125

Ser Gly Ser Thr Ile Ser Pro Thr Lys Pro Pro Glu Pro Ser Thr Pro
    130                 135                 140

Pro Gly Gly Ser Asn Thr Ser Met Ile Trp Thr Arg Lys Lys Ile Ile
145                 150                 155                 160

Trp Leu Ile Thr Leu Ile Ala Leu Ser Val Ala Ile Ile Val Val Val
                165                 170                 175

Ile Thr Val Pro Val Val Leu Leu Leu Asp Asp Lys His Asn Asp Asp
            180                 185                 190

Pro Ser Tyr Tyr Asn Ser Ala Asn Arg Pro Val Arg Val Val His Asp
        195                 200                 205

Phe Pro Asp Pro Gly Leu Ile Gln Val Asn Ser Thr Trp Tyr Ala Tyr
    210                 215                 220

Ala Thr Val Ala Thr Pro Asp Asn Pro Asp Val Pro His Val Pro Val
225                 230                 235                 240

Ser Thr Ser Arg Asn Phe Ser Ser Trp Thr Trp Leu Gln Gly Tyr Asp
                245                 250                 255

Val Met Pro Ala Ile Ser Ser Trp Glu Thr Asn Met Asn Gln Tyr Ala
            260                 265                 270

Pro Asp Val Ile Gln Arg Lys Asp Gly His Phe Val Leu Tyr Tyr Ser
        275                 280                 285

Gly Glu Leu Lys Asp Trp Leu Arg His His Cys Val Gly Ala Ala Val
    290                 295                 300
```

pectinases.ST251

```
Ser Asn Gly Thr Ser Pro Leu Gly Pro Tyr Ile Pro His Asn Thr Thr
305                 310                 315                 320

Leu Ala Cys Pro Arg Asp His Gly Gly Ala Ile Asp Pro Ala Pro Phe
                325                 330                 335

Arg Asp Val Asn Gly Thr Leu Tyr Val Val Tyr Lys Val Asp Gly Asn
            340                 345                 350

Ser Ile Gly His Gly Gly Gly Cys Asn Asn Gly Lys Lys Pro Ile Val
        355                 360                 365

Ser Thr Pro Ile Met Leu Gln Gln Leu Lys Asp Asp Gly Val Thr Pro
    370                 375                 380

Val Gly Asp Pro Val Glu Ile Leu Thr Asn Glu Lys Val Asp Gly Pro
385                 390                 395                 400

Leu Val Glu Ala Pro Ala Ile Ile Arg Thr Asp Arg Gly Ile Tyr Tyr
                405                 410                 415

Leu Phe Phe Ser Ser His Cys Phe Thr Ser Ser Lys Tyr Ser Val Lys
            420                 425                 430

Tyr Ala Trp Ser Thr Ser Leu Lys Gly Pro Tyr Thr Arg Ala Glu Arg
        435                 440                 445

Pro Leu Phe Arg Ser Gly Asp Phe Gly Leu Lys Ser Pro Gly Gly Ala
    450                 455                 460

Thr Ala Ser Val Asp Gly Ser Arg Ile Val Phe His Ala Phe Cys Gly
465                 470                 475                 480

Asp Tyr Arg Cys Arg Val Ser Gly Lys Pro Ser Ile Leu Ser
                485                 490
```

<210> 91
<211> 251

pectinases.ST251

<212> PRT
<213> Aspergillus niger

<400> 91

Met Trp Thr His Leu Pro Ser Leu Cys Ala Leu Gly Leu Thr Leu Ile
1 5 10 15

Thr Ser Val Ile Thr Ser Pro Ile Glu Ile Arg Ala Thr Gly Pro Trp
20 25 30

Leu Ala Leu Asp Thr Asp Phe Pro Asp Pro Gly Phe Val Gln Ala Asp
35 40 45

Asp Gly Thr Trp Tyr Ala Phe Gly Thr Asn Gly Asn Asn Arg Thr Val
50 55 60

Gln Val Ala Lys Ser Ala Asp Phe Lys Thr Trp Thr Leu Leu Asp Lys
65 70 75 80

Glu Ala Leu Pro Thr Leu Ala Gly Trp Glu Thr Gln Ile Asp His Trp
85 90 95

Ala Pro Asp Val Val Arg Arg Val His Ser Pro Ser His Pro Ile Pro
100 105 110

Phe Pro Gln Ile Gln Thr Asn His His His His Pro Gln Asn Asp Gly
115 120 125

Lys Tyr Val Leu Tyr Tyr Ser Gly Glu Ala Gln Gln Met Leu Arg His
130 135 140

His Cys Ile Gly Thr Ala Val Ser Glu Ser Thr Asp Pro Ser Gly Pro
145 150 155 160

Tyr Ile Pro Asn Pro Thr Pro Leu Ser Cys Arg Leu Asp Gln Gly Gly
165 170 175

Ser Ile Asp Ala Ser Gly Phe Leu Asp Lys Asp Gly Ser Arg Tyr Val
180 185 190

pectinases.ST251

```
Val Phe Lys Val Asp Gly Asn Ser Ile Gly Asn Gly Gly Asp Cys Asn
        195                 200                 205

Asn Gly Ile Ala Pro Leu Lys Pro Thr Pro Ile Leu Leu Gln Lys Arg
    210                 215                 220

Arg Ala Val Gly Gly Gly Pro Glu Phe Asp Ser Ala Trp Gly His Val
225                 230                 235                 240

Leu Phe Val Leu Leu Asp Ala Leu Leu Tyr Gly
                245                 250
```

<210> 92
<211> 646
<212> PRT
<213> Aspergillus niger

<400> 92

```
Met Ile Ala Val Ala Lys Trp Leu Tyr Val Ala Val Thr Ala Tyr Phe
1               5                   10                  15

His Lys Leu Arg Pro Val Ser His Asp Tyr Pro Ala Leu Ala Ala Arg
            20                  25                  30

Asp Asn Gly Thr Asn Gly Ser Val Ala Ser Pro Ile Thr Leu Thr Val
        35                  40                  45

Glu Glu Ser Gly Gly Asn Gln Ser Ser Pro Leu Leu Tyr Gly Val Met
    50                  55                  60

Phe Glu Val Ile Tyr Ser Gly Asp Gly Gly Ile His Gly Gln Leu Leu
65                  70                  75                  80

Gln Asn Asn Gly Phe Gln Gly Asp Asp Pro Gly Leu Thr Ala Tyr Lys
                85                  90                  95

Ala Val Gly Pro Val Asp Leu Met Gln Asp Leu Ile Asn Pro Val Ser
            100                 105                 110
```

pectinases.ST251

Gly Ala Ile Thr Ser Ser Leu Gln Val Ser Val Asp Phe Glu Ala Thr
115 120 125

Gly Phe Val Gly Phe Ala Asn Thr Gly Tyr Ser Gly Ile Pro Val Met
130 135 140

Asn Ala Thr Tyr Ser Cys Gln Phe Trp Met Met Gly Glu Tyr Ser Gly
145 150 155 160

Thr Ile Met Leu Gln Leu Ala Gly Ser Thr Asn Asp Thr Ile Tyr Ala
165 170 175

Ser His Asn Ile Thr Val Lys Ser Ser Trp Gly Lys Phe Thr His Tyr
180 185 190

Gln Thr Ser Phe Asn Ser Ser Ala Ala Pro Asp Gly Asp Asn Glu Trp
195 200 205

Arg Leu Leu Phe Asn Gly Ser Lys Met Ala Gly Gly Met Leu Asn Phe
210 215 220

Gly Leu Val Gln Leu Phe Pro Pro Thr Tyr Lys Ser Arg Ser Asn Gly
225 230 235 240

Leu Arg Asn Asp Val Ala Thr Phe Leu Glu Lys Thr Ala Pro Ser Phe
245 250 255

Leu Arg Phe Pro Gly Gly Asn Asn Leu Glu Gly Leu Gln Ile Asp Ser
260 265 270

Arg Trp Gln Trp Asn Leu Thr Val Gly Pro Val Val Asp Arg Pro Gly
275 280 285

Arg Gln Gly Asp Trp Phe Tyr Pro Asn Thr Asp Ala Leu Gly Leu Asp
290 295 300

Glu Tyr Leu Trp Trp Cys Glu Asp Met Asn Met Glu Pro Val Leu Ala
305 310 315 320

pectinases.ST251

```
Val Trp Asp Gly Lys Ser Tyr Gly Gly Ile Leu Ser Gly Asp Asp Leu
                325                 330                 335

Gln Pro Tyr Ile Asp Asp Ile Met Asn Glu Leu Glu Tyr Leu Leu Gly
            340                 345                 350

Pro Val Asn Ser Thr Tyr Gly Ser Met Arg Ala Gln Asn Gly Arg Ser
        355                 360                 365

Lys Pro Trp Ser Ile Asn Tyr Ile Glu Ile Gly Asn Glu Asp Asp Phe
    370                 375                 380

Thr Gly Gly Cys Asp Thr Tyr Pro Asp Arg Phe Tyr Gln Ile Tyr Asn
385                 390                 395                 400

Ala Ile Ser Asn Ser Tyr Pro Asn Ile Thr Leu Ile Ala Ser Asn Ile
                405                 410                 415

Asp Tyr Leu Cys Leu Pro Ile Glu Pro Pro Pro Gly Leu Ile Tyr Asp
            420                 425                 430

Tyr His Tyr Tyr Arg Lys Pro Asp Asp Leu Val Ala Met Phe Asp Tyr
        435                 440                 445

Trp Asp Asn Gln Pro Arg Thr Gln Pro Ile Met Val Gly Glu Tyr Gly
    450                 455                 460

Cys Arg Asp Thr Ser Glu Ala Asp Gly Ile Phe Trp Ser Ser Met Gln
465                 470                 475                 480

Cys Ser Cys Ser Glu Ala Ala His Met Ile Gly Leu Glu Arg Asn Ser
                485                 490                 495

Asp Val Val Lys Met Ala Ala Tyr Ala Pro Leu Leu Gln His Phe Gly
            500                 505                 510

Tyr Thr Gln Trp Ser Pro Thr Leu Phe Gly Phe Asp Ser Ser Pro Asp
        515                 520                 525
```

pectinases.ST251

Ser Leu Thr Pro Ser Thr Ser Tyr Tyr Val Gln Arg Met Phe Ser Thr
530 535 540

Asn Arg Gly Asp Thr Ile Leu Pro Val Asn Thr Thr Ala Thr Phe Gly
545 550 555 560

Pro Leu Tyr Trp Val Ala Ser Arg Thr Asn Ser Thr Tyr Phe Val Lys
565 570 575

Leu Ala Asn Tyr Gly Ala Gln Asn Gln Thr Val Arg Val Lys Val Pro
580 585 590

Gln Thr Lys Thr Gly His Val Glu Met Leu Tyr Gly Pro Gln Asn Ala
595 600 605

Thr Asn Leu Val His Asn Ile Thr Val Gln Pro Thr Val Lys Asn Val
610 615 620

Thr Ser Ser Arg Gly Ile Tyr Ser Leu Asp Met Pro Pro Trp Gly Val
625 630 635 640

Ala Val Leu Ser Val Trp
645

<210> 93
<211> 396
<212> PRT
<213> Aspergillus niger

<400> 93

Met Pro Ala Thr Glu Ala Ala Ser Thr Val His Ser Pro Pro Val Ser
1 5 10 15

His Ser Ile Pro Pro Pro Met Asn Leu Val Leu His Gly Phe Ser Ser
20 25 30

Val Pro Gly Leu Val Ala Gly Asn Gly His Asp Cys His Phe Pro Ile
35 40 45

His Ala Thr Pro Ala Cys Pro Leu Ala Asp Tyr Arg Cys Leu His Gly

pectinases.ST251

```
    50                  55                  60

Arg Pro Ser Tyr Thr Cys Ser Cys Leu Asn Phe Cys Gln Thr Glu Leu
65                  70                  75                  80

Ser Thr Gln Leu Phe Arg His Ile Arg Ser Ser Ser Ala Gly Gly Ala
                85                  90                  95

Ala Asp Ser Ile Pro Cys Arg Ala His Leu Ser Arg Gly Gly His Phe
            100                 105                 110

Phe Ser Phe Glu Leu Glu Asp Glu Gly Tyr Ser Tyr Lys Asn Leu Lys
        115                 120                 125

Gly Gln Ser Gln Ala Leu Glu Thr Ile Leu Ala Glu Ala Gly Ile Asn
    130                 135                 140

Ser Val Arg Gln Arg Val Trp Gly Gln Phe Gln Ser Trp His Leu Gln
145                 150                 155                 160

Phe Gly Leu Gln Leu Gly Ala Gly Lys Arg Val Lys Ala Ala Gly Met
                165                 170                 175

Ser Ile Tyr Leu Asp Leu His Leu Asn Asn Asn Ile Asp Ile Glu Ile
            180                 185                 190

Ile Ser Ile Gly Asn Glu Ile Arg Ala Gly Leu Leu Trp Pro Leu Gly
        195                 200                 205

Glu Thr Ser Ser Tyr Ser Asn Ile Gly Ala Leu Leu Tyr Ser Gly Ala
    210                 215                 220

Trp Gly Val Lys Asp Ser Asn Leu Ala Thr Leu Pro Lys Ile Ile Ile
225                 230                 235                 240

His Leu Asp Asp Gly Trp Ser Trp Asp Gln Gln Ser Tyr Phe Tyr Lys
                245                 250                 255

Thr Val Leu Ser Thr Gly Glu Leu Leu Asn Thr Asp Phe Asp Tyr Phe
```

pectinases.ST251

260 265 270

Gly Val Leu Tyr Tyr Leu Phe Tyr Ser Ala Ser Val Asn Ile Ala Ser
275 280 285

Leu Lys Thr Ser Leu Ala Asn Leu Gln Ser Ile Tyr Asn Lys Pro Val
290 295 300

Val Val Val Glu Met Asn Trp Pro Val Pro Cys Pro Asn Pro Glu Tyr
305 310 315 320

Ser Cys Phe Ser Asp Pro Arg Leu Ile Ser Phe Ser Val Ala Gly Gln
325 330 335

Gln Gln Phe Leu Glu Lys Leu Ala Ala Val Val Glu Ala Thr Thr Asp
340 345 350

Gly Gln Gly Val Tyr Tyr Trp Glu Pro Ala Trp Ile Thr Asn Ala Gly
355 360 365

Val Gly Ser Ser Cys Asp Glu Asn Leu Met Val Asp Arg Ser Thr Asp
370 375 380

Glu Val Tyr Ala Ser Phe Asp Ile Leu Gly Lys Leu
385 390 395

<210> 94
<211> 379
<212> PRT
<213> Aspergillus niger

<400> 94

Met Lys Tyr Ser Thr Ile Phe Ser Ala Ala Ala Ala Val Phe Ala Gly
1 5 10 15

Ser Ala Ala Ala Val Gly Val Ser Gly Ser Ala Glu Gly Phe Ala Glu
20 25 30

Gly Val Thr Gly Gly Gly Asp Ala Thr Pro Val Tyr Pro Asp Thr Ile
35 40 45

pectinases.ST251

```
Asp Glu Leu Val Ser Tyr Leu Gly Asp Asp Glu Ala Arg Val Ile Val
    50                  55                  60

Leu Thr Lys Thr Phe Asp Phe Thr Asp Ser Glu Gly Thr Thr Thr Gly
65                  70                  75                  80

Thr Gly Cys Ala Pro Trp Gly Thr Ala Ser Ala Cys Gln Val Ala Ile
                85                  90                  95

Asp Gln Asp Asp Trp Cys Glu Asn Tyr Glu Pro Asp Ala Pro Ser Ile
            100                 105                 110

Ser Val Glu Tyr Tyr Asn Ala Gly Val Leu Gly Ile Thr Val Thr Ser
        115                 120                 125

Asn Lys Ser Leu Ile Gly Glu Gly Ser Ser Gly Ala Ile Lys Gly Lys
    130                 135                 140

Gly Leu Arg Ile Val Ser Gly Ala Glu Asn Ile Ile Ile Gln Asn Ile
145                 150                 155                 160

Ala Val Thr Asp Ile Asn Pro Lys Tyr Val Trp Gly Gly Asp Ala Ile
                165                 170                 175

Thr Leu Asp Asp Cys Asp Leu Val Trp Ile Asp His Val Thr Thr Ala
            180                 185                 190

Arg Ile Gly Arg Gln His Tyr Val Leu Gly Thr Ser Ala Asp Asn Arg
        195                 200                 205

Val Ser Leu Thr Asn Asn Tyr Ile Asp Gly Val Ser Asp Tyr Ser Ala
    210                 215                 220

Thr Cys Asp Gly Tyr His Tyr Trp Gly Ile Tyr Leu Asp Gly Asp Ala
225                 230                 235                 240

Asp Leu Val Thr Met Lys Gly Asn Tyr Ile Tyr His Thr Ser Gly Arg
                245                 250                 255
```

pectinases.ST251

```
Ser Pro Lys Val Gln Asp Asn Thr Leu Leu His Cys Val Asn Asn Tyr
            260                 265                 270

Phe Tyr Asp Ile Ser Gly His Ala Phe Glu Ile Gly Glu Gly Gly Tyr
        275                 280                 285

Val Leu Ala Glu Gly Asn Val Phe Gln Asn Val Asp Thr Val Leu Glu
    290                 295                 300

Thr Tyr Glu Gly Ala Ala Phe Thr Val Pro Ser Thr Thr Ala Gly Glu
305                 310                 315                 320

Val Cys Ser Thr Tyr Leu Gly Arg Asp Cys Val Ile Asn Gly Phe Gly
                325                 330                 335

Ser Ser Gly Thr Phe Ser Glu Asp Ser Thr Ser Phe Leu Ser Asp Phe
            340                 345                 350

Glu Gly Lys Asn Ile Ala Ser Ala Ser Ala Tyr Thr Ser Val Ala Ser
        355                 360                 365

Ser Val Val Ala Asn Ala Gly Gln Gly Asn Leu
    370                 375
```

<210> 95
<211> 379
<212> PRT
<213> Aspergillus niger

<400> 95

```
Met His Tyr Lys Leu Leu Phe Ala Ala Ala Ala Ala Ser Leu Ala Ser
1               5                   10                  15

Ala Val Ser Ala Ala Gly Val Val Gly Ala Ala Glu Gly Phe Ala His
            20                  25                  30

Gly Val Thr Gly Gly Gly Ser Ala Ser Pro Val Tyr Pro Thr Thr Thr
        35                  40                  45
```

pectinases.ST251

```
Asp Glu Leu Val Ser Tyr Leu Gly Asp Asn Glu Pro Arg Val Ile Ile
    50                  55                  60

Leu Asp Arg Thr Phe Asp Phe Thr Gly Thr Glu Gly Thr Glu Thr Thr
65                  70                  75                  80

Thr Gly Cys Ala Pro Trp Gly Thr Ala Ser Gln Cys Gln Val Ala Ile
                85                  90                  95

Asn Leu His Ser Trp Cys Asp Asn Tyr Gln Ala Ser Ala Pro Lys Val
            100                 105                 110

Ser Val Thr Tyr Asp Lys Ala Gly Ile Leu Pro Ile Thr Val Asn Ser
        115                 120                 125

Asn Lys Ser Ile Val Gly Gln Gly Thr Lys Gly Val Ile Lys Gly Lys
    130                 135                 140

Gly Leu Arg Val Val Ser Gly Ala Lys Asn Val Ile Ile Gln Asn Ile
145                 150                 155                 160

Ala Val Thr Asp Ile Asn Pro Lys Tyr Val Trp Gly Gly Asp Ala Ile
                165                 170                 175

Thr Val Asp Asp Ser Asp Leu Val Trp Ile Asp His Val Thr Thr Ala
            180                 185                 190

Arg Ile Gly Arg Gln His Ile Val Leu Gly Thr Ser Ala Asp Asn Arg
        195                 200                 205

Val Thr Ile Ser Tyr Ser Leu Ile Asp Gly Arg Ser Asp Tyr Ser Ala
    210                 215                 220

Thr Cys Asn Gly His His Tyr Trp Gly Val Tyr Leu Asp Gly Ser Asn
225                 230                 235                 240

Asp Met Val Thr Leu Lys Gly Asn Tyr Phe Tyr Asn Leu Ser Gly Arg
                245                 250                 255
```

pectinases.ST251

```
Met Pro Lys Val Gln Gly Asn Thr Leu Leu His Ala Val Asn Asn Leu
            260                 265                 270

Phe His Asn Phe Asp Gly His Ala Phe Glu Ile Gly Thr Gly Gly Tyr
        275                 280                 285

Val Leu Ala Glu Gly Asn Val Phe Gln Asp Val Asn Thr Val Val Glu
    290                 295                 300

Thr Pro Ile Ser Gly Gln Leu Phe Ser Ser Pro Asp Ala Asn Thr Asn
305                 310                 315                 320

Gln Gln Cys Ala Ser Val Phe Gly Arg Ser Cys Gln Leu Asn Ala Phe
                325                 330                 335

Gly Asn Ser Gly Ser Met Ser Gly Ser Asp Thr Ser Ile Ile Ser Lys
            340                 345                 350

Phe Ala Gly Lys Thr Ile Ala Ala Ala His Pro Pro Gly Asn Ile Ala
        355                 360                 365

Gln Trp Thr Met Lys Asn Ala Gly Gln Gly Lys
    370                 375
```

<210> 96
<211> 395
<212> PRT
<213> Aspergillus niger

<400> 96

```
Met Lys Val Pro Phe Leu Gln Leu Leu Cys Leu Asn Ala Ala Leu Ala
1               5                   10                  15

Ser Ala Asn Val Val Lys Gly Ala Ala Gln Gly Phe Ala Ala Gly Val
            20                  25                  30

Thr Gly Gly Gly Asp Ile Thr Pro Ser Tyr Pro Lys Thr Asn Glu Glu
        35                  40                  45
```

pectinases.ST251

```
Leu Val Ser Leu Leu Glu Ser Asp Glu Pro Gln Val Val Val Leu Thr
    50                  55                  60

Lys Thr Phe Glu Phe Ile Gly Thr Glu Gly Thr Thr Thr Glu Asn Gly
65                  70                  75                  80

Cys Ala Pro Trp Gly Thr Gly Lys Ser Cys Gln Leu Ala Ile Asn Ser
                85                  90                  95

Asn Gly Trp Cys Gly Lys Asn Pro Val Val Thr Ile Thr Tyr Asp Asn
            100                 105                 110

Ala Ala Lys Asn Gly Ile His Ile Lys Ser Asn Lys Thr Leu Val Gly
        115                 120                 125

Glu Gly Asp Lys Gly Val Leu Ser Gly Lys Gly Leu Tyr Phe Glu Gly
    130                 135                 140

Gly Val Ser Asn Ile Ile Val Gln Asn Ile Lys Ile Thr Asn Leu Asn
145                 150                 155                 160

Pro Gly Phe Val Trp Gly Gly Asp Ala Phe Thr Phe Phe Gly Ala Asp
                165                 170                 175

Leu Ile Trp Ile Asp His Cys Glu Thr Ser Leu Thr Gly Arg Gln His
            180                 185                 190

Tyr Val Thr Gly Phe His Pro Asn Thr Arg Met Thr Trp Ser Asn Asn
        195                 200                 205

Phe Leu Asn Gly Val Thr Thr His Ser Ala Gly Cys Asp Asp His His
    210                 215                 220

Tyr Trp Thr Met Glu Leu Val Gly Pro Gly Asp Gln Ile Thr Phe Gln
225                 230                 235                 240

Asn Asn Tyr Val Tyr His Thr Thr Gly Arg Gly Pro Ala Leu Ser Gly
                245                 250                 255
```

pectinases.ST251

```
Thr Thr Leu Phe His Ala Val Asn Ser Val Trp Ser Ser Ile Pro Gly
            260                 265                 270

His Ala Ile Glu Gly Gly Asp Lys Gly Arg Gly Leu Phe Glu Gly Cys
        275                 280                 285

Phe Phe Glu Asp Val Val Glu Ile Ala Pro Ala Lys Pro Glu Asn Gln
    290                 295                 300

Leu Phe Ser Ala Ser Glu Ala Asn Ala Ala Ser Cys Lys Ser Ala Leu
305                 310                 315                 320

Gly Arg Ala Cys Gln Ala Asn Ser Tyr Ser Lys Ser Gly Ala Phe Gly
                325                 330                 335

Ser Ser Glu Thr Gly Phe Phe Lys Asp Phe Ala Gly Leu Thr Ile Ala
            340                 345                 350

Pro Ala Gly Ser Ala Thr Asp Ala Leu Ala Tyr Val Pro Lys Asn Cys
        355                 360                 365

Val Cys Ile Lys Gln Glu Gly Gly Phe Ser Lys Ala Arg Asn Phe Leu
    370                 375                 380

His Val Lys Gln Pro Asn Trp Leu Asp Pro Thr
385                 390                 395
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/05726

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/40 C12N15/52 C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 447 862 A (MEYHACK BERND ET AL) 5 September 1995 (1995-09-05) see SEQ ID NO:7examples 12.3,12.4,12.5,13.2,14.1,14.2,14.3	1-4, 9-11,18
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Y	MILL P J: "The pectic enzymes of Aspergillus niger. A mercury-activated exopolygalacturonase." THE BIOCHEMICAL JOURNAL. ENGLAND JUN 1966, vol. 99, no. 3, June 1966 (1966-06), pages 557-561, XP009018280 ISSN: 0264-6021 the whole document	5-8, 12-17, 19-21

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 October 2003	15. 01. 04

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Guarinos Viñals, E

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
	-& MILL P J: "The pectic enzymes of Aspergillus niger. A second exopolygalacturonase." THE BIOCHEMICAL JOURNAL. ENGLAND, vol. 99, no. 3, - June 1966 (1966-06) pages 562-565, XP009018279 the whole document	
A	OMRAN H ET AL: "SOME RESULTS OF THE ISOLATION OF DISCRETE ENZYMES EXOPOLYGALACTURONASE AND PECTINESTERASE FROM A COMMERCIAL PECTIC ENZYME PREPARATION I. ISOLATION PURIFICATION AND CHARACTERIZATION OF AN EXOPOLYGALACTURONASE EXOPG" LEBENSMITTEL-WISSENSCHAFT & TECHNOLOGIE, vol. 19, no. 6, 1986, pages 457-463, XP009018347 ISSN: 0023-6438 the whole document	
A	HARA T ET AL: "CLARIFICATION OF APPLE JUICE WITH POLYGALACTURONASE OF ASPERGILLUS-NIGER AND PECTINESTERASE OF ASPERGILLUS-ORYZAE AND THEIR PROTOPECTIN ADSORPTION AND DEGRADATION BEHAVIOR" JOURNAL OF THE JAPANESE SOCIETY FOR FOOD SCIENCE AND TECHNOLOGY, vol. 33, no. 5, 1986, pages 336-341, XP009018288 ISSN: 0029-0394 table 1	
A	US 6 197 566 B1 (HALKIER TORBEN ET AL) 6 March 2001 (2001-03-06)	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/05726

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Information on patent family members

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/05726

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

s. PCT/ISA/210 annex

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-21 partially

a polynucleotide identified by SEQ NO:1 or SEQ ID NO:33, encoding the polypeptide identified by SEQ ID NO:65 (PEC1), a vector comprising said polynucleotides, a method for manufacturing said polynucleotides or said polypeptide, a recombinant host cell comprising said polynucleotide, antibodies reactive with said polypeptide and a fusion protein comprising said polypeptide.

Inventions 2-32: claims 1-21 partially

as invention 1 but related to SEQ ID NOs: 2-32, SEQ ID NOs: 34-64 and SEQ ID NOs: 66-96, wherein
invention 2 relates to SEQ ID NOs: 2, 34 and 66 (PEC2)

invention 3 relates to SEQ ID NOs: 3, 35 and 67 (PEC3)
.....
invention 32 relates to SEQ ID NOs: 32, 64 and 96 (PEC 32).
