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DESCRIPTION

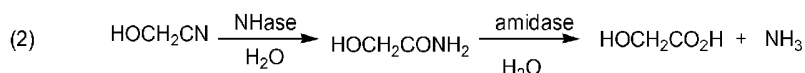
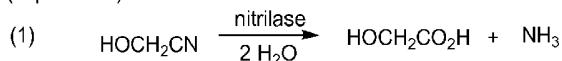
FIELD OF THE INVENTION

[0001] This invention relates to the field of organic acid synthesis and microbiology. More specifically, the present invention provides a process for preparing an enzyme catalyst having improved retention of initial nitrilase activity during hydrolysis of glycolonitrile to glycolic acid, said process comprising pretreating the enzyme catalyst with glutaraldehyde prior to immobilization and cross-linking. The glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst has improved specific activity, as stated above, when compared to non-glutaraldehyde-pretreated immobilized and cross-linked enzyme catalysts, and thereby, has improved overall catalyst activity, catalyst productivity and volumetric productivity for the conversion of glycolonitrile to glycolic acid.

BACKGROUND OF THE INVENTION

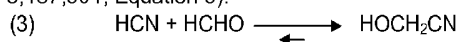
[0002] Glycolic acid (HOCH_2COOH ; CAS Registry Number is 79-14-1) is the simplest member of the α -hydroxy acid family of carboxylic acids. Its properties make it ideal for a broad spectrum of consumer and industrial applications, including use in water well rehabilitation, the leather industry, the oil and gas industry, the laundry and textile industry, as a monomer in the preparation of polyglycolic acid (PGA), and as a component in personal care products. Glycolic acid also is a principle ingredient for cleaners in a variety of industries (dairy and food processing equipment cleaners, household and institutional cleaners, industrial cleaners [for transportation equipment, masonry, printed circuit boards, stainless steel boiler and process equipment, cooling tower/heat exchangers], and metals processing [for metal pickling, copper brightening, etching, electroplating, electropolishing]). It has also been reported that polyglycolic acid is useful as a gas barrier material (*i.e.*, exhibits high oxygen barrier characteristics) for packing foods and carbonated drinks (WO 2005/106005 A1). However, traditional chemical synthesis of glycolic acid produces a significant amount of impurities that must be removed prior to use. New technology to commercially produce glycolic acid, especially one that produces glycolic acid in high purity and at low cost, would be eagerly received by industry.

[0003] Microbial enzyme catalysts can hydrolyze a nitrile (e.g., glycolonitrile) directly to the corresponding carboxylic acids (e.g., glycolic acid) using a nitrilase (EC 3.5.5.7), where there is no intermediate production of the corresponding amide (Equation 1), or by a combination of nitrile hydratase (EC 4.2.1.84) and amidase (EC 3.5.1.4) enzymes, where a nitrile hydratase (NHase) initially converts a nitrile to an amide, and then the amide is subsequently converted by the amidase to the corresponding carboxylic acid (Equation 2):



[0004] It has been demonstrated that the enzyme catalyst specific activity measured as micromoles glycolic acid produced per minute per g dry cell weight of catalyst, decreases by from 35% to 50% of initial activity after a single use in consecutive batch reactions with biocatalyst recycle. This represents a significant loss in specific activity of the enzyme catalyst in batch reactions, and, in turn, overall enzyme activity and productivity. For a commercially feasible enzymatic process for producing glycolic acid, this loss in enzyme activity needs to be addressed.

[0005] One aspect of the loss of enzyme activity may be attributable to impurities and other components present when reacting the enzyme catalyst with glycolonitrile for glycolic acid production. Methods to synthesize glycolonitrile by reacting aqueous solutions of formaldehyde and hydrogen cyanide have previously been reported (US 2,175,805; US 2,890,238; and US 5,187,301; Equation 3).



[0006] WO 2006/069114 describes a process for producing glycolic acid from formaldehyde and hydrogen cyanide. More specifically, heat-treated formaldehyde and hydrogen cyanide are reacted to produce glycolonitrile having low concentrations of impurities. The glycolonitrile is subsequently converted to an aqueous solution of ammonium glycolate using an enzyme catalyst

having nitrilase activity derived from *Acidovorax facilis* 72W (ATCC 57746).

[0007] However, these methods typically result in an aqueous glycolonitrile reaction product that requires significant purification (e.g., distillative purification) as many of the impurities and/or byproducts of the reaction (including excess reactive formaldehyde) may interfere with the enzymatic conversion of glycolonitrile to glycolic acid, including suppression of catalyst activity (i.e., decreased specific activity). In particular, it is well known that formaldehyde can create undesirable modifications in proteins by reacting with amino groups from N-terminal amino acid residues and the side chains of arginine, cysteine, histidine, and lysine residues (Metz et al., J. Biol. Chem., 279 (8): 6235-6243 (2004)). Suppression of catalyst activity decreases the overall productivity of the catalyst (i.e., total grams of glycolic acid formed per gram of catalyst), adding a significant cost to the overall process that may make enzymatic production economically non-viable when compared to chemical synthesis. As such, reaction conditions are needed that can help to protect the enzymatic activity against undesirable impurities that decrease the activity of the catalyst.

[0008] A method of producing high purity glycolonitrile has been reported by subjecting the formaldehyde to a heat treatment prior to the glycolonitrile synthesis reaction (US 2006/0160196 and US 2006/0247467; Equation 3). However, glycolonitrile can reversibly disassociate into formaldehyde and hydrogen cyanide. As such, there remains a need to protect nitrilase activity against the undesirable effects of both formaldehyde and hydrogen cyanide produced by dissociation of glycolonitrile.

[0009] US 5,508,181 also describes similar difficulties related to rapid enzyme catalyst inactivation when converting nitrile compounds to α -hydroxy acids. Specifically, U.S. 5,508,181 provides that α -hydroxy nitrile compounds partially disassociate into the corresponding aldehydes, according to the disassociation equilibrium. These aldehydes were reported to inactivate the enzyme within a short period of time by binding to the protein, thus making it difficult to obtain α -hydroxy acid or α -hydroxy amide in a high concentration with high productivity from α -hydroxy nitriles (col. 2, lines 16-29). As a solution to prevent enzyme inactivation due to accumulation of aldehydes, phosphate or hypophosphite ions were added to the reaction mixture. Similarly, US 5,326,702 describes the use of sulfite, disulfite, or dithionite ions to sequester aldehyde and prevent enzyme inactivation, but concludes that the concentration of α -hydroxy acid produced and accumulated even by using such additives is not sufficient for most commercial purposes.

[0010] Moreover, U.S. 6,037,155 teaches that low accumulation of α -hydroxy acid product is related to enzyme inactivation within a short time due to the disassociated-aldehyde accumulation. These inventors suggest that enzymatic activity is inhibited in the presence of hydrogen cyanide (Asano et al., Agricultural Biological Chemistry, Vol. 46, pages 1165-1174 (1982)) generated in the partial disassociation of the α -hydroxy nitrile in water together with the corresponding aldehyde or ketone (Mowry, David T., Chemical Reviews, Vol. 42, pages 189-283 (1948)). The inventors address the problem of aldehyde-induced enzyme inactivation by using microorganisms whose enzyme activity could be improved by adding a cyanide substance to the reaction mixture. The addition of a cyanide substance limited the disassociation of α -hydroxy nitrile to aldehyde and hydrogen cyanide. While this tactic provides a benefit to the system, it only addresses one aspect associated with enzyme inactivation in conversion of glycolonitrile to glycolic acid, in that, as stated above, glycolonitrile is known to reversibly disassociate to hydrogen cyanide and formaldehyde, and both are known to negatively effect enzyme catalyst activity.

[0011] Ben-Basset et al. Adv. Synth. Catal. 2008, 350, 1761-1769 describes a chemoenzymatic process that employs an immobilized microbial nitrilase biocatalyst for the conversion of glycolonitrile to high-purity glycolic acid.

[0012] A separate process has been developed to protect the specific activity of an enzyme catalyst having nitrilase activity when converting glycolonitrile to glycolic acid in the presence of formaldehyde where significant improvements in catalyst activity and stability were achieved by adding an amine protectant to the reaction mixture, or by immobilization of the nitrilase catalyst in or on a matrix that is comprised of an amine protectant, e.g. PEI, polyallylamine, PVOH/polyvinylamine, etc. In that system, the specific activity of the catalyst in the presence of formaldehyde is improved, but does not address, altogether, issues related to the loss in specific activity of recovered catalyst activity in consecutive batch reactions with catalyst recycle.

[0013] EP 1233057 describes a sterilized microbial cell which is sterilized by adding a surfactant in a process for sterilizing a viable microbial cell having produced therein an industrially useful enzyme without deactivating the enzyme.

[0014] US 4,355,105 describes glutaraldehyde polyethylenimine immobilization of whole microbial cells.

[0015] Panova et al. Adv. Synth. Catal. 2007, 349, 1462-1472 describes a chemoenzymatic process for the production of high-purity glycolic acid starting with the reaction of formaldehyde and hydrogen cyanide, using a glutaraldehyde/polyethylenimine cross-linked carrageenan-immobilized *E. coli* MG1655 transformant expressing the *A. facilis* 72W nitrilase mutant.

[0016] US 4,288,552 describes enzymes immobilized by reacting microbial cell material with glutaraldehyde in the presence of polyamine which is preferably a branched polyethylene imine.

[0017] U.S. 4,288,552 discloses (column 1, lines 46-49, and column 2, lines 50 - 55) that glutaraldehyde-sensitive enzymes (such as thiol-enzymes (e.g. nitrilase) and others with an SH group in or very near the active site of the enzyme molecule) are inactivated by thiol-reactive agents such as glutaraldehyde. Therefore, use of glutaraldehyde to improve the retention of initial catalyst activity during hydrolysis of glycolonitrile to glycolic acid was heretofore unpredictable. Said unpredictable benefit is demonstrated herein.

[0018] Therefore there is a need for a process that provides improved retention of initial nitrilase activity during hydrolysis of glycolonitrile to glycolic acid, thereby improving overall catalyst activity, catalyst productivity, and volumetric productivity for the conversion of glycolonitrile to glycolic acid.

SUMMARY OF THE INVENTION

[0019] The present invention resolves the need described above by providing a process for preparing an enzyme catalyst having nitrilase activity with improved retention of initial catalyst activity for hydrolysis of glycolonitrile to glycolic acid, said process comprising pretreating the enzyme catalyst with glutaraldehyde prior to immobilization and cross-linking. The glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst has improved specific activity during the conversion of glycolonitrile to glycolic acid when compared to immobilized and cross-linked enzyme catalysts prepared without glutaraldehyde-pretreatment, and thereby, has improved overall catalyst activity, catalyst productivity and volumetric productivity for the conversion of glycolonitrile to glycolic acid.

[0020] In one aspect, the present invention provides a process for producing a glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst, said process comprising:

1. (a) producing an enzyme catalyst having nitrilase activity by fermentation;
2. (b) pretreating said enzyme catalyst with glutaraldehyde;
3. (c) optionally inactivating unreacted glutaraldehyde with bisulfite following glutaraldehyde pretreatment;
4. (d) recovering the enzyme catalyst from (b) or (c) and immobilizing said enzyme catalyst in carrageenan;
5. (e) cross-linking the resulting carrageenan-immobilized enzyme catalyst of (d) with glutaraldehyde and polyethylenimine; and
6. (f) producing a glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst, wherein said glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst has improved retention of initial specific activity during conversion of glycolonitrile to glycolic acid as compared to non-glutaraldehyde-pretreated immobilized and cross-linked enzyme catalysts under the same reaction conditions;

wherein said enzyme catalyst comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, and 57.

[0021] Also described is a process for producing an enzyme catalyst having nitrilase activity with improved retention of the initial specific activity during the conversion of glycolonitrile to glycolic acid, said process comprising:

1. (a) producing an enzyme catalyst having nitrilase activity by fermentation;
2. (b) pretreating said enzyme catalyst with glutaraldehyde;
3. (c) optionally inactivating unreacted glutaraldehyde with bisulfite following glutaraldehyde pretreatment;
4. (d) recovering the enzyme catalyst from (b) or (c), and immobilizing said enzyme catalyst in carrageenan; and
5. (e) cross-linking the resulting carrageenan-immobilized enzyme catalyst of (d) with glutaraldehyde and polyethylenimine, whereby a glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst is produced and wherein said glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst has improved retention of initial specific activity during conversion of glycolonitrile to glycolic acid as compared to non-glutaraldehyde-pretreated immobilized and cross-linked enzyme catalysts under the same reaction conditions.

[0022] A further aspect of the invention comprises contacting the glutaraldehyde-pretreated immobilized and cross-linked

enzyme catalyst of (f) above with glycolonitrile in an aqueous solution, under suitable reaction conditions whereby glycolic acid is produced. And further, recovering said glycolic acid.

[0023] Another aspect of the invention is directed to the glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst that is produced by the process of steps a) through f) above. Further, said glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst, having nitrilase activity, retains a significantly-greater percentage of its initial specific activity (μ moles of glycolonitrile hydrolyzed per minute per gram of catalyst) when used for the conversion of glycolonitrile to glycolic acid (as the ammonium salt) as compared to a comparable enzyme catalyst without glutaraldehyde pretreatment.

[0024] Another aspect of the invention is directed to a glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst having nitrilase activity that is produced by the process of steps a) through f) above, wherein said enzyme catalyst retains at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, or at least about 97% of its initial specific activity after at least four consecutive batch recycles.

[0025] Another aspect of the invention is directed to a glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst having nitrilase activity that is produced by the process of steps a) through f) above, wherein said enzyme catalyst retains at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, or at least about 97% of its initial specific activity after the production of at least 40 g of glycolic acid per gram dry cell weight of glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst in a continuous reaction for the production of glycolic acid from glycolonitrile, for example, when running the reaction in a continuously stirred tank reactor (CSTR), or in a fixed-bed plug flow reactor, or in a fluidized-bed or semi-fluidized bed reactor.

[0026] A further aspect of the invention is directed to a process for hydrolyzing glycolonitrile to glycolic acid comprising:

1. (a) producing an enzyme catalyst having nitrilase activity by fermentation;
2. (b) pretreating said enzyme catalyst with glutaraldehyde;
3. (c) optionally inactivating unreacted glutaraldehyde with bisulfite following glutaraldehyde pretreatment;
4. (d) recovering the enzyme catalyst from (b) or (c) and immobilizing said enzyme catalyst in carrageenan;
5. (e) cross-linking the resulting carrageenan-immobilized enzyme catalyst of (d) with glutaraldehyde and polyethylenimine, whereby a cross-linked immobilized enzyme catalyst is produced, wherein said glutaraldehyde-pretreated immobilized and cross-linked enzyme catalyst has improved retention of initial specific activity during conversion of glycolonitrile to glycolic acid as compared to non-glutaraldehyde-pretreated immobilized and cross-linked enzyme catalysts under the same reaction conditions; and
6. (f) contacting the cross-linked immobilized enzyme catalyst of (e) with glycolonitrile in an aqueous solution under suitable reaction conditions whereby glycolic acid is produced;

wherein said enzyme catalyst comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, and 57. Step (f) may occur in at least two consecutive batch recycles.

[0027] Further, the process may include recovering the glycolic acid produced by said improved process.

[0028] Also described is an improved process for hydrolyzing glycolonitrile to glycolic acid comprising improving the retention of the initial specific activity of an immobilized enzyme catalyst having nitrilase activity during conversion of glycolonitrile to glycolic acid, said process comprising:

1. (a) producing an enzyme catalyst having nitrilase activity by fermentation;
2. (b) pretreating said enzyme catalyst with glutaraldehyde;
3. (c) optionally inactivating unreacted glutaraldehyde with bisulfite following glutaraldehyde pretreatment;
4. (d) recovering the enzyme catalyst from (b) or (c) and immobilizing said enzyme catalyst in carrageenan;
5. (e) cross-linking the resulting carrageenan-immobilized enzyme catalyst of (d) with glutaraldehyde and polyethylenimine, whereby a cross-linked immobilized enzyme catalyst is produced; and
6. (f) contacting the cross-linked immobilized enzyme catalyst of (e) with glycolonitrile in an aqueous solution under suitable reaction conditions whereby glycolic acid is produced,

wherein step (f) occurs in at least two consecutive batch recycles.

[0029] Further, the process may include recovering the glycolic acid produced by said improved process.

BRIEF DESCRIPTION OF THE FIGURE, SEQUENCE LISTING, AND**THE BIOLOGICAL DEPOSITS**

[0030] The invention can be more fully understood from the Figure, sequence listing, biological deposits, and detailed description, that together form this application.

FIGURE

[0031] Figure 1, panels A-G, is a CLUSTALW alignment (version 1.83 using default parameters) of various nitrilase sequences. The conserved catalyst signature sequence surrounding the catalyst cysteine residue is highlighted in gray shading. The amino acids representing the catalytic triad (Glu₄₈, Lys₁₃₀, and Cys₁₆₄; numbering based on the amino acid sequence SEQ ID NO: 4) are underlined.

SEQUENCE LISTING

[0032] The following sequence descriptions and sequences listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825. The Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IYUB standards described in Nucleic Acids Research 13:3021-3030 (1985) and in the Biochemical Journal 219 (No. 2):345-373 (1984). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

SEQ ID NO: 1 is the amino acid sequence of the catalytic signature motif encompassing the essential cysteine residue of nitrilase enzymes (Formula 1).

SEQ ID NO: 2 is the amino acid sequence of a preferred catalyst signature motif encompassing the essential cysteine residue of nitrilase enzymes (Formula 2).

SEQ ID NO: 3 is the nucleotide sequence of the *Acidovorax facilis* 72W nitrilase coding sequence comprising a change in the start codon from TTG to ATG to facilitate recombinant expression in *E. coli*.

SEQ ID NO: 4 is the deduced amino acid sequence of the *Acidovorax facilis* 72W nitrilase (ATCC 55746).

SEQ ID NO: 5 is the amino acid sequence of the *Alcaligenes faecalis* JM3 nitrilase (GENBANK® BAA02684.1).

SEQ ID NO: 6 is the amino acid sequence of the *Rhodococcus rhodochrous* J1 nitrilase (GENBANK® Q03217).

SEQ ID NO: 7 is the amino acid sequence of the *Rhodococcus rhodochrous* K22 nitrilase (GENBANK® Q02068).

SEQ ID NO: 8 is the amino acid sequence of the *Nocardia* sp. C-14-1 nitrilase (GENBANK® AAX18182.1).

SEQ ID NO: 9 is the amino acid sequence of the *Bordetella bronchiseptica* RB50 nitrilase (GENBANK® NP_887662.1).

SEQ ID NO: 10 is the amino acid sequence of the *Arabidopsis thaliana* nitrilase (GENBANK® AAB60275.1 and AAA19627.1).

SEQ ID NO: 11 is the amino acid sequence of the *Synechococcus elongatus* PCC 7942 nitrilase (GENBANK® YP_399857.1).

SEQ ID NO: 12 is the amino acid sequence of the *Synechococcus elongatus* PCC 6301 nitrilase (GENBANK® YP_171411.1).

SEQ ID NO: 13 is the amino acid sequence of the *Synechocystis* sp. PCC 6803 nitrilase (GENBANK® NP_442646.1).

SEQ ID NO: 14 is the amino acid sequence of the *Pseudomonas entomophila* L48 nitrilase (GENBANK® YP_609048.1).

SEQ ID NO: 15 is the amino acid sequence of the *Zymomonas mobilis* nitrilase (GENBANK® YP_162942.1).

SEQ ID NO: 16 is the amino acid sequence of the *Bacillus* sp. OxB-1 nitrilase (GENBANK® BAA90460.1).

SEQ ID NO: 17 is the amino acid sequence of the *Comamonas testosteroni* nitrilase (GENBANK® AAA82085.1).

SEQ ID NO: 18 is the amino acid sequence of the *Synechococcus* sp. CC9605 nitrilase (GENBANK® YP_381420.1).

SEQ ID NO: 19 is the amino acid sequence of the *Pseudomonas fluorescens* Pf-5 nitrilase (GENBANK® YP_260015.1).

SEQ ID NO: 20 is the amino acid sequence of the *Nocardia farcinica* IFM 10152 nitrilase (GENBANK® YP_119480.1).

SEQ ID NO: 21 is the amino acid sequence of the *Alcaligenes faecalis* 1650 nitrilase (GENBANK® AAY06506.1).

SEQ ID NO: 22 is the amino acid sequence of the *Pseudomonas syringae* pv. *syringae* B728a nitrilase (GENBANK® AAY35081.1).

SEQ ID NO: 23 is the amino acid sequence of the *Bradyrhizobium* sp. BTail nitrilase (GENBANK® ZP_00859948.1).

SEQ ID NO: 24 is the amino acid sequence of the *Rhodococcus rhodochrous* NCIMB 11216 nitrilase (GENBANK® CAC88237).

SEQ ID NO: 25 is the amino acid sequence of *Rhodococcus rhodochrous* ATCC™ 39484

SEQ ID NO: 26 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201Q; Leu → Gln).

SEQ ID NO: 27 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 26) comprising a single amino acid substitution at residue position 201 (Leu201 → Gln) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 28 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201A; Leu → Ala).

SEQ ID NO: 29 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 28) comprising a single amino acid substitution at residue position 201 (Leu201 → Ala) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 30 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201C; Leu → Cys).

SEQ ID NO: 31 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 30) comprising a single amino acid substitution at residue position 201 (Leu201 → Cys) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 32 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201T; Leu → Thr).

SEQ ID NO: 33 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 32) comprising a single amino acid substitution at residue position 201 (Leu201 → Thr) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 34 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201 G; Leu → Gly).

SEQ ID NO: 35 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 34) comprising a single amino acid substitution at residue position 201 (Leu201 → Gly) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 36 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201 H; Leu → His).

SEQ ID NO: 37 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 36) comprising a single amino acid substitution at residue position 201 (Leu201 → His) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 38 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201 K; Leu → Lys).

SEQ ID NO: 39 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 38) comprising a single amino acid substitution at residue position 201 (Leu201 → Lys) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 40 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201 N; Leu → Asn).

SEQ ID NO: 41 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 40) comprising a single amino acid substitution at residue position 201 (Leu201 → Asn) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 42 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 201 (L201S; Leu → Ser).

SEQ ID NO: 43 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 42) comprising a single amino acid substitution at residue position 201 (Leu201 → Ser) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 44 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 168 (F168K; Phe → Lys).

SEQ ID NO: 45 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 44) comprising a single amino acid substitution at residue position 168 (Phe168 → Lys) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 46 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 168 (F168M; Phe → Met).

SEQ ID NO: 47 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 46) comprising a single amino acid substitution at residue position 168 (Phe168 → Met) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 48 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 168 (F168T; Phe → Thr).

SEQ ID NO: 49 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 48) comprising a single amino acid substitution at residue position 168 (Phe168 → Thr) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 50 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 168 (F168V; Phe → Val).

SEQ ID NO: 51 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 50) comprising a single amino acid substitution at residue position 168 (Phe168 → Val) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 52 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 168 (T210A; Thr → Ala).

SEQ ID NO: 53 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 52) comprising a single amino acid substitution at residue position 210 (Thr210 → Ala) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 54 is the nucleotide sequence of an *A. facilis* 72W nitrilase mutant comprising a codon change which resulted in a single amino acid substitution at residue position 168 (T210C; Thr → Cys).

SEQ ID NO: 55 is the deduced amino acid sequence of the mutant nitrilase (SEQ ID NO: 54) comprising a single amino acid substitution at residue position 210 (Thr210 → Cys) of the *A. facilis* 72W nitrilase.

SEQ ID NO: 56 is the nucleotide sequence of the *A. facilis* 72W nitrilase expressed in *E. coli* strain SS1001 (ATCC PTA-1177).

SEQ ID NO: 57 is the deduced amino acid sequence of the mutant *A. facilis* 72W nitrilase expressed in *E. coli* SS1001 (ATCC PTA-1177).

BIOLOGICAL DEPOSITS

[0033] The following biological deposits have been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure:

Depositor Identification Reference	Int'l. Depository Designation	Date of Deposit
<i>Acidovorax facilis</i> 72W	ATCC 55746	8 March 1996
<i>E. coli</i> SS1001	ATCC PTA-1177	11 January 2000

[0034] As used herein, "ATCC" refers to the American Type Culture Collection International Depository Authority located at ATCC, 10801 University Blvd., Manassas, VA 20110-2209, USA. The "International Depository Designation" is the accession

number to the culture on deposit with ATCC.

[0035] The listed deposits will be maintained in the indicated international depository for at least thirty (30) years and will be made available to the public upon the grant of a patent disclosing it. The availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by government action.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The present invention provides a process for preparing an immobilized and cross-linked enzyme catalyst having nitrilase activity for hydrolysis of glycolonitrile to glycolic acid with improved retention of initial enzyme catalyst activity during conversion of glycolonitrile to glycolic acid, said process comprising pretreating the enzyme catalyst with glutaraldehyde prior to immobilization. The glutaraldehyde-pretreated immobilized enzyme catalyst has improved retention of initial specific activity, as stated above, when compared to the retention of initial specific activity of non-glutaraldehyde-pretreated immobilized and cross-linked enzyme catalysts during the conversion of glycolonitrile to glycolic acid, and thereby, has improved overall catalyst activity, catalyst productivity and volumetric productivity.

Definitions:

[0037] In this disclosure, a number of terms and abbreviations are used. The following definitions apply unless specifically stated otherwise.

[0038] As used herein, the term "comprising" means the presence of the stated features, integers, steps, or components as referred to in the claims, but that it does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

[0039] As used herein, the term "about" modifying the quantity of an ingredient or reactant of the invention employed refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or use solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the compositions or carry out the methods; and the like. The term "about" also encompasses amounts that differ due to different equilibrium conditions for a composition resulting from a particular initial mixture. Whether or not modified by the term "about", the claims include equivalents to the quantities. In one embodiment, the term "about" means within 10% of the reported numerical value, preferably within 5% of the reported numerical value.

[0040] As used herein, the term "glycolonitrile" is abbreviated as "GLN" and is synonymous with hydroxyacetonitrile, 2-hydroxyacetonitrile, hydroxymethylnitrile, and all other synonyms of CAS Registry Number 107-16-4.

[0041] As used herein, the term "glycolic acid" is abbreviated as "GLA" and is synonymous with hydroxyacetic acid, hydroxyethanoic acid, and all other synonyms of CAS Registry Number 79-14-1. The glycolic acid produced by the present processes may in the form of the protonated carboxylic acid and/or the corresponding ammonium salt.

[0042] As used herein, the term "ammonium glycolate" is abbreviated "NH₄GLA".

[0043] As used herein, the term "glycolamide" is the amide derived from the reaction of ammonia with glycolic acid and refers to all other synonyms of compounds having CAS Registry Number 598-42-5.

[0044] As used herein, the term "glycolide" refers to the compound of CAS Registry Number 502-97-6.

[0045] As used herein, the term "formaldehyde" is abbreviated as "FA" and is synonymous with formic aldehyde, methyl aldehyde, oxomethane, and all other synonyms of CAS Registry Number 50-00-0. Commercially available formaldehyde is typically comprised of a mixture of monomeric formaldehyde ("free formaldehyde") and various oligomers of formaldehyde along with some methanol (typically about 1 wt% to about 15 wt %).

[0046] As used herein, the term "hydrogen cyanide" is synonymous with prussic acid, hydrocyanic acid, and all other synonyms of CAS Registry Number 200-821-6.

[0047] As used herein, the term "glutaraldehyde" is abbreviated "GA" and is synonymous with pentanedial, 1,5-pentanedial, 1,5-pentanedione, diglutaric aldehyde, glutaral, glutardialdehyde, glutaric acid dialdehyde, glutaric dialdehyde, and all other synonyms of CAS Registry Number 111-30-8.

[0048] As used herein, the term "bisulfite" or "sodium bisulfite" is synonymous with sulfurous acid sodium salt, sulfurous acid monosodium salt, hydrogen sodium sulfite, hydrogen sulfite sodium, monosodium sulfite, sodium acid sulfite, sodium bisulfite, sodium bisulphate, sodium hydrogen sulfite, sodium sulfite (NaHSO₃), and all other synonyms of CAS Registry Number 7631-90-5.

[0049] As used herein, the term "recovering" means isolating, purifying, or transferring the product formed by the present process. Methods to isolate and purify the product(s) from the reaction mixture are well known in the art may include, but are not limited to selective precipitation, crystallization, filtration, reactive solvent extraction, ion exchange, electrodialysis, polymerization, distillation, thermal decomposition, alcoholysis, column chromatography, and combinations thereof. In one embodiment, the term "recovering" may also include transferring the product mixture (typically after filtering out the enzyme catalyst) to another reaction to create one or more additional products. In a preferred embodiment, ion exchange is used to recover the glycolic acid.

[0050] As used herein, the terms "enzyme catalyst", "nitrilase catalyst" or "microbial cell catalyst" refers to a catalyst that is characterized by a nitrilase activity (*i.e.*, comprises at least one polypeptide having nitrilase activity) for converting glycolonitrile to glycolic acid and ammonia. A nitrilase enzyme directly converts a nitrile (preferably, an aliphatic nitrile) to the corresponding carboxylic acid, without forming the corresponding amide as intermediate (see Equation 1). Nitrilases share several conserved signature domains known in the art including a signature domain herein referred to as the "catalytic signature sequence" or "signature sequence". This region comprises an essential cysteine residue (*e.g.*, Cys₁₆₄ of SEQ ID NO: 4). As such, polypeptides having nitrilase activity can be identified by the existence of the catalytic domain signature sequence (SEQ ID NO: 1). In a preferred embodiment, the signature sequence is SEQ ID NO: 2. The enzyme catalyst may be in the form of whole microbial cells or permeabilized microbial cells. As used herein, "recycled enzyme catalyst" refers to an enzyme catalyst that is reused as an enzyme catalyst in batch or continuous reactions. Depending on the step in the process of producing or using the enzyme catalyst as described herein, the enzyme catalyst may be glutaraldehyde pretreated, immobilized, and cross-linked.

[0051] As used herein, the terms "*Acidovorax facilis*" and "*A. facilis*" are used interchangeably and refer to *Acidovorax facilis* 72W deposited to the American Type Culture Collection (an international depository authority) having accession number 55746 ("ATCC 55746"). The mutant nitrilases derived from *A. facilis* 72W characterized by improved nitrilase activity when converting glycolonitrile to glycolic acid have been previously reported (see co-owned U.S. patent 7,198,927). Examples of these *A. facilis* 72W-derived mutant nitrilases are provided by SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55.

[0052] As used herein, the terms "*Escherichia coli*" and "*E. coli*" are used interchangeably. Several strains of *E. coli* suitable for recombinant expression are described herein including, but not limited to *E. coli* MG1655 having international depository number ATCC 47076, *E. coli* FM5 having international depository number ATCC 53911, *E. coli* W3110 having international depository number ATCC 27325, *E. coli* MC4100 having international depository number ATCC 35695, and *E. coli* W1485 having international depository number ATCC 12435. In one embodiment, suitable *Escherichia coli* strains include *E. coli* FM5 (ATCC 53911) and *E. coli* MG1655 (ATCC 47076).

[0053] As used herein, the terms "*E. coli* SS1001" or "SS1001" refer to a transformed *E. coli* strain expressing the *Acidovorax facilis* 72W nitrilase having ATCC Accession No. PTA-1177 (see U.S. patent 6,870,038). The recombinantly expressed *E. coli* SS1001 nitrilase (SEQ ID NO: 57) contains 2 minor sequence changes in comparison to the wild-type 72W nitrilase sequence (SEQ ID NO: 4). The start codon was changed from GTG to ATG to facilitate recombinant expression and an artifact was introduced during cloning that resulted in a single amino acid change near the C-terminal (Pro367 [CCA] → Ser [TCA]).

[0054] As used herein, the terms "suitable aqueous glycolonitrile reaction mixture" and "suitable aqueous reaction mixture" refer to the materials (including at least one amine protectant) and water in which the glycolonitrile and enzyme catalyst come into contact. The components of the suitable aqueous reaction mixture are provided herein and those skilled in the art appreciate the range of component variations suitable for this process.

[0055] As used herein, the terms "aqueous ammonium glycolate solution", "aqueous solution comprising ammonium glycolate", and "aqueous solution of ammonium glycolate" will be used to describe an aqueous solution comprising ammonium glycolate produced by the enzymatic hydrolysis of glycolonitrile under typical enzymatic reaction conditions (*i.e.*, a pH range of about 6 to about 8). The aqueous solution of ammonium glycolate comprises ammonium glycolate at a concentration of at least about 0.1

weight percent (wt %) to about 99 wt % ammonium glycolate. In another embodiment, the aqueous solution of ammonium glycolate is comprised of at least about 10 wt % to about 75 wt % ammonium glycolate. In a further embodiment, the aqueous solution of ammonium glycolate is comprised of at least about 20 wt % to about 50 wt % ammonium glycolate. The pH of the aqueous solution of ammonium glycolate can be about 2 to about 12, preferably 5 to about 10, more preferably 6 to about 8. The pH may be adjusted as needed prior to initiating process steps related to recovering glycolic acid (in the form of the acid or salt) from the aqueous ammonium glycolate solution.

[0056] As used herein, the terms "catalyst productivity" and "enzyme catalyst productivity" refer to the total amount of product produced per gram of enzyme catalyst dry cell weight. In the present invention, the enzyme catalyst comprises a nitrilase enzyme (EC 3.5.5.7) and the product formed is glycolic acid and/or ammonium glycolate (depending upon the pH of the reaction). In general, the processes produced pursuant to producing glycolic acid are conducted under essentially pH neutral conditions so that the glycolic acid produced is predominantly in the form of the corresponding salt of glycolic acid (i.e. ammonium glycolate). Generally, in batch reactions with catalyst recycle, the catalyst activity decreases with each recycle reaction (enzyme inactivation).

[0057] As used herein, the term "volumetric productivity" refers to the volumetric production of glycolic acid in the reaction, expressed as grams of glycolic acid produced per volume of reaction mixture per unit of time. Typically, volumetric productivity is expressed as grams glycolic acid/L/h.

[0058] The term "nitrilase activity" or "specific activity" refers to the enzyme activity per unit mass (for example, milligram) of protein, dry cell weight, or bead weight (immobilized catalyst) when converting glycolonitrile to glycolic acid (or the corresponding ammonium glycolate). Comparisons in nitrilase activity were measured proportional to the dry cell weight or bead weight.

[0059] As used herein, the term "one unit of enzyme activity" or "one unit of nitrilase activity" or "U" is defined as the amount of enzyme activity required for the production of 1 μ mol of glycolic acid product per minute (GLA U/g dry cell weight or bead weight) at a specified temperature (e.g. 25 °C).

[0060] As used herein, the terms "relative nitrilase activity", "improved nitrilase activity", and "relative improvement in nitrilase activity" refers to the nitrilase activity expressed as a multiple (or fraction) of a reference (control) nitrilase activity. The nitrilases described herein exhibit a significant improvement in nitrilase activity relative to the nitrilase activity observed with native *Acidovorax facilis* 72W nitrilase. A "significant improvement" in relative nitrilase activity is an improvement of at least 1.5-fold higher nitrilase activity in comparison to the nitrilase activity of a control under identical reaction conditions. In another embodiment, the improvement is at least 2-fold higher nitrilase activity in comparison to the nitrilase activity of the control under identical reaction conditions. In a further embodiment, the improvement is at least 4-fold higher nitrilase activity in comparison to the nitrilase activity of the control under identical reaction conditions.

[0061] As used herein, the term "initial reaction rate" is a measurement of the rate of conversion of glycolonitrile to glycolic acid under the stated reaction conditions, where the measurement of reaction rate begins upon the initial addition of glycolonitrile to the reaction mixture, and where the reaction rate is measured over a period of time where the concentration of glycolonitrile remains above ca. 50 millimolar (mM) during the course of the reaction. The reaction rate is measured as the change in concentration of glycolic acid produced per unit time (e.g., mole glycolic acid/L/min or mM glycolic acid/hour).

[0062] As used herein, the term "improved retention of initial specific activity" refers to a comparison of a glutaraldehyde pretreated, immobilized and cross-linked enzyme catalyst with a non-glutaraldehyde pretreated, immobilized and cross-linked enzyme catalyst, both having nitrilase activity, during conversion of glycolonitrile to glycolic acid under the stated reaction conditions, measured as micromoles of glycolic acid produced per minute per g dry cell weight of enzyme catalyst, or micromoles glycolic acid produced per minute per g immobilized and crosslinked enzyme catalyst, wherein the specific activity as measured in a first or "initial" reaction is retained to a greater extent for the glutaraldehyde pretreated immobilized and cross-linked enzyme catalyst than for the non-glutaraldehyde pretreated, immobilized and cross-linked enzyme catalyst, for one or more subsequent reactions. The most notable improvement, as described herein, is for the amount of activity retained for the reaction immediately following an initial batch reaction, measured in one or more subsequent batch reactions with catalyst recycle. A second notable improvement, as described herein, is for the amount of activity retained during the course of running the reaction in a continuously stirred tank reactor (CSTR), or in a fixed-bed plug flow reactor, or in a fluidized-bed or semi-fluidized bed reactor.

[0063] As used herein, the terms "recombinant organism", "transformed host", "transformant", "transgenic organism", and "transformed microbial host" refer to a host organism having been transformed with heterologous or foreign DNA. The recombinant organisms of the present invention express foreign coding sequences or genes that encode active nitrilase enzyme. "Transformation" refers to the transfer of a DNA fragment into the host organism. The transferred DNA fragment can be chromosomally or extrachromosomally incorporated (i.e., via a vector) into the host organism. As used herein, the term

"transformation cassette" refers to a specific fragment of DNA containing a set of genetic elements conveniently arranged for insertion into a host cell, usually as part of a plasmid. As used herein, the term "expression cassette" refers to a specific fragment of DNA containing a set of genetic elements conveniently arranged for insertion into a host cell, usually as part of a plasmid that also allows for enhanced gene expression in the host.

[0064] As used herein, the terms "nucleic acid fragment" and "nucleic acid molecule" refer to DNA molecule that may encode an entire gene, coding sequence, and/or regulatory sequences preceding (5', upstream) or following (3', downstream) the coding sequence. In one aspect, the present nucleic acid molecules encode for polypeptides having nitrilase activity.

[0065] As used herein, the term "gene" refers to a nucleic acid molecule that expresses a specific protein. As used herein, it may or may not including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

[0066] As used herein, the term "coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. As used herein, "suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, and stem-loop structures.

[0067] "Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. Promoters that cause a gene to be expressed in most cell types at most times or under most environmental conditions are commonly referred to as "constitutive promoters". Promoters that cause a gene to be expressed only in the presence of a particular compound or environmental condition are commonly referred to as "inducible promoters". Since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

[0068] As used herein, the term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid molecule so that the function of one sequence is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (*i.e.*, that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

[0069] As used herein, the term "3' non-coding sequences" refers to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences (normally limited to eukaryotes) and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal (normally limited to eukaryotes) is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

[0070] The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in using nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its codon usage reflects the preferred codon bias of the host cell. A survey of genes derived from the host cell where sequence information is available can determine its codon bias. Codon-optimization is well known in the art and has been described for various systems including, but not limited to yeast (Outchkourov et al., *Protein Expr Purif*, 24(1):18-24 (2002)) and *E. coli* (Feng et al., *Biochemistry*, 39(50):15399-15409 (2000)).

Enzyme Catalysts Having Nitrilase Activity

[0071] All nitrilases (EC 3.5.5.7) share a conserved catalytic triad (Glu, Lys, and Cys) (Chauhan et al., *Appl. Microbiol.*

Biotechnol. 61:118-122 (2003); Pace, H. and Brenner, C., Genome Biol. [online computer file] 2(1):reviews0001.1-0001.9 (2001)). All known nitrilases have a nucleophilic cysteine in the enzyme active site (Cowan et al., Extremophiles, 2:207-216 (1998); Pace, H. and Brenner, C., *supra*; and Chauhan *et al.*, *supra*) and all are susceptible to inactivation by thiol reagents (1.0 mM concentrations of copper chloride, silver nitrate, mercuric acetate, or ferric chloride each produced major decreases in *A. facilis* 72W nitrilase enzyme activity). Cysteine residues are also capable of being irreversibly oxidized to sulfinic acids, resulting in a loss of enzyme activity. Despite the sensitivity of nitrilase enzymes to various inactivating mechanisms, immobilized *A. facilis* 72W cells are robust, capable of retaining much of their nitrilase activity after numerous recycle reactions (US 6,870,038; U.S. 7,148,051; U.S. 7,198,927; and Chauhan et al., *supra*). Nitrilase catalysts derived from the *A. facilis* 72W nitrilase also been shown to catalyze the conversion of α -hydroxynitriles (*i.e.*, glycolonitrile) to α -hydroxycarboxylic acids (*i.e.*, glycolic acid) (see US 6,383,786; US 6,416,980; and U.S. 7,198,927).

[0072] Sequence comparisons of the *A. facilis* 72W nitrilase to other bacterial nitrilases have been reported (US 6,870,038; Chauhan et al., *supra*). The 72W nitrilase has several conserved signature domains including a 16-amino acid region near the amino terminus (amino acid residues 40-55 of SEQ ID NO: 4) and a 12 amino acid catalytic region (amino acid residues 160-171 of SEQ ID NO: 4) containing the essential cysteine residue. This essential cysteine residue (Cys₁₆₄ of SEQ ID NO: 4), along with conserved glutamic acid (Glu₄₈ of SEQ ID NO:4) and lysine residues (Lys₁₃₀ of SEQ ID NO:4), form the catalytic triad motif found in all nitrilases (Pace, H., and Brenner, C., *supra*).

[0073] The regions surrounding each of the catalytic triad residues are highly conserved, especially the region surrounding the catalytic cysteine residue. The essential catalytic cysteine residue is located with a highly conserved region referred to as the "catalytic signature motif" or "signature motif". As such, the process described herein is useful for protecting the enzymatic activity of any nitrilase comprising the catalytic signature motif defined by Formula 1 (bold indicates strictly conserved amino acid residues, italicized residues are those that exhibit minimal variability [*i.e.* minimal variation of 3 or fewer amino acid residues], the catalytic cysteine residue is underlined):

Formula 1 (SEQ ID NO: 1). Gly-Xaa₁-Xaa₂-Xaa₃-**Cys**-Trp-Glu-Xaa₄-Xaa₅-Xaa₆-Xaa₇-Xaa₈

wherein

Xaa₁ = Ala or Gly;

Xaa₂ = Leu, Val, or Ala;

Xaa₃ = Ala, Asn, Ile, Cys, Val, or Gln;

Xaa₄ = His or Asn;

Xaa₅ = Leu, Tyr, Phe, Ala, Met, Lys, Val, Thr, or Arg;

Xaa₆ = Asn, Gln, Met, Leu, or Ser;

Xaa₇ = Pro or Thr; and

Xaa₈ = Leu or Val.

[0074] In a preferred embodiment, the nitrilase signature motif of Formula 1 is Xaa₁ = Ala or Gly; Xaa₂ = Leu; Xaa₃ = Ala, Asn, Ile, Cys, Val, or Gln; Xaa₄ = His; Xaa₅ = Leu, Tyr, Phe, Ala, Met, Lys, Val, Thr or Arg; Xaa₆ = Ser, Gln, Asn, or Met; Xaa₇ = Pro; and Xaa₈ = Leu; resulting in the catalytic signature motif represented by the following:

Gly-Xaa₁-Leu-Xaa₃-Cys-Trp-Glu-His-Xaa₅-Xaa₆-Pro-Leu (SEQ ID NO: 2)

[0075] Examples of nitrilases, including the sequences and position of the corresponding catalytic signature motif sequence, are provided in Table 1.

Table 1. Conserved Catalytic Cysteine Region - Catalytic Signature Motifs

Nitrilase Source	GenBank® Accession Number	Amino Acid SEQ ID NO.	Sequence of Signature Motif (amino acid residue positions)
<i>Acidovorax Facilis</i> 72W	ABD98457.1	4	GGLNCWEHFQPL (160-171)
<i>Alcaligenes faecalis</i> JM3	BAA02684.1	5	GALCCWEHLSPL (159-170)
<i>Rhodococcus rhodochrous</i> J1	Q03217	6	GALNCWEHFQTL (161-172)
<i>Rhodococcus rhodochrous</i> K22	Q02068	7	GGLNCWEHFQPL (166-177)
<i>Nocardia</i> sp. C-14-1	AAX18182.1	8	GGLNCWEHFQPL (154-165)
<i>Bordetella bronchiseptica</i> RB50	NP_887662.1	9	GAVVCWENYMPL (161-172)
<i>Arabidopsis thaliana</i>	AAB60275.1 AAA19627.1	10	GAAICWENRMPL (175-186)
<i>Synechococcus elongatus</i> PCC 7942	YP_399857.1	11	GALACWEHYNPL (157-168)
<i>Synechococcus elongatus</i> PCC 6301	YP_171411.1	12	GALACWEHYNPL (157-168)
<i>Synechocystis</i> sp. PCC 6803	NP_442646.1	13	GALACWEHYNPL (165-176)
<i>Pseudomonas entomophila</i> L48	YP_609048.1	14	GAAVCWENYMPL (161-172)
<i>Zymomonas mobilis</i>	YP_162942.1	15	GAAICWENYMPV (161-172)
<i>Bacillus</i> sp. OxB-1	BAA90460.1	16	GGLQCWEHFLPL (158-169)
<i>Comamonas testosteroni</i>	AAA82085.1	17	GGLQCWEHALPL (159-170)
<i>Synechococcus</i> sp. CC9605	YP_381420.1	18	GALACWEHYNPL (156-167)
<i>Pseudomonas fluorescens</i> Pf-5	YP_260015.1	19	GAVICWENMMPL (161-172)
<i>Nocardia farcinica</i> IFM 10152	YP_119480.1	20	GALCCWEHLQPL (159-170)
<i>Alcaligenes faecalis</i> 1650	AA06506.1	21	GALCCWEHLSPL (159-170)
<i>Pseudomonas syringae</i> pv. <i>syringae</i> B728a	AA03508.1	22	GALCCWEHLQPL (157-168)
<i>Bradyrhizobium</i> sp. BTail	ZP_00859948.1	23	GALCCWEHLQPL (163-174)
<i>Rhodococcus rhodochrous</i> NCIMB 11216	CAC88237	24	GALNCWEHFQTL (161-172)
<i>Rhodococcus rhodochrous</i> ATCC 39484™	N/A	25	GALNCWEHFQTL (161-172)

[0076] Also disclosed herein, the nitrilase catalyst comprises a polypeptide having nitrilase activity isolated from a genera selected from the group consisting of *Acidovorax*, *Rhodococcus*, *Nocardia*, *Bacillus*, and *Alcaligenes*. In another example, the nitrilase catalyst comprises a polypeptide having nitrilase activity isolated from a genera selected from the group consisting of *Acidovorax* and *Rhodococcus*.

[0077] In another embodiment, the polypeptide having nitrilase activity is derived from *Acidovorax facilis* 72W (ATCC 55746) or a polypeptide (having nitrilase activity) that is substantially similar to the *Acidovorax facilis* 72W nitrilase (SEQ ID NO: 4) or the *A. facilis* 72W derived enzyme represented by SEQ ID NO: 51.

[0078] In one embodiment, the nitrilase catalyst is a microbial host cell transformed to express at least one polypeptide having nitrilase activity. In one embodiment the transformed host cell is selected from the group consisting of *Comamonas* sp., *Corynebacterium* sp., *Brevibacterium* sp., *Rhodococcus* sp., *Azotobacter* sp., *Citrobacter* sp., *Enterobacter* sp., *Clostridium* sp., *Klebsiella* sp., *Salmonella* sp., *Lactobacillus* sp., *Aspergillus* sp., *Saccharomyces* sp., *Yarrowia* sp., *Zygosaccharomyces* sp., *Pichia* sp., *Kluyveromyces* sp., *Candida* sp., *Hansenula* sp., *Dunaliella* sp., *Debaryomyces* sp., *Mucor* sp., *Torulopsis* sp., *Methylobacteria* sp., *Bacillus* sp., *Escherichia* sp., *Pseudomonas* sp., *Rhizobium* sp., and *Streptomyces* sp. In a preferred

embodiment, the microbial host cell is selected from the group consisting of *Bacillus sp.*, *Pseudomonas sp.*, and *Escherichia sp.*. In a preferred embodiment, the catalyst is an *Escherichia coli* host cell recombinantly expressing one or more of the polypeptides having nitrilase activity.

[0079] Also disclosed herein, the nitrilase catalyst comprises a polypeptide having nitrilase activity wherein said polypeptide having nitrilase activity has at least 60% identity to SEQ ID NO: 51, preferably at least 70% identity to SEQ ID NO: 51, even more preferably at least 80% identity to SEQ ID NO: 51, yet even more preferably at least 90% identity to SEQ ID NO: 51, and most preferably at least 95% identity to SEQ ID NO: 51.

[0080] Working examples of several catalysts having nitrilase activity derived from various sources are described herein, including a catalyst derived from the *A. facilis* 72W nitrilase. Various mutants derived from the *Acidovorax facilis* 72W nitrilase enzyme have been reported in the art (U.S. Patent 7,148,051 and U.S. 7,198,927).

[0081] In one embodiment, the polypeptide having nitrilase activity is selected from the group consisting of SEQ ID NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, and 57. In another embodiment, the polypeptide having nitrilase activity is selected from the group consisting of 4, 24, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, and 57. In another embodiment, the polypeptide having nitrilase activity is selected from the group consisting of 4, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, and 57. In another embodiment, the polypeptide having nitrilase activity is selected from the group consisting of 4, 24, 25, and 51. In another embodiment, the nitrilase catalyst comprises the polypeptide of SEQ ID NO: 51.

Acidovorax facilis 72W (ATCC 55746 Nitrilase)

[0082] The *A. facilis* 72W nitrilase (EC 3.5.5.1) is a robust catalyst for producing carboxylic acids from aliphatic or aromatic nitriles (WO 01/75077; US 6,870,038; and Chauhan et al., *supra*). It has also been shown to catalyze the conversion of α -hydroxynitriles (*i.e.*, glycolonitrile) to α -hydroxycarboxylic acids (*i.e.*, glycolic acid) (see US 6,383,786 and US 6,416,980). However, nitrilase catalysts having improved nitrilase activity and/or stability (relative to the *A. facilis* 72W nitrilase) when converting glycolonitrile to glycolic acid would reduce the cost of manufacturing glycolic acid. As such, a method of producing glycolic acid using an improved nitrilase catalyst is useful to reduce the cost of manufacturing glycolic acid, however *A. facilis* 72W nitrilase is an enzyme catalyst for purposes of the processes herein, as well as said improved nitrilases described in detail above.

Industrial Production of the Enzyme Catalyst

[0083] Where commercial production of the enzyme catalysts described herein is desired, a variety of culture methodologies may be used. Fermentation runs may be conducted in batch, fed-batch, or continuous mode, methods well-known in the art (Thomas D. Brock in *Biotechnology: A Textbook of Industrial Microbiology*, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA, (1989); Deshpande, Mukund V., *Appl. Biochem. Biotechnol.* 36(3): 227-234 (1992)).

[0084] A classical batch culturing method is a closed system where the composition of the media is set at the beginning of the culture and not subject to artificial alterations during the culturing process. Thus, at the beginning of the culturing process the media is inoculated with the desired organism or organisms and growth or metabolic activity is permitted to occur adding nothing to the system. Typically, however, a "batch" culture is batch with respect to the addition of carbon source and attempts are often made at controlling factors such as pH and oxygen concentration. In batch systems the metabolite and biomass compositions of the system change constantly up to the time the culture is terminated. Within batch cultures cells moderate through a static lag phase to a high growth log phase and finally to a stationary phase where growth rate is diminished or halted. If untreated, cells in the stationary phase will eventually die. Cells in log phase are often responsible for the bulk of production of end product or intermediate in some systems. Stationary or post-exponential phase production can be obtained in other systems.

[0085] A variation on the standard batch system is the Fed-Batch system. Fed-Batch culture processes are also suitable in the present invention and comprise a typical batch system with the exception that the substrate is added in increments as the culture progresses. Fed-Batch systems are useful when catabolite repression is apt to inhibit the metabolism of the cells and where it is desirable to have limited amounts of substrate in the media. Measurement of the actual substrate concentration in Fed-Batch systems is difficult and is therefore estimated on the basis of the changes of measurable factors such as pH, dissolved oxygen, and the partial pressure of waste gases such as CO₂. Batch and Fed-Batch culturing methods are common and well known in the art and examples may be found in Brock (*supra*) and Deshpande (*supra*).

[0086] Commercial production of the present enzyme catalysts having nitrilase activity may also be accomplished with a continuous culture. Continuous cultures are an open system where a defined culture media is added continuously to a bioreactor and an equal amount of conditioned media is removed simultaneously for processing. Continuous cultures generally maintain the cells at a constant high-liquid-phase density where cells are primarily in log phase growth. Alternatively, continuous culture may be practiced with immobilized cells where carbon and nutrients are continuously added and valuable products, by-products or waste products are continuously removed from the cell mass. Cell immobilization may be performed using a wide range of solid supports composed of natural and/or synthetic materials.

[0087] Continuous or semi-continuous culture allows for the modulation of one factor or any number of factors that affect cell growth or end cell concentration. For example, one method will maintain a limiting nutrient such as the carbon source or nitrogen level at a fixed rate and allow all other parameters to moderate. In other systems a number of factors affecting growth can be altered continuously while the cell concentration, measured by media turbidity, is kept constant. Continuous systems strive to maintain steady-state growth conditions and thus the cell loss due to media being drawn off must be balanced against the cell growth rate in the culture. Methods of modulating nutrients and growth factors for continuous culture processes, as well as techniques for maximizing the rate of cell formation, are well known in the art of industrial microbiology and a variety of methods are detailed by Brock (*supra*).

[0088] Fermentation media in the present invention must contain suitable carbon substrates. Suitable substrates may include, but are not limited to monosaccharides such as glucose and fructose, disaccharides such as lactose or sucrose, polysaccharides such as starch or cellulose or mixtures thereof, and unpurified mixtures from renewable feedstocks such as cheese whey permeate, cornsteep liquor, sugar beet molasses, and barley malt. Hence, it is contemplated that the source of carbon utilized in the present invention may encompass a wide variety of carbon-containing substrates and will only be limited by the choice of organism.

Glutaraldehyde Pretreatment of the Enzyme Catalyst Prior to Immobilization

[0089] Treatment of an enzyme catalyst fermentation culture with glutaraldehyde can be a convenient way to kill the microbes in the culture, thus avoiding containment and safety issues for handling, storage and transportation associated with live recombinant cultures. It has now been discovered that pretreatment with glutaraldehyde, or glutaraldehyde pretreatment followed by bisulfite treatment, can preserve nitrilase activity in cells in suspension and in an immobilized form.

[0090] Preservation of nitrilase activity with glutaraldehyde pretreatment of an enzyme catalyst is affected by time, temperature, glutaraldehyde concentration, pH and the concentration of inhibitory products like ammonia and other amines (e.g., amino acids and peptides) in the media that interact with glutaraldehyde. A preferred glutaraldehyde pretreatment method treats cells from high-density fermentation (100-150 OD₅₅₀) with 5 -10 wt % glutaraldehyde in water that is preferably delivered with adequate mixing at 50 mg to 500 mg glutaraldehyde /L-min, more preferably delivered with adequate mixing at 50 mg to 200 mg glutaraldehyde/L-min, most preferably delivered with adequate mixing at 50 mg to 100 mg glutaraldehyde/L-min, resulting in a final concentration of about 3 g to about 5 g glutaraldehyde /L (about 0.025 g to about 0.042 g glutaraldehyde per OD₅₅₀), more preferably about 3.6 g to about 5 g glutaraldehyde /L (about 0.030 g to about 0.042 g glutaraldehyde per OD₅₅₀). The glutaraldehyde pretreated culture may be held in the fermenter for about 1 to 5 hours. A 10 wt % solution of sodium bisulfite in water is then optionally added at 1 g/L to inactivate the residual glutaraldehyde.

[0091] The preferred pH for the glutaraldehyde pretreatment of the enzyme catalyst in the fermentation broth or cell suspension is from pH 5.0 to 9.0, more preferably from pH 5.0 to 8.0, even more preferably from pH 5.0 to 7.0, still more preferably pH 5.0 to 6.0, and most preferably pH 5.0 to 5.5. The residual glutaraldehyde concentration after glutaraldehyde pretreatment is typically low, in the range of 10 - 200 ppm, and can be inactivated as stated above, with the addition of sodium bisulfite to a final concentration of about 1 g/L. Glutaraldehyde and bisulfite pretreatment were found to have no significant detrimental effect on the nitrilase activity. The glutaraldehyde or glutaraldehyde/ bisulfite pretreated cell suspension is optionally chilled to 5 - 10 °C, and optionally washed (by concentration and re-dilution of the cell suspension or fermentation broth) with water or an appropriate storage buffer to remove residual bisulfite and unreacted glutaraldehyde.

Immobilization of Glutaraldehyde Pretreated Enzyme Catalyst and Chemical Cross-linking

[0092] Methods for the immobilization of enzyme catalysts have been widely reported and are well known to those skilled in the art (Methods in Biotechnology, Vol. 1: Immobilization of Enzymes and Cells; Gordon F. Bickerstaff, Editor; Humana Press, Totowa, NJ, USA; 1997). The immobilization of the *A. facilis* 72W nitrilase catalyst has also been previously reported (US 6,870,038).

[0093] Further, a method for immobilization in carrageenan and subsequent glutaraldehyde/polyethylenimine cross-linking of the immobilized enzyme catalyst follows (and as disclosed in US 6,870,038, and as described in detail in US 6,551,804 B), however, one of ordinary skill in the art would recognize and readily apply variations to accomplish immobilization and cross-linking. Said variations are contemplated herein and are within the scope of the instant process. Further, the amounts or concentrations of components used for immobilization and chemical cross-linking will vary depending on the amount and type of enzyme catalyst and fermentative production of enzyme catalyst. One of ordinary skill in the art would recognize these factors and adjust the immobilization and chemical cross-linking procedures accordingly. With regard to cross-linking with glutaraldehyde and polyethylenimine, US 6,551,804 (supra), describes the processes and procedures for chemically cross-linking alginate immobilized cells. Said description applies here for carrageenan immobilized cells as well.

Hydrolysis of Glycolonitrile to Glycolic Acid Using a Nitrilase Catalyst

[0094] The enzymatic conversion of glycolonitrile to glycolic acid (in the form of the acid and/or the corresponding ammonium salt) may be performed by contacting an enzyme catalyst, immobilized enzyme catalyst, or cross-linked immobilized enzyme catalyst having nitrilase activity under suitable reaction conditions as described below (i.e. in an aqueous reaction mixture at certain pH range, temperatures, concentrations, etc.). In one embodiment, whole recombinant microbial cells are immobilized in carrageenan, cross-linked, and the resulting enzyme catalyst used directly for the conversion of glycolonitrile to glycolic acid, or unimmobilized cells can be maintained separately from the bulk reaction mixture using hollow-fiber membrane cartridges or ultrafiltration membranes. In a second embodiment, whole recombinant microbial cells are immobilized in polyacrylamide gel, and the resulting enzyme catalyst used directly for the conversion of glycolonitrile to glycolic acid.

[0095] The concentration of enzyme catalyst in an aqueous reaction mixture depends on the specific activity of the enzyme catalyst and is chosen to obtain the desired rate of reaction. The wet cell weight of the microbial cells used as catalyst in hydrolysis reactions typically ranges from 0.001 grams to 0.250 grams of wet cells per mL of total reaction volume, preferably from 0.002 grams to 0.050 grams of wet cells per mL. The indicated wt % of wet cells per volume of total reaction volume may be present in the reaction mixture in the form of an immobilized enzyme catalyst prepared as previously described (supra), where the weight of wet cells as a percentage of the total weight of the immobilized enzyme catalyst is known from the method of preparation of the immobilized enzyme catalyst.

[0096] The temperature of the glycolonitrile hydrolysis reaction is chosen to control both the reaction rate and the stability of the enzyme catalyst activity. The temperature of the reaction may range from just above the freezing point of the reaction mixture (approximately 0 °C) to about 65 °C, with a preferred range of reaction temperature of from about 5 °C to about 35 °C. An enzyme catalyst suspension may be prepared by suspending the immobilized cells in distilled water, or in an aqueous solution of a buffer which will maintain the initial pH of the reaction between about 5.0 and about 10.0, preferably between about 5.5 and about 8.0, more preferably between about 5.5 and about 7.7, and most preferably about 6.0 to about 7.7. As the reaction proceeds, the pH of the reaction mixture may change due to the formation of an ammonium salt of the carboxylic acid from the corresponding nitrile functionality. The reaction can be run to complete conversion of glycolonitrile with no pH control, or a suitable acid or base can be added over the course of the reaction to maintain the desired pH.

[0097] Glycolonitrile was found to be completely miscible with water in all proportions at 25 °C. In cases where reaction conditions are chosen such that the solubility of the substrate (i.e., an α -hydroxynitrile) is also dependent on the temperature of the solution and/or the salt concentration (buffer or product glycolic acid ammonium salt, also known as ammonium glycolate) in the aqueous phase, the reaction mixture may initially be composed of two phases: an aqueous phase containing the enzyme catalyst and dissolved α -hydroxynitrile, and an organic phase (the undissolved α -hydroxynitrile). As the reaction progresses, the α -hydroxynitrile dissolves into the aqueous phase, and eventually a single phase product mixture is obtained. The reaction may also be run by adding the α -hydroxynitrile to the reaction mixture at a rate approximately equal to the enzymatic hydrolysis reaction rate, thereby maintaining a single-phase aqueous reaction mixture, and avoiding the potential problem of substrate inhibition of the enzyme at high starting material concentrations.

[0098] Glycolic acid may exist in the product mixture as a mixture of the protonated carboxylic acid and/or its corresponding ammonium salt (dependent on the pH of the product mixture; pKa of glycolic acid is about 3.83), and may additionally be present as a salt of the carboxylic acid with any buffer that may additionally be present in the product mixture. Typically, the glycolic acid

produced is primarily in the form of the ammonium salt (pH of the glycolonitrile hydrolysis reaction is typically between about 5.5 and about 7.7). The glycolic acid product may be isolated from the reaction mixture as the protonated carboxylic acid, or as a salt of the carboxylic acid, as desired.

[0099] The final concentration of glycolic acid in the product mixture at complete conversion of glycolonitrile may range from 0.001 M to the solubility limit of the glycolic acid product. In one embodiment, the concentration of glycolic acid will range from about 0.10 M to about 5.0 M. In another embodiment, the concentration of glycolic acid will range from about 0.2 M to about 3.0 M.

[0100] Glycolic acid may be recovered in the form of the acid or corresponding salt using a variety of techniques including, but not limited to ion exchange, electrodialysis, reactive solvent extraction, polymerization, thermal decomposition, alcoholysis, and combinations thereof.

[0101] Further, when an amount, concentration, or other value or parameter is given either as a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the invention be limited to the specific values recited when defining a range.

GENERAL METHODS

[0102] The following examples are provided to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus may be considered to constitute preferred modes for its practice.

[0103] Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in Manual of Methods for General Bacteriology (1994) (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds.), American Society for Microbiology, Washington, DC.) or by Thomas D. Brock, in Biotechnology: A Textbook of Industrial Microbiology, (1989) Second Edition, (Sinauer Associates, Inc., Sunderland, MA). Methods to immobilize enzymatic catalysts can be found in Bickerstaff, G.F., *supra*).

[0104] Procedures required for genomic DNA preparation, PCR amplification, DNA modifications by endo- and exo-nucleases for generating desired ends for cloning of DNA, ligations, and bacterial transformation are well known in the art. Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Maniatis, *supra*; and by T. J. Silhavy, M. L. Bannan, and L. W. Enquist, Experiments with Gene Fusions, (1984) Cold Spring Harbor Laboratory Press, Cold Spring, NY; and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, (1994-1998) John Wiley & Sons, Inc., New York.

[0105] All reagents and materials were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma/Aldrich Chemical Company (St. Louis, MO) unless otherwise specified.

[0106] The abbreviations in the specification correspond to units of measure, techniques, properties, or compounds as follows: "sec" means second(s), "min" means minute(s), "h" or "hr" means hour(s), "d" means density in g/mL, "μL" means microliters, "mL" means milliliters, "L" means liters, "mM" means millimolar, "M" means molar, "mmol" means millimole(s), "wt" means weight, "wt%" means weight percent, "g" means grams, "μg" means micrograms, HPLC means high performance liquid chromatography, "O.D." means optical density at the designated wavelength, "dcw" means dry cell weight, "U" means units of nitrilase activity, "EDTA" means ethylenediaminetetraacetic acid, and "DTT" means dithiothreitol. One U of nitrilase activity corresponds to the hydrolysis of 1 μmol glycolonitrile/min.

Analytical Methodology

HPLC Analysis

[0107] Unless otherwise noted, the following HPLC method was used. The reaction product mixtures were analyzed by the following HPLC method. Aliquots (0.01 mL) of the reaction mixture were added to 1.50 mL of water, and analyzed by HPLC (HPX 87H column, 30 cm x 7.8 mm; 0.01 N H₂SO₄ mobile phase; 1.0 mL/min flow at 50 °C; 10 µL injection volume; RI detector, 20 min analysis time). The method was calibrated for glycolonitrile at a series of concentrations using commercially available glycolonitrile purchased from Aldrich.

Quantitative ¹³C NMR Analysis

[0108] Quantitative ¹³C NMR spectra were obtained using a Varian Unity Inova spectrometer (Varian, Inc., Palo Alto, CA) operating at 400 MHz. Samples were prepared by taking 3.0 mL of the reaction product along with 0.5 mL of D₂O in a 10 mm NMR tube. ¹³C NMR spectra were typically acquired using a spectral width of 26 KHz with the transmitter located at 100 ppm, 128K points, and a 90-degree pulse (pw90 = 10.7 microseconds at a transmitter power of 56 db). The longest ¹³C T₁ (23 sec) was associated with the GLN nitrile carbon, and the total recycle time was set greater than ten times this value (recycle delay d1 = 240 sec, acquisition time at = 2.52 sec). Signal averaging of 360 scans gave a total experiment time of 26.3 hours. The Nuclear Overhauser Enhancement (NOE) was suppressed by gating on the Waltz-modulated ¹H decoupling only during the acquisition time (at).

EXAMPLE 1

Fermentation of *E. coli* MG1655/pSW138-168V

[0109] Seed cultures of *E. coli* MG1655/pSW138-168V were grown in 500 mL LB media supplemented with 0.1 mg ampicillin per mL for 6-10 h (OD₅₅₀ = 1-2) at 30 °C with shaking (300 rpm) prior to inoculation of the fermentor. Growth of *E. coli* MG1655/pSW138-168V nitrilase strain was in 14-L Braun Biostat C fermenters (B. Braun Biotech International GmbH, Melsungen, Germany) using mineral medium with glucose, ammonia, and salts, and lactose was used for induction. Pre-sterilization fermenter media (7.5 L) is described in Table 2. Post-sterilization additions include filter-sterilized trace elements (Table 3), 0.1 mg ampicillin per mL, 2 g casamino acids (Difco) per L, 4 g glucose per L, and 500 mL seed culture.

[0110] Fermentation set points are described in Table 4. NH₄OH (40% w/v) and H₃PO₄ (20% w/v) were used for pH control. The dissolved oxygen concentration was controlled at 25% of air saturation with the agitation set to rise first with increase in oxygen demand, with aeration to follow. The fermentation feed protocol used with lactose induction is given in Table 5. Glucose feed rates were reduced if glucose accumulated above 5 g/L. After 40-56 h, the fermentation broth was chilled to 5-10 °C and the cells harvested by centrifugation. Cell paste was frozen and stored at -70 °C. The cell paste was designated as NIT 60 (1910 GLN U/g dew).

Table 2. Fermentation media, pre-sterilization.

(NH ₄) ₂ SO ₄	5.0 g/L
K ₂ HPO ₄	4.0 g/L
KH ₂ PO ₄	3.5 g/L
MgSO ₄ *7H ₂ O	0.6 g/L
Na ₃ Citrate*2H ₂ O	1.0 g/L
NZ Amine AS (Quest)	2.5 g/L
Antifoam - Biospumex 153K	0.25 ml/L

Table 3. Fermentation trace elements

	Concentration
Citric acid	10 g/L
CaCl ₂ *2H ₂ O	1.5 g/L
FeSO ₄ *7H ₂ O	5 g/L

	Concentration
ZnSO ₄ *7H ₂ O	0.39 g/L
CuSO ₄ *5H ₂ O	0.38 g/L
CoCl ₂ *6H ₂ O	0.2 g/L
MnCl ₂ *4H ₂ O	0.3 g/L

Table 4. Fermentation set points

	Initial Set-Point	Minimum	Maximum
Stirrer (rpm)	400	400	1000
Airflow (slpm)	2	2	10
pH	6.8	6.8	6.8
Pressure (kPa)	0.5	0.5	0.5
DO	25%	25%	25%
Temperature °C	30	30	30

Table 5. Fermentation feed protocol used with lactose induction

EFT (h)	Feed Rate (g/min)	Substrate
0	0	Glucose (batched)
5	0.27	Glucose (50% w/w)
14	1.3	Lactose (25% w/w)

EXAMPLE 2**Immobilization of *E. coli* MG1655/pNM18-168V in GA/PEI-cross-linked Carrageenan Beads**

[0111] With rapid stirring, 12 g of carrageenan (FMC GP911) was slowly added to 228 g deionized distilled water at 50 °C, the resulting mixture heated to 80 °C until the carrageenan was completely dissolved, and the resulting solution cooled with stirring to 52 °C. In a separate beaker equipped with stir bar, 83.2 g of frozen *E. coli* MG1655/pNM18-168V cells (25.2 % dcw) were added to 84.8 g of 0.35 M Na₂HPO₄ (pH 7.3) at ca. 25 °C and mixed until the cells were suspended, then a deoxyribonuclease I solution (10 µL of 12,500 U/mL DNase (Sigma)/100 mL of cell suspension) was added. The cell suspension was filtered consecutively through a 230 micron and 140 micron Nupro TF strainer element filter, and heated with stirring to 50 °C. With stirring, 160.0 g of *E. coli* MG1655/pNM18-168V cell suspension at 50 °C was added to the carrageenan solution at 52 °C, and the resulting cell/carrageenan suspension was pumped through an electrically-heated 20 gauge needle at 47 °C and dripped into 0.25 M KHCO₃ (pH = 7.3) with stirring at ca. 37-38 °C; the flow rate through the needle was set at 5 - 8 mL/min. The resulting beads were allowed to harden in this same buffer for 1 h at room temperature with stirring, and were stored in 0.25 M potassium bicarbonate (pH 7.3).

[0112] Chemical cross-linking of the immobilized cell/carrageenan beads was performed by addition of 0.5 g of 25 % glutaraldehyde (GA) in water (Sigma M 752-07) to 20 g beads suspended in 48 mL of 0.25 M potassium bicarbonate (pH 7.3), and stirring for 1 h at room temperature. To the suspension of beads was then added 2.0 g of 12.5 wt % polyethylenimine (PEI, BASF LUPASOL PS) in water, and the bead suspension stirred for an additional 18 h at room temperature. The GA/PEI-cross-linked beads were recovered from the suspension, stirred twice for 15 min in 48 mL of 0.25 M potassium bicarbonate (pH 7.3), then stored in 1.0 M ammonium bicarbonate (pH 7.3) at 5 °C. Prior to use as catalyst for conversion of glycolonitrile to glycolic acid (as the ammonium salt), the beads were washed twice for 15 min with 180 mL of 0.1 M ammonium glycolate (pH 7.3) at room temperature to remove the 1.0 M ammonium bicarbonate (pH 7.3) storage buffer. The resulting immobilized cell catalyst was identified as immobilized NIT 60.

EXAMPLE 3:

Pretreatment of *E. coli* MG1655/pSW138-168V with Glutaraldehyde Prior to Immobilization

[0113] A 200-L fermentation was performed to produce a broth containing *E. coli* MG1655/pSW138-168V cells that were subsequently pretreated with glutaraldehyde in-situ prior to immobilization. A pre-seed culture was first prepared by charging a 2-L shake flask with 0.5 L seed medium containing yeast extract (Ambrex 695, 5.0 g/L), K_2HPO_4 (10.0 g/L), KH_2PO_4 (7.0 g/L), sodium citrate dihydrate (1.0 g/L), $(NH_4)_2SO_4$ (4.0 g/L), $MgSO_4$ heptahydrate (1.0 g/L) and ferric ammonium citrate (0.10 g/L). The pH of the medium was adjusted to 6.8 and the medium was sterilized in the flask. Post sterilization additions included glucose (10 mL, 50 wt %) and 1 mL ampicillin (25 mg/mL). The pre-seed medium was inoculated with a 1-mL frozen stock culture of *E. coli* MG1655/pSW138-168V in 20% glycerol, and cultivated at 35 °C and 300 rpm. The seed culture was transferred at ca. 2 OD₅₅₀ to a 14L seed fermentor (Braun) with 8 L of medium containing KH_2PO_4 (3.50 g/L), $FeSO_4$ heptahydrate (0.05 g/L), $MgSO_4$ heptahydrate (2.0 g/L), sodium citrate dihydrate (1.90 g/L), yeast extract (Ambrex 695, 5.0 g/L), Biospumex153K antifoam (0.25 mL/L, Cognis Corporation), NaCl (1.0 g/L), $CaCl_2$ dihydrate (10 g/L), and NIT trace elements solution (10 mL/L). The trace elements solution contained citric acid monohydrate (10 g/L), $MnSO_4$ hydrate (2 g/L), NaCl (2 g/L), $FeSO_4$ heptahydrate (0.5 g/L), $ZnSO_4$ heptahydrate (0.2 g/L), $CuSO_4$ pentahydrate (0.02 g/L) and $NaMoO_4$ dihydrate (0.02 g/L). Post sterilization additions included 120 g glucose solution (50% w/w) and ampicillin 16 mL stock solution (25 mg/mL).

[0114] The dissolved oxygen (dO) concentration was controlled at 25% of air saturation. The dO was controlled first by impeller agitation rate (400 to 1400 rpm) and later by aeration rate (2 to 10 slpm). The pH was controlled at 6.8. NH_4OH (29% w/w) and H_2SO_4 (20% w/v) were used for pH control. The temperature was controlled at 35 °C and the head pressure was 0.5 bars. At ca 6 OD₅₅₀ the culture was transferred to the 200L Biostat-D Braun fermenter. The medium used was the same as in the seed fermenter, the initial working volume was 140 L and 50% w/w glucose was charged to 8 g/L. The fermentation started as a batch operation, and once the glucose was depleted (<0.5 g/L) a fed batch operation with 50% w/w glucose was initiated with a predetermined rate (Table 6), at ca 25 OD₅₅₀ the feed was switched to 25% D-lactose solution with a pre-determined rate (Table 7).

[0115] The temperature was controlled at 35.0 °C, the head pressure at 0.5 bar, the pH at 1st stage (glucose phase) at 6.8 and at the 2nd stage (lactose phase) at 7.2, NH_4OH (29% w/w) and H_2SO_4 (20% w/v) were used for pH control, the dO controlled at 1st stage at 25% of air saturation and 2nd stage at 10%, the dO was controlled by agitation first (250-450 rpm) and later by aeration (25-35 slpm). Glucose and lactose levels were monitored during the fed operation and if the levels of glucose exceeds 0.1 g/L or lactose above 1 g/L the feed program was either temporarily halted or reduced. The run was ended 40 h after the initiation of lactose feed, and cells were either harvested by centrifugation or microfiltration or kept in the vessel for treatment with glutaraldehyde. The fermentation produced about 8 kg dry cell weight with a nitrilase specific activity of 2819 BZN U/g dcw (1788 GLN U/g dcw).

Table 7: Feed protocol

Feed time intervals (h)	Feed rate g/min	Substrate	Stage
0	6.13	50% w/w glucose	1st
1	7.13	50% w/w glucose	1st
2	8.28	50% w/w glucose	1st
3	9.62	50% w/w glucose	1st
4	11.18	50% w/w glucose	1st
5	11.18	50% w/w glucose	1st
6	11.18	50% w/w glucose	1st
7	11.18	50% w/w glucose	1st
8	11.18	50% w/w glucose	1st
0	11.22	25% w/w lactose	2nd
2	24.42	25% w/w lactose	2nd

Feed time intervals (h)	Feed rate g/min	Substrate	Stage
20	16.72	25% w/w lactose	2nd
30	18.7	25% w/w lactose	2nd
40	18.7	25% w/w lactose	2nd

[0116] At the end of the fermentation, the agitation was reduced to 150 rpm, the aeration stopped and the temperature maintained at 35 °C. Part of the fermentation broth was withdrawn, leaving ca. 180 kg in the fermenter. This remaining broth was titrated to pH 5.2 and maintained at this pH with 20% H₂SO₄ (20% w/w) and NaOH (50% w/w) while 9.0 L of aqueous glutaraldehyde (GA, 10% w/w) was added with stirring at a rate of ~90 mL/min; this rate of addition was equivalent to 50 mg glutaraldehyde/L fermentation broth/min, and the final concentration of glutaraldehyde was ca. 5 g glutaraldehyde/L (0.035 g glutaraldehyde/ OD₅₅₀). After 5 h from initiation of glutaraldehyde addition to the broth, the pH was adjusted to 7.0, and 1.8 L of aqueous sodium bisulfite (10% w/w, pH 7) was added (ca. 1 g sodium bisulfite/L final concentration) with stirring, and the broth stirred for an additional 15 min. The temperature of the broth was then decreased to 10 °C, and the agitation decreased to 100 rpm. The broth was concentrated to 40 kg of cell suspension using a Diskstack centrifuge (Alfa Laval), then 50 kg DI water (20 °C) was added to the suspension and the mixture was concentrated by centrifugation to produce 40 kg of washed cell suspension. The suspension (identified as NIT 188A-C2) was stored at 5 °C, and a portion of the cell suspension was used directly for the preparation of an immobilized cell catalyst (Example 6). Nitrilase specific activity during each process step is summarized in Table 8.

Table 8: Nitrilase activity during different stages of GA and bisulfite treatment

fermentation stage	BZN U/g dcw
pre GA treatment	2819
post GA	3300
post NaHSO ₃	2493

EXAMPLE 4

Immobilization of Glutaraldehyde Pretreated *E. coli* MG1655/pNM18-168V in GA/PEI-cross-linked Carrageenan Beads

[0117] The final cell suspension concentrate recovered from the glutaraldehyde and sodium bisulfite-treated fermentation broth of Example 5 was centrifuged at 5 °C. The resulting cell pellet was re-suspended in a 5-fold by weight amount of 0.35 M potassium phosphate buffer (pH 7.2), and centrifugation of the resulting cell suspension at 5 °C produced a wet cell paste that was immobilized and chemically cross-linked with GA and PEI as described in Example 2. The resulting immobilized cell catalyst was identified as immobilized NIT 188A-C2.

EXAMPLE 5

Improvement in Biocatalyst Specific Activity in Consecutive Batch Reactions with Catalyst Recycle using Glutaraldehyde/polyethylenimine Cross-linked Carrageenan-immobilized *E. coli* MG1655/pSW138-F168V Transformant

[0118] In a typical procedure, duplicate sets of batch reactions for the conversion of glycolonitrile to glycolic acid were run in 50-mL jacketed reaction vessels equipped with overhead stirring and temperature control. Each reactor was charged with 8 g of GA/PEI-cross-linked *E. coli* MG1655/pSW138-168V/carrageenan beads (prepared using the process as described in Example 1 (no GA pretreatment prior to immobilization) or Example 4 (GA pretreatment prior to immobilization)) containing 5 % (dcw) transformant expressing the *A. facilis* 72W nitrilase mutant F168V (SEQ ID NO: 51). To the vessel was then added 14.78 mL of distilled water and 6.0 mL of aqueous ammonium glycolate (4.0 M, pH 7.0), and the reaction vessel flushed with nitrogen. The mixture was stirred at 25 °C while programmable syringe pumps were used to simultaneously add 1.07 mL of 60 wt % glycolonitrile (GLN) in water (12.0 mmol GLN, 0.084 mmol formaldehyde; Fluka (redistilled, stabilized with 0.5 wt% glycolic acid)) and 0.150 mL

of aqueous ammonium hydroxide (1.875 wt%); one equivalent volume of GLN and ammonium hydroxide solutions was added simultaneously every 2 h (for a total of eight equivalent additions each of GLN solution and aqueous ammonium hydroxide) to maintain the concentration of GLN at ≤ 400 mM and the pH within a range of 6.5 - 7.5. Four 0.050-mL reaction samples were removed at pre-determined times after the first GLN addition and analyzed by HPLC to determine the initial reaction rate and the catalyst specific activity (μmol glycolic acid/min/g dcw biocatalyst). At completion of the reaction, there was 100% conversion of GLN to produce glycolic acid (as the ammonium salt) in $> 99\%$ yield.

[0119] At the end of the first reaction, the aqueous product mixture was decanted from the catalyst (under nitrogen), leaving ca. 10.3 g of a mixture of immobilized cell catalyst (8.0 g) and remaining product mixture (ca. 2.3 g). To the reaction vessel then added 20.78 mL of distilled, deionized water, and a second reaction was performed at 25 °C by the addition of aliquots of aqueous GLN and ammonium hydroxide as described immediately above. The specific activities of recovered biocatalyst in consecutive batch reactions with catalyst recycle are listed in Table 7.

Table 7. Dependence of recovered biocatalyst specific activity in consecutive batch reactions with catalyst recycle on glutaraldehyde pretreatment of cells prior to immobilization.

		biocatalyst specific activity (GLN U/g dcw) in consecutive batch reactions				
immobilized cell biocatalyst	glutaraldehyde pretreatment of cells	reaction 1	reaction 2	reaction 3	reaction 4	decrease in specific activity, rxn1 to rxn4 (%)
NIT 188C2	yes	1826	1518	1596	1759	4
NIT 188C2	yes	1857	1656	1581	1947	0
NIT 027	no	1312	872	898	816	38
NIT 027	no	1416	856	931	621	56
NIT 49	no	1694	702	718	886	48
NIT 49	no	1665	868	869	946	43
NIT 60-A	no	1319	730	744	563	57
NIT 60-A	no	1410	750	725	699	50
NIT 60-B	no	1222	757	857	802	34
NIT 60-B	no	1335	722	839	808	39

EXAMPLE 6

Storage Stability of Glutaraldehyde/polyethylenimine Cross-linked Carrageenan-immobilized *E. coli* MG1655/pSW138-F168V Prepared using Glutaraldehyde Pretreated Cells.

[0120] Freshly-prepared glutaraldehyde/polyethylenimine cross-linked carrageenan-immobilized *E. coli* MG1655/pSW138-F168V prepared using glutaraldehyde pretreated cells (as described in Example 4) were stored for 32 days in 1.0 M ammonium bicarbonate (pH 7.3) at 5 °C. Prior to use, the beads were washed twice for 15 min with 180 mL of 0.1 M ammonium glycolate (pH 7.0) at room temperature. Biocatalyst stored for either 4 days or 32 days at 5 °C were each evaluated in duplicate sets of consecutive batch reactions with biocatalyst recycle using the procedure described in Example 5 (Table 8).

Table 8. Specific activity of biocatalyst prepared using glutaraldehyde pretreatment of cells prior to immobilization in consecutive batch reactions with catalyst recycle, using biocatalyst stored at 5 °C for 4 or 32 days.

		biocatalyst specific activity (GLN U/g dcw) in consecutive batch reactions				
immobilized cell biocatalyst	days stored at 5 °C	reaction 1	reaction 2	reaction 3	reaction 4	decrease in specific activity, rxn 1 to rxn4 (%)
NIT 188C2	4	1826	1518	1596	1759	4
NIT 188C2	4	1857	1656	1581	1947	0

		biocatalyst specific activity (GLN U/g dcw) in consecutive batch reactions				
immobilized cell biocatalyst	days stored at 5 °C	reaction 1	reaction 2	reaction 3	reaction 4	decrease in specific activity, rxn 1 to rxn4 (%)
NIT 188C2	32	1910	1455	1580	1543	19
NIT 188C2	32	1987	1434	1472	1783	10

SEQUENCE LISTING

[0121]

<110> E.I. duPont de Nemours and Company, Inc.

<120> IMPROVEMENT IN IMMOBILIZED MICROBIAL NITRILASE FOR PRODUCTION OF GLYCOLIC ACID

<130> CL3888PCT

<160> 57

<170> PatentIn version 3.4

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ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc	96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile	
20 25 30	
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa	144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu	
35 40 45	
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag	192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys	
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tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta	240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu	
65 70 75 80	
ggg gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa	288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys	
85 90 95	
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat	336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr	
100 105 110	
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat ccg	384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg	
115 120 125	
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc	432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly	
130 135 140	
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt	480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly	
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gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg	528
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tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc	576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser	
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cct ctt cag ccg gat gtt ttc caa ctg agc atc gaa gcc aac gcg acg	624
Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr	
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gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg	672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser	
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Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225                230                235                240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac      768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245                250                255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag      816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260                265                270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag      864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275                280                285

gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg      912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290                295                300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att      960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305                310                315                320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga     1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
325                330                335

ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag ccg ctc gga     1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340                345                350

acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca     1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
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aag tag
Lys
1110

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20          25          30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35          40          45

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

Ile Ala Glu Ala Ala Arg Asn Gly Cys Glu Leu Val Ala Phe Pro Glu
35      40      45

Val Phe Ile Pro Gly Tyr Pro Tyr His Ile Trp Val Asp Ser Pro Leu
50      55      60

Ala Gly Met Ala Lys Phe Ala Val Arg Tyr His Glu Asn Ser Leu Thr
65      70      75      80

Met Asp Ser Pro His Val Gln Arg Leu Leu Asp Ala Ala Arg Asp His
      85      90      95

Asn Ile Ala Val Val Val Gly Ile Ser Glu Arg Asp Gly Gly Ser Leu
100      105      110

Tyr Met Thr Gln Leu Val Ile Asp Ala Asp Gly Gln Leu Val Ala Arg
115      120      125

Arg Arg Lys Leu Lys Pro Thr His Val Glu Arg Ser Val Tyr Gly Glu
130      135      140

Gly Asn Gly Ser Asp Ile Ser Val Tyr Asp Met Pro Phe Ala Arg Leu
145      150      155      160

Gly Ala Leu Asn Cys Trp Glu His Phe Gln Thr Leu Thr Lys Tyr Ala
165      170      175

Met Tyr Ser Met His Glu Gln Val His Val Ala Ser Trp Pro Gly Met
180      185      190

Ser Leu Tyr Gln Pro Glu Val Pro Ala Phe Gly Val Asp Ala Gln Leu
195      200      205

Thr Ala Thr Arg Met Tyr Ala Leu Glu Gly Gln Thr Phe Val Val Cys
210      215      220

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Thr Thr Gln Val Val Thr Pro Glu Ala His Glu Phe Phe Cys Asp Asn
225 230 235 240

Asp Glu Gln Arg Lys Leu Ile Gly Arg Gly Gly Gly Phe Ala Arg Ile
245 250 255

Ile Gly Pro Asp Gly Arg Asp Leu Ala Thr Pro Leu Ala Glu Asp Glu
260 265 270

Glu Gly Ile Leu Tyr Ala Asp Ile Asp Leu Ser Ala Ile Thr Leu Ala
275 280 285

Lys Gln Ala Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu
290 295 300

Ser Leu Asn Phe Asn Gln Arg His Thr Thr Pro Val Asn Thr Ala Ile
305 310 315 320

Ser Thr Ile His Ala Thr His Thr Leu Val Pro Gln Ser Gly Ala Leu
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Asp Gly Val Arg Glu Leu Asn Gly Ala Asp Glu Gln Arg Ala Leu Pro
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Leu Ala Phe Pro Glu Val Trp Ile Pro Gly Tyr Pro Tyr Trp Ala Trp
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Ile Gly Asp Val Lys Trp Ala Val Ser Asp Phe Ile Pro Lys Tyr His
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Asp Gly Ala Ser Arg Tyr Leu Ser Gln Val Phe Ile Asp Gln Asn Gly
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Asp Ile Val Ala Asn Arg Arg Lys Leu Lys Pro Thr His Val Glu Arg
130         135         140

Thr Ile Tyr Gly Glu Gly Asn Gly Thr Asp Phe Leu Thr His Asp Phe
145         150         155         160

Gly Phe Gly Arg Val Gly Gly Leu Asn Cys Trp Glu His Phe Gln Pro
165         170         175

Leu Ser Lys Tyr Met Met Tyr Ser Leu Asn Glu Gln Ile His Val Ala
180         185         190

Ser Trp Pro Ala Met Phe Ala Leu Thr Pro Asp Val His Gln Leu Ser
195         200         205

Val Glu Ala Asn Asp Thr Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln
210         215         220

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

Leu Phe Ala Gly Asp Asp Ala Lys Arg Ala Leu Leu Pro Leu Gly
245         250         255

Gln Gly Trp Ala Arg Ile Tyr Gly Pro Asp Gly Lys Ser Leu Ala Glu
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Pro Leu Pro Glu Asp Ala Glu Gly Leu Leu Tyr Ala Glu Leu Asp Leu
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Glu Gln Ile Ile Leu Ala Lys Ala Ala Ala Asp Pro Ala Gly His Tyr
290         295         300

Ser Arg Pro Asp Val Leu Ser Leu Lys Ile Asp Thr Arg Asn His Thr
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Pro Val Gln Tyr Ile Thr Ala Asp Gly Arg Thr Ser Leu Asn Ser Asn
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Ser Arg Val Glu Asn Tyr Arg Leu His Gln Leu Ala Asp Ile Glu Lys
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Tyr Glu Asn Ala Glu Ala Ala Thr Leu Pro Leu Asp Ala Pro Ala Pro
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 Tyr Trp Ala Trp Ile Gly Asp Val Lys Trp Ala Val Ser Glu Phe Ile
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 Tyr Ser Glu Lys Asp Gly Ala Ser Arg Tyr Leu Ser Gln Val Phe Ile
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 130 135 140

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 145 150 155 160
 His Phe Gln Pro Leu Ser Lys Tyr Met Met Tyr Ser Leu Asn Glu Gln
 165 170 175
 Ile His Val Ala Ser Trp Pro Ala Met Phe Ala Leu Thr Pro Asp Val
 180 185 190
 His Gln Leu Ser Val Glu Ala Asn Asp Thr Val Thr Arg Ser Tyr Ala
 195 200 205
 Ile Glu Gly Gln Thr Phe Val Leu Ala Ala Thr His Val Ile Gly Lys
 210 215 220
 Ala Thr Gln Asp Leu Phe Ala Gly Asp Asp Glu Ala Lys Arg Ala Leu
 225 230 235 240
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 245 250 255
 Ser Leu Ala Glu Pro Leu Ala Glu Asn Ala Glu Gly Leu Leu Tyr Ala
 260 265 270
 Glu Leu Asp Leu Glu Gln Ile Ile Val Ala Lys Ala Ala Ala Asp Pro
 275 280 285
 Ala Gly His Tyr Ser Arg Pro Asp Val Leu Ser Leu Lys Val Asp Thr
 290 295 300
 Arg Asn His Thr Pro Val Gln Tyr Val Thr Glu Asp Gly Gly Ser Ser
 305 310 315 320
 Leu Asn Ser Asn Ser Arg Val Glu Asn Tyr Arg Leu Arg Gln Leu Ala
 325 330 335
 Asp Ile Glu Lys Tyr Glu Asn Ala Asp Ser Ala Thr Val Pro Leu Asp
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 Lys Ala Gln Gly Ala Arg Leu Ala Leu Phe Pro Glu Ala Phe Val Gly
 35 40 45
 Gly Tyr Pro Lys Gly Ala Asp Phe His Ile Phe Leu Gly Gly Arg Thr
 50 55 60
 Pro Gln Gly Arg Ala Gln Tyr Gln Arg Tyr Ala Glu Thr Ala Ile Ala
 65 70 75 80
 Val Pro Gly Pro Val Thr Glu Arg Ile Gly Gln Ile Ala Ala Glu Gln
 85 90 95
 Asp Met Phe Ile Val Val Gly Val Ile Glu Arg Asp Gly Gly Thr Leu
 100 105 110
 Tyr Cys Thr Ile Leu Phe Phe Ser Pro Glu Gly Glu Leu Leu Gly Lys
 115 120 125
 His Arg Lys Leu Met Pro Thr Ala Leu Glu Arg Leu Leu Trp Gly Tyr
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 Gly Asp Gly Ser Thr Phe Pro Val Tyr Asp Thr Pro Leu Gly Lys Leu
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 Gly Ala Val Val Cys Trp Glu Asn Tyr Met Pro Leu Leu Arg Met Ala
 165 170 175
 Met Tyr Gly Lys Gln Ile Gln Ile Tyr Cys Ala Pro Thr Ala Asp Asp
 180 185 190
 Lys Pro Thr Trp Val Ser Thr Met Gln His Val Ala Leu Glu Gly Arg
 195 200 205
 Cys Phe Val Leu Ser Ala Cys Gln His Leu Arg Gly Lys Asp Phe Pro
 210 215 220
 Pro Glu Phe His Asn Ala Leu Asp Val Gln Pro Asp Thr Val Leu Met
 225 230 235 240
 Arg Gly Gly Ser Cys Ile Val Asp Pro Met Gly Gln Leu Leu Ala Gly
 245 250 255
 Pro Val Tyr Asp Glu Asp Ala Ile Leu Val Ala Asp Ile Asp Leu Asp
 260 265 270
 Ala Val Thr Arg Gly Lys Met Asp Phe Asp Val Val Gly His Tyr Ala
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<211> 339

<212> PRT

<213> *Arabidopsis thaliana*

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Ile Val Arg Ala Thr Ile Val Gln Ala Ser Thr Val Tyr Asn Asp Thr
20 25 30

Pro Ala Thr Leu Glu Lys Ala Asn Lys Phe Ile Val Glu Ala Ala Ser
35 40 45

Lys Gly Ser Glu Leu Val Val Phe Pro Glu Ala Phe Ile Gly Gly Tyr
50 55 60

Pro Arg Gly Phe Arg Phe Gly Leu Gly Val Gly Val His Asn Glu Glu
65 70 75 80

Gly Arg Asp Glu Phe Arg Lys Tyr His Ala Ser Ala Ile Lys Val Pro
85 90 95

Gly Pro Glu Val Glu Lys Leu Ala Glu Leu Ala Gly Lys Asn Asn Val
100 105 110

Tyr Leu Val Met Gly Ala Ile Glu Lys Asp Gly Tyr Thr Leu Tyr Cys
115 120 125

Thr Ala Leu Phe Phe Ser Pro Gln Gly Gln Phe Leu Gly Lys His Arg
130 135 140

Lys Leu Met Pro Thr Ser Leu Glu Arg Cys Ile Trp Gly Gln Gly Asp
145 150 155 160

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

Ala Ile Cys Trp Glu Asn Arg Met Pro Leu Tyr Arg Thr Ala Leu Tyr
180 185 190

Ala Lys Gly Ile Glu Leu Tyr Cys Ala Pro Thr Ala Asp Gly Ser Lys
195 200 205

Glu Trp Gln Ser Ser Met Leu His Ile Ala Ile Glu Gly Gly Cys Phe
210 215 220

Val Leu Ser Ala Cys Gln Phe Cys Leu Arg Lys Asp Phe Pro Asp His
225 230 235 240

Pro Asp Tyr Leu Phe Thr Asp Trp Tyr Asp Asp Lys Glu Pro Asp Ser
245 250 255

Ile Val Ser Gln Gly Gly Ser Val Ile Ile Ser Pro Leu Gly Gln Val
260 265 270

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

Asp Leu Gly Asp Val Ala Arg Ala Lys Leu Tyr Phe Asp Ser Val Gly
290 295 300

His Tyr Ser Arg Pro Asp Val Leu His Leu Thr Val Asn Glu His Pro
305 310 315 320

Lys Lys Pro Val Thr Phe Ile Ser Lys Val Glu Lys Ala Glu Asp Asp
325 330 335

Ser Asn Lys

<210> 11

<211> 334

<212> PRT

<213> Synechococcus elongatus PCC 7942

<400> 11

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Met Ala Asp Lys Ile Ile Val Ala Ala Ala Gln Ile Arg Pro Val Leu
1      5      10      15

Phe Ser Leu Glu Gly Ser Val Ala Arg Val Leu Ala Ala Met Ala Glu
20     25     30

Ala Ala Ala Ala Gly Val Gln Leu Ile Val Phe Pro Glu Thr Phe Leu
35     40     45

Pro Tyr Tyr Pro Tyr Phe Ser Phe Val Glu Pro Pro Val Leu Met Gly
50     55     60

Arg Ser His Leu Lys Leu Tyr Glu Gln Ala Phe Thr Met Thr Gly Pro
65     70     75     80

Glu Leu Gln Gln Ile Ala Arg Ala Ala Arg Gln His Arg Leu Phe Val
85     90     95

Leu Leu Gly Val Asn Glu Arg Asp Gly Gly Ser Leu Tyr Asn Thr Gln
100    105    110

Leu Leu Ile Ser Asp Gln Gly Asp Leu Leu Leu Lys Arg Arg Lys Ile
115    120    125

Thr Pro Thr Tyr His Glu Arg Met Val Trp Gly Gln Gly Gly Gly Ala
130    135    140

Gly Leu Thr Val Val Glu Thr Val Leu Gly Lys Val Gly Ala Leu Ala
145    150    155    160

Cys Trp Glu His Tyr Asn Pro Leu Ala Arg Phe Ser Leu Met Thr Gln
165    170    175

Gly Glu Glu Ile His Cys Ala Gln Phe Pro Gly Ser Leu Val Gly Pro
180    185    190

Ile Phe Ser Glu Gln Thr Ala Val Thr Leu Arg His His Ala Leu Glu
195    200    205

Ala Gly Cys Phe Val Leu Ser Ser Thr Ala Trp Leu Asp Pro Ala Asp
210    215    220

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

Gly Cys His Thr Ala Ile Ile Ser Pro Glu Gly Arg Tyr Leu Ala Gly
245    250    255

Pro Leu Pro Glu Gly Glu Gly Leu Ala Ile Ala Glu Leu Asp Lys Ser
260    265    270

Leu Ile Thr Lys Arg Lys Arg Met Met Asp Ser Val Gly His Tyr Ser
275    280    285

Arg Pro Asp Leu Leu Ser Leu Arg Ile Asn Arg Ser Pro Ala Thr Gln
290    295    300

Val Gln Ala Ile Gly Ser Ala Ala Ala Leu Pro Glu Leu Pro Asn Leu
305    310    315    320

Glu Ala Ala Pro Ala Glu Thr Ala Glu Asp Tyr Leu His Ala
325    330

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<210> 12

<211> 334

<212> PRT

<213> *Synechococcus elongatus* PCC 6301

<400> 12

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

<210> 13

<211> 346

<212> PRT

<213> Synechocystis sp. PCC 6803

<400> 13

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

<210> 14

<211> 307

<212> PRT

<213> Pseudomonas entomophila L48

<400> 14

Met Pro Lys Ser Ile Val Ala Ala Leu Gln Val Gly Ser Leu Pro Glu
1 5 10 15

Gly Lys Ala Ala Thr Leu Glu Gln Ile Leu Gly Tyr Glu Gln Ala Ile
20 25 30

Arg Glu Ala Gly Ala Arg Leu Val Val Met Pro Glu Ala Leu Leu Gly
35 40 45

Gly Tyr Pro Lys Gly Glu Gly Phe Gly Thr Gln Leu Gly Tyr Arg Leu
50 55 60

Pro Glu Gly Arg Glu Ala Phe Ala Arg Tyr Phe Ala Asn Ala Ile Asp
65 70 75 80

Val Pro Gly Ser Glu Thr Ala Ala Leu Ala Gly Leu Ser Ala Arg Thr
85 90 95

Gly Ala Ser Leu Val Leu Gly Val Ile Glu Arg Ser Gly Asn Thr Leu
100 105 110

Tyr Cys Thr Val Leu Phe Phe Glu Pro Glu Gly Gly Leu Val Ala Lys
115 120 125

His Arg Lys Leu Met Pro Thr Gly Thr Glu Arg Leu Ile Trp Gly Lys
130 135 140

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

Gly Ala Ala Val Cys Trp Glu Asn Tyr Met Pro Leu Leu Arg Thr Ala
165 170 175

Met Tyr Ala Lys Gly Val Gln Leu Trp Cys Ala Pro Thr Val Asp Glu
180 185 190

Arg Glu Leu Trp Gln Val Ser Met Arg His Val Ala Ala Glu Gly Arg
195 200 205

Cys Phe Val Ile Ser Ala Cys Gln Val Gln Asp Ser Pro Ala Ala Leu
210 215 220

Gly Met Glu Val Ala Asn Trp Pro Ala Glu Arg Pro Leu Ile Asn Gly
225 230 235 240

Gly Ser Leu Ile Val Gly Pro Leu Gly Asp Val Leu Ala Gly Pro Leu
245 250 255

Leu Gly Ala Arg Gly Leu Val Cys Ala Glu Val Asp Thr Asp Glu Leu
260 265 270

Val Arg Ala Arg Tyr Asp Phe Asp Val Val Gly His Tyr Ala Arg Pro
275 280 285

Asp Val Phe Glu Leu Ser Val Asp Glu Arg Pro Arg Pro Gly Val Arg
290 295 300

Phe Ile Gly
305

<210> 15

<211> 329

<212> PRT

<213> *Zymomonas mobilis* subsp. *mobilis* ZM4

<400> 15

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

<210> 16

<211> 339

<212> PRT

<213> Bacillus sp. OxB-1

<400> 16

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Met Ser Asn Tyr Pro Lys Tyr Arg Val Ala Ala Val Gln Ala Ser Pro
1      5      10      15

Val Leu Leu Asp Leu Asp Ala Thr Ile Asp Lys Thr Cys Arg Leu Val
20      25      30

Asp Glu Ala Ala Ala Asn Gly Ala Lys Val Ile Ala Phe Pro Glu Ala
35      40      45

Phe Ile Pro Gly Tyr Pro Trp Trp Ile Trp Leu Gly Asn Ala Asp Tyr
50      55      60

Gly Met Lys Tyr Tyr Ile Gln Leu Tyr Lys Asn Ser Val Glu Ile Pro
65      70      75      80

Ser Leu Ala Val Gln Lys Leu Ser Ser Ala Gly Thr Asn Lys Val Tyr
85      90      95

Phe Cys Val Ser Val Thr Glu Lys Asp Gly Gly Ser Leu Tyr Leu Thr
100     105     110

Gln Leu Trp Phe Asp Pro Asn Gly Asp Leu Ile Gly Lys His Arg Lys
115     120     125

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

Ser Met Met Pro Val Phe Glu Thr Glu Phe Gly Asn Leu Gly Gly Leu
145     150     155     160

Gln Cys Trp Glu His Phe Leu Pro Leu Asn Val Ala Ala Met Ala Ser
165     170     175

Met Asn Glu Gln Val His Val Ala Ser Trp Pro Ile Gly Met Pro Gln
180     185     190

Glu Gly His Leu Phe Gly Pro Glu Gln Cys Val Thr Ala Thr Lys Tyr
195     200     205

Tyr Ala Ile Ser Asn Gln Val Phe Cys Leu Leu Ser Ser Gln Ile Trp
210     215     220

Thr Glu Glu Gln Arg Asp Lys Ile Cys Glu Thr Glu Glu Gln Arg Asn
225     230     235     240

Phe Met Lys Val Gly His Gly Phe Ser Lys Ile Ile Ala Pro Asn Gly
245     250     255

Met Glu Ile Gly Asn Lys Leu Ala His Asp Glu Glu Gly Ile Thr Tyr
260     265     270

Ala Asp Ile Asp Leu Glu Gln Ile Ile Pro Gly Lys Phe Leu Ile Asp
275     280     285

Ser Ala Gly His Tyr Ser Thr Pro Gly Phe Leu Ser Leu Ser Phe Asp
290     295     300

Arg Thr Glu Lys Lys Pro Ile Lys His Ile Gly Glu Ser Ala Gln Glu
305     310     315     320

Thr Val Thr Tyr Glu Glu Ile Gln Tyr Gly Asn Lys Ala Asn Val Lys
325     330     335

Val His Ser

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<210> 17

<211> 354

<212> PRT

<213> Comamonas testosterone

<400> 17

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Met Lys Asn Tyr Pro Thr Val Lys Val Ala Ala Val Gln Ala Ala Pro
1      5      10      15

Val Phe Met Asn Leu Glu Ala Thr Val Asp Lys Thr Cys Lys Leu Ile
20      25      30

Ala Glu Ala Ala Ser Met Gly Ala Lys Val Ile Gly Phe Pro Glu Ala
35      40      45

Phe Ile Pro Gly Tyr Pro Tyr Trp Ile Trp Thr Ser Asn Met Asp Phe
50      55      60

Thr Gly Met Met Trp Ala Val Leu Phe Lys Asn Ala Ile Glu Ile Pro
65      70      75      80

Ser Lys Glu Val Gln Gln Ile Ser Asp Ala Ala Lys Lys Asn Gly Val
85      90      95

Tyr Val Cys Val Ser Val Ser Glu Lys Asp Asn Ala Ser Leu Tyr Leu
100     105     110

Thr Gln Leu Trp Phe Asp Pro Asn Gly Asn Leu Ile Gly Lys His Arg
115     120     125

Lys Phe Lys Pro Thr Ser Ser Glu Arg Ala Val Trp Gly Asp Gly Asp
130     135     140

Gly Ser Met Ala Pro Val Phe Lys Thr Glu Tyr Gly Asn Leu Gly Gly
145     150     155     160

Leu Gln Cys Trp Glu His Ala Leu Pro Leu Asn Ile Ala Ala Met Gly
165     170     175

Ser Leu Asn Glu Gln Val His Val Ala Ser Trp Pro Ala Phe Val Pro
180     185     190

Lys Gly Ala Val Ser Ser Arg Val Ser Ser Ser Val Cys Ala Ser Thr
195     200     205

Asn Ala Met His Gln Ile Ile Ser Gln Phe Tyr Ala Ile Ser Asn Gln
210     215     220

Val Tyr Val Ile Met Ser Thr Asn Leu Val Gly Gln Asp Met Ile Asp
225     230     235     240

Met Ile Gly Lys Asp Glu Phe Ser Lys Asn Phe Leu Pro Leu Gly Ser
245     250     255

Gly Asn Thr Ala Ile Ile Ser Asn Thr Gly Glu Ile Leu Ala Ser Ile
260     265     270

Pro Gln Asp Ala Glu Gly Ile Ala Val Ala Glu Ile Asp Leu Asn Gln
275     280     285

Ile Ile Tyr Gly Lys Trp Leu Leu Asp Pro Ala Gly His Tyr Ser Thr
290     295     300

Pro Gly Phe Leu Ser Leu Thr Phe Asp Gln Ser Glu His Val Pro Val
305     310     315     320

Lys Lys Ile Gly Glu Gln Thr Asn His Phe Ile Ser Tyr Glu Asp Leu
325     330     335

His Glu Asp Lys Met Asp Met Leu Thr Ile Pro Pro Arg Arg Val Ala
340     345     350

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Thr Ala

<210> 18

<211> 332

<212> PRT

<213> *Synechococcus* sp. CC9605

<400> 18

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Met Thr Thr Val Lys Val Ala Ala Ala Gln Ile Arg Pro Val Leu Phe
1      5      10      15

Ser Leu Asp Gly Ser Leu Gln Lys Val Leu Asp Ala Met Ala Glu Ala
      20      25      30

Ala Ala Gln Gly Val Glu Leu Ile Val Phe Pro Glu Thr Phe Leu Pro
      35      40      45

Tyr Tyr Pro Tyr Phe Ser Phe Val Glu Pro Pro Val Leu Met Gly Arg
      50      55      60

Ser His Leu Ala Leu Tyr Glu Gln Ala Val Val Val Pro Gly Pro Val
      65      70      75      80

Thr Asp Ala Val Ala Ala Ala Ala Ser Gln Tyr Gly Met Gln Val Leu
      85      90      95

Leu Gly Val Asn Glu Arg Asp Gly Gly Thr Leu Tyr Asn Thr Gln Leu
      100      105      110

Leu Phe Asn Ser Cys Gly Glu Leu Val Leu Lys Arg Arg Lys Ile Thr
      115      120      125

Pro Thr Tyr His Glu Arg Met Val Trp Gly Gln Gly Asp Gly Ser Gly
      130      135      140

Leu Lys Val Val Gln Thr Pro Leu Ala Arg Val Gly Ala Leu Ala Cys
      145      150      155      160

Trp Glu His Tyr Asn Pro Leu Ala Arg Tyr Ala Leu Met Ala Gln Gly
      165      170      175

Glu Glu Ile His Cys Ala Gln Phe Pro Gly Ser Leu Val Gly Pro Ile
      180      185      190

Phe Thr Glu Gln Thr Ala Val Thr Met Arg His His Ala Leu Glu Ala
      195      200      205

Gly Cys Phe Val Ile Cys Ser Thr Gly Trp Leu His Pro Asp Asp Tyr
      210      215      220

Ala Ser Ile Thr Ser Glu Ser Gly Leu His Lys Ala Phe Gln Gly Gly
      225      230      235      240

Cys His Thr Ala Val Ile Ser Pro Glu Gly Arg Tyr Leu Ala Gly Pro
      245      250      255

Leu Pro Asp Gly Glu Gly Leu Ala Ile Ala Asp Leu Asp Leu Ala Leu
      260      265      270

Ile Thr Lys Arg Lys Arg Met Met Asp Ser Val Gly His Tyr Ser Arg
      275      280      285

Pro Glu Leu Leu Ser Leu Gln Ile Asn Ser Ser Pro Ala Val Pro Val
      290      295      300

Gln Asn Met Ser Thr Ala Ser Val Pro Leu Glu Pro Ala Thr Ala Thr
      305      310      315      320

Asp Ala Leu Ser Ser Met Glu Ala Leu Asn His Val
      325      330

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<210> 19

<211> 306

<212> PRT

<213> *Pseudomonas fluorescens* Pf-5

<400> 19

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Met Pro Lys Ser Val Val Ala Ala Leu Gln Ile Gly Ala Leu Pro Glu
1      5      10      15

Gly Lys Ala Ala Thr Leu Glu Gln Ile Leu Ser Tyr Glu Ala Ala Ile
20      25      30

Ile Glu Ala Gly Ala Gln Leu Val Val Met Pro Glu Ala Leu Leu Gly
35      40      45

Gly Tyr Pro Lys Gly Glu Gly Phe Gly Thr Gln Leu Gly Tyr Arg Leu
50      55      60

Pro Glu Gly Arg Glu Ala Phe Ala Arg Tyr Phe Ala Asn Ala Ile Glu
65      70      75      80

Val Pro Gly Val Glu Thr Asp Ala Leu Ala Ala Leu Ser Ala Arg Thr
85      90      95

Gly Ala Asn Leu Val Leu Gly Val Ile Glu Arg Ser Gly Ser Thr Leu
100     105     110

Tyr Cys Thr Ala Leu Tyr Phe Asp Pro Gln Gln Gly Leu Ser Gly Lys
115     120     125

His Arg Lys Leu Met Pro Thr Gly Thr Glu Arg Leu Ile Trp Gly Lys
130     135     140

Gly Asp Gly Ser Thr Leu Pro Val Leu Asp Thr Gln Val Gly Arg Val
145     150     155     160

Gly Ala Val Ile Cys Trp Glu Asn Met Met Pro Leu Leu Arg Thr Ala
165     170     175

Met Tyr Ala Gln Gly Ile Glu Val Trp Cys Ala Pro Thr Val Asp Glu
180     185     190

Arg Glu Met Trp Gln Val Ser Met Arg His Ile Ala His Glu Gly Arg
195     200     205

Cys Phe Val Val Ser Ala Cys Gln Val Gln Ala Ser Pro Glu Glu Leu
210     215     220

Gly Leu Glu Ile Ala Asn Trp Pro Ala Gln Arg Pro Leu Ile Ala Gly
225     230     235     240

Gly Ser Val Ile Val Gly Pro Met Gly Asp Val Leu Ala Gly Pro Leu
245     250     255

Val Gly Arg Ala Gly Leu Ile Ser Ala Gln Ile Asp Thr Ala Asp Leu
260     265     270

Val Arg Ala Arg Tyr Asp Tyr Asp Val Val Gly His Tyr Ala Arg Pro
275     280     285

Asp Val Phe Glu Leu Thr Val Asp Gln Arg Pro Arg Pro Gly Val Arg
290     295     300

Phe Thr
305

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<210> 20

<211> 336

<212> PRT

<213> *Nocardia farcinica* IFM 10152]

<400> 20

Met Ser Gln Arg Asp Ser Phe Arg Ala Ala Ala Val Gln Ala Ala Pro
1 5 10 15

Val Trp Leu Asp Gly Ala Ala Thr Val Asp Lys Cys Val Ala Leu Ile
20 25 30

Glu Glu Ala Ala Asp Asn Gly Ala Ala Leu Ile Ala Phe Pro Glu Thr
35 40 45

Phe Val Pro Gly Tyr Pro Trp Trp Leu Trp Leu Asp Ser Pro Ala Trp
50 55 60

Gly Met Gln Phe Val Ala Arg Tyr Phe Asp Asn Ser Leu Ala Leu Asp
65 70 75 80

Gly Pro Leu Phe Ala Arg Leu Arg Glu Ala Ala Arg Arg Ser Ala Ile
85 90 95

Thr Val Val Thr Gly His Ser Glu Arg Asp Gly Gly Ser Leu Tyr Met
100 105 110

Gly Gln Ala Ile Ile Gly Ala Asp Gly Glu Val Leu Ala Ala Arg Arg
115 120 125

Lys Leu Lys Pro Thr His Val Glu Arg Thr Val Phe Gly Glu Ser Asp
130 135 140

Gly Ser Asn Leu Thr Val Val Asp Thr Glu Leu Gly Arg Leu Gly Ala
145 150 155 160

Leu Cys Cys Trp Glu His Leu Gln Pro Leu Thr Lys Tyr Ala Met Tyr
165 170 175

Ser Gln His Glu Gln Ile His Val Ala Ala Trp Pro Ser Phe Ser Val
180 185 190

Tyr Arg Gly Ala Ala Tyr Ala Leu Gly Pro Glu Val Asn Thr Gly Ala
195 200 205

Ala Arg Gln Tyr Ala Val Glu Gly Gln Cys Phe Val Leu Ser Pro Cys
210 215 220

Ala Val Ile Asp Glu Ala Gly Val Glu Leu Phe Cys Asp Thr Pro Ala
225 230 235 240

Lys Arg Glu Leu Leu Leu Pro Gly Gly Gly Phe Ala Gln Ile Tyr Gly
245 250 255

Pro Asp Gly Arg Glu Leu Gly Thr Ala Leu Pro Glu Thr Glu Glu Gly
260 265 270

Leu Val Tyr Ala Asp Leu Glu Ala Ser Ala Val Ala Val Ala Tyr Ser
275 280 285

Ala Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Gln Leu
290 295 300

Leu Trp Asp Pro Arg Pro Arg Ser Val Val Arg Gln Val Ala Leu Ser
305 310 315 320

Val Ala Ser Pro Ala Glu Ser Ala Asp Asp Ala Glu Pro Ala Val Arg
325 330 335

<210> 21

<211> 356

<212> PRT

<213> *Alcaligenes faecalis* 1650

<400> 21

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

<210> 22

<211> 336

<212> PRT

<213> *Pseudomonas syringae* pv. *syringae* B728a

<400> 22

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Met Lys Glu Pro Leu Lys Val Ala Cys Val Gln Ala Ala Pro Val Phe
1      5      10      15

Leu Asp Leu Asp Ala Thr Val Asp Lys Thr Ile Thr Leu Met Glu Gln
      20      25      30

Ala Ala Ala Ala Gly Ala Gly Leu Ile Ala Phe Pro Glu Thr Trp Ile
      35      40      45

Pro Gly Tyr Pro Trp Phe Leu Trp Leu Asp Ala Pro Ala Trp Asn Met
      50      55      60

Pro Leu Val Gln Arg Tyr His Gln Gln Ser Leu Val Leu Asp Ser Val
      65      70      75      80

Gln Ala Arg Arg Ile Ser Asp Ala Ala Arg His Leu Gly Leu Tyr Val
      85      90      95

Val Leu Gly Tyr Ser Glu Arg Asn Lys Ala Ser Leu Tyr Ile Gly Gln
      100     105     110

Trp Ile Ile Asp Asp His Gly Glu Thr Val Gly Val Arg Arg Lys Leu
      115     120     125

Lys Ala Thr His Val Glu Arg Thr Met Phe Gly Glu Gly Asp Gly Ala
      130     135     140

Ser Leu Arg Thr Phe Glu Thr Pro Val Gly Val Leu Gly Ala Leu Cys
      145     150     155     160

Cys Trp Glu His Leu Gln Pro Leu Ser Lys Tyr Ala Met Tyr Ala Gln
      165     170     175

Asn Glu Gln Ile His Val Ala Ala Trp Pro Ser Phe Ser Leu Tyr Arg
      180     185     190

Asn Ala Thr Ser Ala Leu Gly Pro Glu Val Asn Thr Ala Ala Ser Arg
      195     200     205

Val Tyr Ala Ala Glu Gly Gln Cys Phe Val Leu Ala Pro Cys Ala Ile
      210     215     220

Val Ser Pro Glu Met Ile Glu Met Leu Cys Asp Ser Asp Ala Lys Arg
      225     230     235     240

Ser Leu Leu Gln Ala Gly Gly Gly His Ala Arg Ile Phe Gly Pro Asp
      245     250     255

Gly Ser Asp Leu Ala Thr Pro Leu Gly Glu His Glu Glu Gly Leu Leu
      260     265     270

Tyr Ala Thr Leu Asp Pro Ala Ala Leu Thr Leu Ala Lys Val Ala Ala
      275     280     285

Asp Pro Ala Gly His Tyr Ser Arg Pro Asp Val Thr Arg Leu Met Phe
      290     295     300

Asn Pro Asn Pro Thr Pro Cys Val Val Asp Leu Pro Asp Leu Pro Ile
      305     310     315     320

Ser Ser Glu Ser Ile Glu Leu Leu Arg Pro Asp Ile Ala Leu Glu Val
      325     330     335

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<210> 23

<211> 345

<212> PRT

<213> Bradyrhizobium sp. BTAi1

<400> 23

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Met Gly Leu Ala His Pro Lys Tyr Lys Val Ala Val Val Gln Ala Ala
1      5      10      15

Pro Ala Trp Leu Asp Leu Asp Ala Ser Ile Lys Lys Thr Ile Ala Leu
20     25     30

Ile Glu Glu Ala Ala Asp Lys Gly Ala Lys Leu Ile Ala Phe Pro Glu
35     40     45

Val Phe Ile Pro Gly Tyr Pro Trp His Ile Trp Met Asp Ser Pro Ala
50     55     60

Trp Cys Ile Gly Arg Gly Phe Val Gln Arg Tyr Phe Asp Asn Ser Leu
65     70     75     80

Ala Tyr Asp Ser Pro Gln Ala Glu Ala Leu Arg Ala Ala Val Arg Lys
85     90     95

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

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

Lys Arg Arg Lys Leu Arg Pro Thr His Ala Glu Arg Thr Val Tyr Gly
130    135    140

Glu Gly Asp Gly Ser Asp Leu Ala Val His Glu Arg Pro Asp Ile Gly
145    150    155    160

Arg Ile Gly Ala Leu Cys Cys Trp Glu His Leu Gln Pro Leu Ser Lys
165    170    175

Tyr Ala Met Tyr Ala Gln Asn Glu Gln Val His Val Ala Ala Trp Pro
180    185    190

Ser Phe Ser Leu Tyr Asp Pro Phe Ala Pro Ala Leu Gly Ala Glu Val
195    200    205

Asn Asn Ala Ala Ser Arg Val Tyr Ala Val Glu Gly Ser Cys Phe Val
210    215    220

Leu Ala Pro Cys Ala Thr Val Ser Gln Ala Met Ile Asp Glu Leu Cys
225    230    235    240

Asp Arg Pro Asp Lys His Ala Leu Leu His Ala Gly Gly Gly His Ala
245    250    255

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

Asp Gln Glu Gly Leu Leu Ile Ala Glu Ile Asp Leu Gly Met Ile Gly
275    280    285

Ile Ala Lys Asn Ala Ala Asp Pro Ala Gly His Tyr Ser Arg Pro Asp
290    295    300

Val Thr Arg Leu Leu Leu Asn Lys Lys Pro Leu Asn Arg Val Glu His
305    310    315    320

Phe Ser Leu Pro Val Asp Ser Ala Ala Ala Ala Leu Pro Gly Glu Ala
325    330    335

Ala Val Ala Arg Pro Asp Gln Ser Ile
340    345

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<210> 24

<211> 366

<212> PRT

<213> Rhodococcus rhodochrous NCIMB 11216

<400> 24

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Met Val Glu Tyr Thr Asn Thr Phe Lys Val Ala Ala Val Gln Ala Gln
1      5      10      15

Pro Val Trp Phe Asp Ala Ala Lys Thr Val Asp Lys Thr Val Ser Ile
      20      25      30

Ile Ala Glu Ala Ala Arg Asn Gly Cys Glu Leu Val Ala Phe Pro Glu
35      40      45

Val Phe Ile Pro Gly Tyr Pro Tyr His Ile Trp Val Asp Ser Pro Leu
50      55      60

Ala Gly Met Ala Lys Phe Ala Val Arg Tyr His Glu Asn Ser Leu Thr
65      70      75      80

Met Asp Ser Pro His Val Gln Arg Leu Leu Asp Ala Ala Arg Asp His
      85      90      95

Asn Ile Ala Val Val Val Gly Ile Ser Glu Arg Asp Gly Gly Ser Leu
      100      105      110

Tyr Met Thr Gln Leu Ile Ile Asp Ala Asp Gly Gln Leu Val Ala Arg
115      120      125

Arg Arg Lys Leu Lys Pro Thr His Val Glu Arg Ser Val Tyr Gly Glu
130      135      140

Gly Asn Gly Ser Asp Ile Ser Val Tyr Asp Met Pro Phe Ala Arg Leu
145      150      155      160

Gly Ala Leu Asn Cys Trp Glu His Phe Gln Thr Leu Thr Lys Tyr Ala
165      170      175

Met Tyr Ser Met His Glu Gln Val His Val Ala Ser Trp Pro Gly Met
180      185      190

Ser Leu Tyr Gln Pro Glu Val Pro Ala Phe Gly Val Asp Ala Gln Leu
195      200      205

Thr Ala Thr Arg Met Tyr Ala Leu Glu Gly Gln Thr Phe Val Val Cys
210      215      220

Thr Thr Gln Val Val Thr Pro Glu Ala His Glu Phe Phe Cys Glu Asn
225      230      235      240

Glu Glu Gln Arg Lys Leu Ile Gly Arg Gly Gly Gly Phe Ala Arg Ile
245      250      255

Ile Gly Pro Asp Gly Arg Asp Leu Ala Thr Pro Leu Ala Glu Asp Glu
260      265      270

Glu Gly Ile Leu Tyr Ala Asp Ile Asp Leu Ser Ala Ile Thr Leu Ala
275      280      285

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Lys Gln Ala Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu
290 295 300

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

Ser Thr Ile His Ala Thr His Thr Phe Val Pro Gln Phe Gly Ala Leu
325 330 335

Asp Gly Val Arg Glu Leu Asn Gly Ala Asp Glu Gln Arg Ala Leu Pro
340 345 350

Ser Thr His Ser Asp Glu Thr Asp Arg Ala Thr Ala Thr Leu
355 360 365

<210> 25

<211> 366

<212> PRT

<213> Rhodococcus rhodochrous ATCC 39484

<400> 25

Met Val Glu Tyr Thr Asn Thr Phe Lys Val Ala Ala Val Gln Ala Gln
1 5 10 15

Pro Val Trp Phe Asp Ala Ala Lys Thr Val Asp Lys Thr Val Ser Ile
20 25 30

Ile Ala Glu Ala Ala Arg Asn Gly Cys Glu Leu Val Ala Phe Pro Glu
35 40 45

Val Phe Ile Pro Gly Tyr Pro Tyr His Ile Trp Val Asp Ser Pro Leu
50 55 60

Ala Gly Met Ala Lys Phe Ala Val Arg Tyr His Glu Asn Ser Leu Thr
65 70 75 80

Met Asp Ser Pro His Val Gln Arg Leu Leu Asp Ala Ala Arg Asp His
85 90 95

Asn Ile Ala Val Val Val Gly Ile Ser Glu Arg Asp Gly Gly Ser Leu
100 105 110

Tyr Met Thr Gln Leu Ile Ile Asp Ala Asp Gly Gln Leu Val Ala Arg
115 120 125

Arg Arg Lys Leu Lys Pro Thr His Val Glu Arg Ser Val Tyr Gly Glu
130 135 140

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

<210> 26

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 26

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu 1 5 10 15	48
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile 20 25 30	96
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu 35 40 45	144
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys 50 55 60	192
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu 65 70 75 80	240
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys 85 90 95	288
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr 100 105 110	336
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg 115 120 125	384
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly 130 135 140	432
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly 145 150 155 160	480
gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa cag agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln Gln Ser Ile Glu Ala Asn Ala Thr 195 200 205	624
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg	672

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Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210                215                220

acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac      720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225                230                235                240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac      768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245                250                255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag      816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260                265                270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag      864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275                280                285

gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg      912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290                295                300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att      960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305                310                315                320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga      1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
325                330                335

ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga      1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340                345                350

acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca      1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
355                360                365

aag tag
Lys
1110

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<210> 27

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 27

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1          5          10          15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20         25         30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35         40         45

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

Lys

<210> 28

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 28

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atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag      48
Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc      96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20     25     30

atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa     144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35     40     45

gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag     192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50     55     60

tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta     240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65     70     75     80

ggg gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa     288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85     90     95

atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat     336

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100    105    110

ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg     384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
115    120    125

cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc     432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130    135    140

aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt     480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145    150    155    160

gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg     528
Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
165    170    175

tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc     576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180    185    190

cct ctt cag ccg gat gtt ttc caa gct agc atc gaa gcc aac gcg acg     624
Pro Leu Gln Pro Asp Val Phe Gln Ala Ser Ile Glu Ala Asn Ala Thr
195    200    205

gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg     672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210    215    220

acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac     720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225    230    235    240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac     768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245    250    255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag     816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260    265    270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag     864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275    280    285

gct gga gcc gat ccg gtc ggg cac tat tcg cgg cct gac gtg ctg tcg     912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290    295    300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att     960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305    310    315    320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga     1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
325    330    335

ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga     1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly

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          340          345          350
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca 1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
          355          360          365

aag tag 1110
Lys

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<210> 29

<211> 369

<212> PRT

<213> *Acidovorax facilis* 72W

<400> 29

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1          5          10          15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
          20          25          30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
          35          40          45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
          50          55          60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65          70          75          80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
          85          90          95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
          100          105          110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
          115          120          125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
          130          135          140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145          150          155          160

Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
          165          170          175

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

Lys

<210> 30

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 30

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu 1 5 10 15	48
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile 20 25 30	96
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu 35 40 45	144
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys 50 55 60	192
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu 65 70 75 80	240
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys 85 90 95	288
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr 100 105 110	336
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg 115 120 125	384
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly 130 135 140	432
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly 145 150 155 160	480
gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa tgt agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln Cys Ser Ile Glu Ala Asn Ala Thr 195 200 205	624
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser 210 215 220	672
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp 225 230 235 240	720

225	230	235	240	
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac				768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr				
	245	250	255	
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag				816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu				
	260	265	270	
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag				864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys				
	275	280	285	
gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg				912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser				
	290	295	300	
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att				960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile				
	305	310	315	320
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga				1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg				
	325	330	335	
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga				1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly				
	340	345	350	
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca				1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala				
	355	360	365	
aag tag				1110
Lys				

<210> 31

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 31

Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu			
1	5	10	15
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile			
20	25	30	
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu			
35	40	45	
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys			
50	55	60	

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

Lys

<210> 32

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 32

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu 1 5 10 15	48
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile 20 25 30	96
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu 35 40 45	144
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys 50 55 60	192
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu 65 70 75 80	240
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys 85 90 95	288
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr 100 105 110	336
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg 115 120 125	384
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly 130 135 140	432
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc glc ggt Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly 145 150 155 160	480
gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa acc agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln Thr Ser Ile Glu Ala Asn Ala Thr 195 200 205	624
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser 210 215 220	672
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp 225 230 235 240	720
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr 245 250 255	768
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu 260 265 270	816
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys 275 280 285	864
gct gga gcc gat ccg gtc ggg cac tat tcg cgg cct gac gtg ctg tcg Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser 290 295 300	912
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile 305 310 315 320	960
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg 325 330 335	1008
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly 340 345 350	1056
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala 355 360 365	1104
aag tag Lys	1110

<210> 33

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 33

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
      20      25      30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
      35      40      45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
      50      55      60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
      65      70      75      80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
      85      90      95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
      100      105      110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
      115      120      125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
      130      135      140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
      145      150      155      160

Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
      165      170      175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
      180      185      190

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

Lys

<210> 34

<211> 1110

<212> DNA

<213> *Acidovorax facilis* 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 34

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag
 Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu

48

1	5	10	15	
cgc gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc				96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile	20	25	30	
atc gaa gaa gct gcc caa aag ggc gcg agt ctc atc gct ttc ccg gaa				144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu	35	40	45	
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag				192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys	50	55	60	
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta				240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu	65	70	75	80
ggc gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa				288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys	85	90	95	
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat				336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr	100	105	110	
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat ccg				384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg	115	120	125	
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc				432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly	130	135	140	
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt				480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly	145	150	155	160
gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg				528
Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met	165	170	175	
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc				576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser	180	185	190	
cct ctt cag ccg gat gtt ttc caa gga agc atc gaa gcc aac gcg acg				624
Pro Leu Gln Pro Asp Val Phe Gln Gly Ser Ile Glu Ala Asn Ala Thr	195	200	205	
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg				672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser	210	215	220	
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac				720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp	225	230	235	240
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac				768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr	245	250	255	
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag				816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu	260	265	270	
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag				864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys	275	280	285	
gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg				912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser	290	295	300	
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att				960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile	305	310	315	320
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga				1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg	325	330	335	
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag ccg ctc gga				1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly	340	345	350	
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca				1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala	355	360	365	
aag tag				1110
Lys				

<210> 35

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 35

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20     25     30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35     40     45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50     55     60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65     70     75     80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85     90     95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100    105    110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
115    120    125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130    135    140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145    150    155    160

Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
165    170    175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180    185    190

Pro Leu Gln Pro Asp Val Phe Gln Gly Ser Ile Glu Ala Asn Ala Thr
195    200    205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210    215    220

Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225    230    235    240

Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245    250    255

Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260    265    270

Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275    280    285

Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290    295    300

Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305    310    315    320

Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg

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[illegible]

aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly 145 150 155 160	480
gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa cac agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln His Ser Ile Glu Ala Asn Ala Thr 195 200 205	624
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser 210 215 220	672
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp 225 230 235 240	720
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr 245 250 255	768
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu 260 265 270	816
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys 275 280 285	864
gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser 290 295 300	912
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile 305 310 315 320	960
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg 325 330 335	1008
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly 340 345 350	1056
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala 355 360 365	1104
aag tag Lys	1110

<210> 37

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 37

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20      25      30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35      40      45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50      55      60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65      70      75      80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85      90      95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100     105     110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
115     120     125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130     135     140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145     150     155     160

Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
165     170     175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180     185     190

Pro Leu Gln Pro Asp Val Phe Gln His Ser Ile Glu Ala Asn Ala Thr
195     200     205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser

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210 215 220
 Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
 225 230 235 240
 Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
 245 250 255
 Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
 260 265 270
 Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
 275 280 285
 Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
 290 295 300
 Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
 305 310 315 320
 Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
 325 330 335
 Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
 340 345 350
 Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
 355 360 365

Lys

<210> 38

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 38

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag 48
 Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
 1 5 10 15
 ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc 96
 Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
 20 25 30

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atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa 144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
      35              40              45

gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag 192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
      50              55              60

tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta 240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
      65              70              75              80

ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa 288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
      85              90              95

atc gca ctc gtc atg ggc tat tcg gag ccg gaa gcc gga tcg cgc tat 336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
      100              105              110

ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat ccg 384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
      115              120              125

cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc 432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
      130              135              140

aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt 480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
      145              150              155              160

gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg 528
Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
      165              170              175

tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc 576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
      180              185              190

cct ctt cag ccg gat gtt ttc caa aag agc atc gaa gcc aac gcg acg 624
Pro Leu Gln Pro Asp Val Phe Gln Lys Ser Ile Glu Ala Asn Ala Thr
      195              200              205

gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg 672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
      210              215              220

acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac 720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
      225              230              235              240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac 768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
      245              250              255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag 816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
      260              265              270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag 864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
      275              280              285

gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg 912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
      290              295              300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att 960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
      305              310              315              320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga 1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
      325              330              335

ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag ccg ctc gga 1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
      340              345              350

acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca 1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
      355              360              365

aag tag 1110
Lys

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<210> 39

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 39

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20     25     30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35     40     45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50     55     60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65     70     75     80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85     90     95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100    105    110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Gln Ile Val Ala Asn Arg
115    120    125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130    135    140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145    150    155    160

Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
165    170    175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180    185    190

Pro Leu Gln Pro Asp Val Phe Gln Lys Ser Ile Glu Ala Asn Ala Thr
195    200    205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210    215    220

Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225    230    235    240

Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245    250    255

Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260    265    270

Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275    280    285

Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290    295    300

Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305    310    315    320

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

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340    345    350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
355    360    365

Lys

```

<210> 40

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 40

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag	48
Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu	
1 5 10 15	
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc	96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile	
20 25 30	
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa	144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu	
35 40 45	
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag	192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys	
50 55 60	
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta	240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu	
65 70 75 80	
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa	288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys	
85 90 95	
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat	336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr	
100 105 110	
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg	384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg	
115 120 125	
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc	432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly	
130 135 140	
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt	480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly	
145 150 155 160	

gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa aat agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln Asn Ser Ile Glu Ala Asn Ala Thr 195 200 205	624
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser 210 215 220	672
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp 225 230 235 240	720
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr 245 250 255	768
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu 260 265 270	816
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys 275 280 285	864
gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser 290 295 300	912
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile 305 310 315 320	960
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg 325 330 335	1008
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly 340 345 350	1056
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala 355 360 365	1104
aag tag Lys	1110

<210> 41

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 41

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20      25      30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35      40      45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50      55      60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65      70      75      80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85      90      95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100     105     110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
115     120     125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130     135     140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145     150     155     160

Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
165     170     175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180     185     190

Pro Leu Gln Pro Asp Val Phe Gln Asn Ser Ile Glu Ala Asn Ala Thr
195     200     205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210     215     220

Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225     230     235     240

Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245     250     255

Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260     265     270

Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275     280     285

Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290     295     300

Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305     310     315     320

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

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340     345     350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
355     360     365

Lys

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<210> 42

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 42

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atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag      48
Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc      96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20     25     30

atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa      144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35     40     45

gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag      192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50     55     60

tac agc cta age ttt act tca cgc tat cac gag aat tog ttg gag cta      240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65     70     75     80

ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa      288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85     90     95

atc gca ctc gtc atg ggc tat tgc gag cgg gaa gcc gga tcg cgc tat      336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100    105    110

ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg      384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
115    120    125

cgc aag ctg aag ccc aca cac gll gag cgl acg atc lac ggc gaa ggc      432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130    135    140

aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt      480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145    150    155    160

gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg      528
Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
165    170    175

tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc      576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180    185    190

cct ctt cag ccg gat gtt ttc caa tct agc atc gaa gcc aac gcg acg      624
Pro Leu Gln Pro Asp Val Phe Gln Ser Ser Ile Glu Ala Asn Ala Thr
195    200    205

gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg      672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210    215    220

acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac      720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225    230    235    240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac      768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245    250    255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag      816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260    265    270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag      864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275    280    285

gct gga gcc gat ccg gtc ggg cac tat tcg cgg cct gac gtg ctg tcg      912

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Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
 290 295 300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att 960
 Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
 305 310 315 320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga 1008
 Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
 325 330 335

ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga 1056
 Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
 340 345 350

acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca 1104
 Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
 355 360 365

aag tag 1110
 Lys

<210> 43

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 43

Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
 1 5 10 15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
 20 25 30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
 35 40 45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
 50 55 60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
 65 70 75 80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
 85 90 95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
 100 105 110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
 115 120 125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
 130 135 140
 Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
 145 150 155 160
 Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
 165 170 175
 Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
 180 185 190
 Pro Leu Gln Pro Asp Val Phe Gln Ser Ser Ile Glu Ala Asn Ala Thr
 195 200 205
 Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
 210 215 220
 Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
 225 230 235 240
 Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
 245 250 255
 Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
 260 265 270
 Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
 275 280 285
 Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
 290 295 300
 Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
 305 310 315 320
 Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
 325 330 335
 Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
 340 345 350
 Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
 355 360 365

Lys

<210> 44

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 44

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag	48
Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu	
1 5 10 15	
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc	96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile	
20 25 30	
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa	144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu	
35 40 45	
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag	192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys	
50 55 60	
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta	240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu	
65 70 75 80	
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa	288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys	
85 90 95	
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat	336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr	
100 105 110	
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg	384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg	
115 120 125	
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc	432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly	
130 135 140	
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt	480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly	
145 150 155 160	
gga ttg aac tgc tgg gaa cat aaa caa ccg ctc agc aag ttc atg atg	528
Gly Leu Asn Cys Trp Glu His Lys Gln Pro Leu Ser Lys Phe Met Met	
165 170 175	
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc	576

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser	
180 185 190	
cct ctt cag ccg gat gtt ttc caa ctg agc atc gaa gcc aac gcg acg	624
Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr	
195 200 205	
gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg	672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser	
210 215 220	
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac	720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp	
225 230 235 240	
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac	768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr	
245 250 255	
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag	816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu	
260 265 270	
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag	864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys	
275 280 285	
gct gga gcc gat ccg gtc ggg cac tat tcg cgg cct gac gtg ctg tcg	912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser	
290 295 300	
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att	960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile	
305 310 315 320	
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga	1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg	
325 330 335	
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga	1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly	
340 345 350	
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca	1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala	
355 360 365	
aag tag	1110
Lys	

<210> 45

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 45

Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1 5 10 15

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

Lys

<210> 46

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 46

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atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag      48
Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1      5      10      15

ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc      96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20     25     30

atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa     144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35     40     45

gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag     192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
50     55     60

tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta     240

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
65     70     75     80

ggg gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa     288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
85     90     95

atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat     336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
100    105    110

ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat ccg     384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
115    120    125

cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc     432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
130    135    140

aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt     480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145    150    155    160

gga ttg aac tgc tgg gaa cat atg caa ccg ctc agc aag ttc atg atg     528
Gly Leu Asn Cys Trp Glu His Met Gln Pro Leu Ser Lys Phe Met Met
165    170    175

tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc     576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180    185    190

cct ctt cag ccg gat gtt ttc caa ctg agc atc gaa gcc aac gcg acg     624
Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr
195    200    205

gtc acc cgc tcg tac gca atc gaa gcc caa acc ttt gtg ctt tgc tcg     672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210    215    220

acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac     720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225    230    235    240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac     768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245    250    255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag     816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260    265    270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag     864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275    280    285

gct gga gcc gat ccg gtc ggg cac tat tcg cgg cct gac gtg ctg tcg     912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290    295    300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att     960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile

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305	310	315	320	
gac ggt cgc ttg gat	gtg aat acc cgc agt	cgc gtg gag aat ttc cga		1008
Asp Gly Arg Leu Asp	Val Asn Thr Arg Ser	Arg Val Glu Asn Phe Arg		
	325	330	335	
ctg cga caa gcg gct	gag cag gag cgt	cag gca tcc aag cgg	ctc gga	1056
Leu Arg Gln Ala Ala	Glu Gln Glu Arg	Gln Ala Ser Lys Arg	Leu Gly	
	340	345	350	
acg aaa ctc ttt gaa	caa tcc ctt ctg	gct gaa gaa ccg	gtc cca gca	1104
Thr Lys Leu Phe Glu	Gln Ser Leu Leu	Ala Glu Glu Pro	Val Pro Ala	
	355	360	365	
aag tag				1110
Lys				

<210> 47

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 47

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

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Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
145                150                155                160

Gly Leu Asn Cys Trp Glu His Met Gln Pro Leu Ser Lys Phe Met Met
165                170                175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180                185                190

Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr
195                200                205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210                215                220

Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225                230                235                240

Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245                250                255

Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260                265                270

Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275                280                285

Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290                295                300

Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305                310                315                320

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

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340                345                350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
355                360                365

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Lys

<210> 48

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 48

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu 1 5 10 15	48
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile 20 25 30	96
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu 35 40 45	144
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys 50 55 60	192
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu 65 70 75 80	240
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys 85 90 95	288
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr 100 105 110	336
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg 115 120 125	384
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly 130 135 140	432
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly 145 150 155 160	480
gga ttg aac tgc tgg gaa cat acc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Thr Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa ctg agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr 200 205 210 215	624

195	200	205	
gtc acc cgc tgc tac gca atc gaa ggc caa acc ttt gtg ctt tgc tgc			672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser			
210	215	220	
acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac			720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp			
225	230	235	240
gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac			768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr			
245	250	255	
ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag			816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu			
260	265	270	
ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag			864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys			
275	280	285	
gct gga gcc gat ccg gtc ggg cac tat tgc cgg cct gac gtg ctg tgc			912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser			
290	295	300	
gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att			960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile			
305	310	315	320
gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga			1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg			
325	330	335	
ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga			1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly			
340	345	350	
acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca			1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala			
355	360	365	
aag tag			1110
Lys			

<210> 49

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 49

Met	Val	Ser	Tyr	Asn	Ser	Lys	Phe	Leu	Ala	Ala	Thr	Val	Gln	Ala	Glu
1				5					10					15	

Pro	Val	Trp	Leu	Asp	Ala	Asp	Ala	Thr	Ile	Asp	Lys	Ser	Ile	Gly	Ile
	20						25						30		

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

Lys

<210> 50

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 50

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atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag      48
Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1          5          10          15

ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc      96
Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
          20          25          30

atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa     144
Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
          35          40          45

gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag     192
Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
          50          55          60

tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta     240
Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
          65          70          75          80

ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa     288
Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
          85          90          95

atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat     336
Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
          100          105          110

ctg agc caq gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat ccg     384
Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
          115          120          125

cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc     432
Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
          130          135          140

aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt     480
Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
          145          150          155          160

gga ttg aac tgc tgg gaa cat gtg caa ccg ctc agc aag ttc atg atg     528
Gly Leu Asn Cys Trp Glu His Val Gln Pro Leu Ser Lys Phe Met Met
          165          170          175

tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc     576
Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
          180          185          190

cct ctt cag ccg gat gtt ttc caa ctg agc atc gaa gcc aac gcg acg     624
Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr
          195          200          205

gtc acc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg     672
Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
          210          215          220

acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac     720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
          225          230          235          240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac     768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
          245          250          255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag     816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
          260          265          270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag     864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
          275          280          285

gcl gga gcc gal ccg qlc ggg cac lal lcq cgg ccl qac qlq clq lcq     912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
          290          295          300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att     960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
          305          310          315          320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga     1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
          325          330          335

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ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag cgg ctc gga 1056
 Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
 340 345 350

acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca 1104
 Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
 355 360 365

aag tag 1110
 Lys

<210> 51

<211> 369

<212> PRT

<213> *Acidovorax facilis* 72W

<400> 51

Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
 1 5 10 15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
 20 25 30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
 35 40 45

Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
 50 55 60

Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
 65 70 75 80

Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
 85 90 95

Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
 100 105 110

Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
 115 120 125

Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
 130 135 140

Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
 145 150 155 160

Gly Leu Asn Cys Trp Glu His Val Gln Pro Leu Ser Lys Phe Met Met
165 170 175

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser
180 185 190

Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr
195 200 205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser
210 215 220

Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225 230 235 240

Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245 250 255

Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260 265 270

Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275 280 285

Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290 295 300

Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305 310 315 320

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

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340 345 350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
355 360 365

Lys

<210> 52

<211> 1110

<212> DNA

<213> Acidovorax facilis 72W

<220>

<221> CDS

<222> (1)..(1110)

<400> 52

atg gtt tcg tat aac agc aag ttc ctc gcg gca acc gtt cag gca gag Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu 1 5 10 15	48
ccg gta tgg ctc gac gca gac gca acg atc gac aag tcg atc ggc atc Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile 20 25 30	96
atc gaa gaa gct gcc caa aag ggc gcg agt ctg atc gct ttc ccg gaa Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu 35 40 45	144
gta ttc att ccg ggc tac ccc tat tgg gcg tgg ctc ggc gac gtg aag Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys 50 55 60	192
tac agc cta agc ttt act tca cgc tat cac gag aat tcg ttg gag cta Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu 65 70 75 80	240
ggt gac gac cgt atg cgt cgc ctc cag ctg gcc gcg cgc cgc aac aaa Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys 85 90 95	288
atc gca ctc gtc atg ggc tat tcg gag cgg gaa gcc gga tcg cgc tat Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr 100 105 110	336
ctg agc cag gtg ttc atc gac gag cgt ggc gag atc gtt gcc aat cgg Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg 115 120 125	384
cgc aag ctg aag ccc aca cac gtt gag cgt acg atc tac ggc gaa ggc Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly 130 135 140	432
aac gga acc gat ttc ctc acg cac gac ttc gcg ttc gga cgc gtc ggt Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly 145 150 155 160	480
gga ttg aac tgc tgg gaa cat ttc caa ccg ctc agc aag ttc atg atg Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met 165 170 175	528
tac agc ctc ggt gag cag gtc cac gtt gca tcg tgg ccg gcg atg tcc Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser 180 185 190	576
cct ctt cag ccg gat gtt ttc caa ctg agc atc gaa gcc aac gcg acg Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr 195 200 205	624
gtc gcc cgc tcg tac gca atc gaa ggc caa acc ttt gtg ctt tgc tcg Val Ala Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser 210 215 220	672

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acg cag gtg atc gga cct agc gcg atc gaa acg ttc tgc ctc aac gac      720
Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp
225                230                235                240

gaa cag cgc gca ctg ttg ccg caa gga tgt ggc tgg gcg cgc att tac      768
Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr
245                250                255

ggc ccg gat gga agc gag ctt gcg aag cct ctg gcg gaa gat gct gag      816
Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu
260                265                270

ggg atc ttg tac gca gag atc gat ctg gag cag att ctg ctg gcg aag      864
Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys
275                280                285

gct gga gcc gat ccg gtc ggg cac tat tcg ccg cct gac gtg ctg tcg      912
Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser
290                295                300

gtc cag ttc gac ccg cgc aat cat acg cca gtt cat cgc atc ggc att      960
Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile
305                310                315                320

gac ggt cgc ttg gat gtg aat acc cgc agt cgc gtg gag aat ttc cga     1008
Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
325                330                335

ctg cga caa gcg gct gag cag gag cgt cag gca tcc aag ccg ctc gga     1056
Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
340                345                350

acg aaa ctc ttt gaa caa tcc ctt ctg gct gaa gaa ccg gtc cca gca     1104
Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
355                360                365

aag tag
Lys
1110

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<210> 53

<211> 369

<212> PRT

<213> Acidovorax facilis 72W

<400> 53

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Met Val Ser Tyr Asn Ser Lys Phe Leu Ala Ala Thr Val Gln Ala Glu
1          5          10          15

Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
20          25          30

Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
35          40          45

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

Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser

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

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REFERENCES CITED IN THE DESCRIPTION

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- 1 -

FORBEDRING AF IMMOBILISERET MIKROBIEL NITRILASE TIL FREMSTILLING AF
GLYCOLSYRE

PATENTKRAV

1. Fremgangsmåde til fremstilling af en glutaraldehyd-forbehandlet, immobiliseret og tværbundet
5 enzymkatalysator, hvilken fremgangsmåde omfatter:
 - (a) fremstilling af en enzymkatalysator, der har nitrilaseaktivitet, ved fermentering;
 - (b) forbehandling af enzymkatalysatoren med glutaraldehyd;
 - (c) eventuelt inaktivering af uomsat glutaraldehyd med bisulfit efter glutaraldehydforbehandling;
 - 10 (d) genvinding af enzymkatalysatoren fra (b) eller (c) og immobilisering af enzymkatalysatoren i carrageenan;
 - (e) tværbinding af den resulterende carrageenan-immobiliserede enzymkatalysator fra (d) med glutaraldehyd og polyethylenimin og
 - (f) fremstilling af en glutaraldehyd-forbehandlet, immobiliseret og tværbundet
15 enzymkatalysator, hvor den glutaraldehyd-forbehandlede, immobiliserede og tværbundne enzymkatalysator har forbedret retention af oprindelig specifik aktivitet under omdannelse af glycolonitril til glycolsyre sammenlignet med ikke-glutaraldehyd-forbehandlede, immobiliserede og tværbundne enzymkatalysatorer under de samme reaktionsforhold;
hvor enzymkatalysatoren omfatter et polypeptid, der har en aminosyresekvens udvalgt fra gruppen
20 bestående af SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55 og 57.
2. Fremgangsmåde ifølge krav 1, hvor pH'en holdes mellem 5,0 og 9,0 under forbehandlingen med glutaraldehyd.
3. Fremgangsmåde ifølge krav 1, hvor forbehandlingen med glutaraldehyd i trin (b) omfatter
25 tilsætning af glutaraldehyd til en fermenteringsbouillon, der er fremstillet i trin (a), i en mængde, der ligger i området fra ca. 3 g/L (0,025 g GA pr. OD₅₅₀) og ca. 5 g/L (0,042 g GA pr. OD₅₅₀).
4. Fremgangsmåde ifølge krav 1, hvor forbehandlingen med glutaraldehyd i trin (b) omfatter tilsætning af glutaraldehyd til en fermenteringsbouillon fremstillet i trin (a) ved en hastighed på 50 mg/L/t til 500 mg/L/t.
- 30 5. Glutaraldehydforbehandlet, immobiliseret og tværbunden enzymkatalysator, der er fremstillet ved hjælp af fremgangsmåden ifølge krav 1.
6. Enzymkatalysator ifølge krav 5, hvor katalysatoren bibeholder mindst ca. 70 % af dens oprindelige specifikke aktivitet efter mindst fire konsekutive batch-recirkuleringsreaktioner.
7. Fremgangsmåde til hydrolysering af glycolonitril til glycolsyre, hvilken fremgangsmåde
35 omfatter:
 - (a) fremstilling af en enzymkatalysator, der har nitrilaseaktivitet, ved fermentering;
 - (b) forbehandling af enzymkatalysatoren med glutaraldehyd;

- 2 -

(c) eventuelt inaktivering af uomsat glutaraldehyd med bisulfit efter glutaraldehydforbehandling;

(d) genvinding af enzymkatalysatoren fra (b) eller (c) og immobilisering af enzymkatalysatoren i carrageenan;

- 5 (e) tværbinding af den resulterende carrageenan-immobiliserede enzymkatalysator fra (d) med glutaraldehyd og polyethylenimin, hvorved der fremstilles en tværbundet immobiliseret enzymkatalysator, hvor den glutaraldehydforbehandlede immobiliserede og tværbundne enzymkatalysator har forbedret retention af oprindelig specifik aktivitet under omdannelse af glycolonitril til glycolsyre sammenlignet med ikke-glutaraldehyd-forbehandlede, immobiliserede og
- 10 tværbundne enzymkatalysatorer under de samme reaktionsforhold; og

(f) etablering af kontakt mellem den tværbundne immobiliserede enzymkatalysator fra (e) og glycolonitril i en vandig opløsning under egnede reaktionsforhold, hvorved der fremstilles glycolsyre;

- hvor enzymkatalysatoren omfatter et polypeptid, der har en aminosyresekvens udvalgt fra gruppen bestående af SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27,
- 15 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55 og 57.

8. Fremgangsmåde ifølge krav 7, hvilken fremgangsmåde endvidere omfatter trin (g) genvinding af glycolsyre fremstillet i trin (f).

9. Fremgangsmåde ifølge krav 7, hvor den tværbundne immobiliserede enzymkatalysator bibeholder mindst ca. 70 % af dens oprindelige specifikke aktivitet efter fremstilling af mindst 40 g
- 20 glycolsyre pr. gram tørcellevægt af immobiliseret enzymkatalysator, og hvor fremgangsmåden har forbedret produktivitet for glycolsyrefremstilling sammenlignet med en fremgangsmåde til fremstilling af glycolsyre med en tværbunden immobiliseret enzymkatalysator, der omfatter en enzymkatalysator, der har nitrilaseaktivitet, og som ikke er forbehandlet med glutaraldehyd.

DRAWINGS

CLUSTAL W (.83) multiple sequence alignment

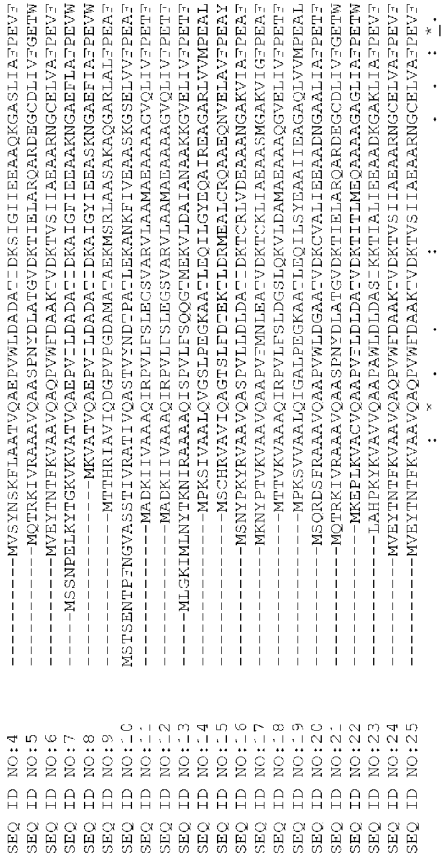


FIGURE 1A

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SEQ ID NO:20	IPGYP-----YAWWGDVWAVSD--PIPKYIENSLTLGGDDRMRLQLAARQNNIALVMGY
SEQ ID NO:21	IPGYP-----YAWWGDVWAVSD--PIPKYIENSLTLGGDDRMRLQLAARQNNIALVMGY
SEQ ID NO:22	IPGYP-----YAWWGDVWAVSD--PIPKYIENSLTLGGDDRMRLQLAARQNNIALVMGY
SEQ ID NO:23	IPGYP-----YAWWGDVWAVSD--PIPKYIENSLTLGGDDRMRLQLAARQNNIALVMGY
SEQ ID NO:24	IPGYP-----YAWWGDVWAVSD--PIPKYIENSLTLGGDDRMRLQLAARQNNIALVMGY
SEQ ID NO:25	IPGYP-----YAWWGDVWAVSD--PIPKYIENSLTLGGDDRMRLQLAARQNNIALVMGY

FIGURE 1B

[illegible]

F-GURF - C

SEQ ID NO:4	GQTFVLSTQVVG---PSAIEETFCLNDE--QRALLPQGGGWAR--YSPDGSELAKPLAED
SEQ ID NO:5	GQCTTIAASSVVT---QETILDMLEVGEIL--NASLLKVGSSSM--TAPDGRDIAPIYLPID
SEQ ID NO:6	GQTFVCTTQVVT---PEAHEPFCNDDE--QRKLIIRGGGZAR--IGPDGRDIAATPLAED
SEQ ID NO:7	GQTFVLASTIVVG---KATQDLFAGDDA-KRALPLGQGGWAR--YSPDGKSLAEPLPED
SEQ ID NO:8	GQTFVLAATHVG---KATQDLFAGDEA-KRALPLGQGGWAR--YSPDGKSLAEPLAEN
SEQ ID NO:9	GRCFVLGACQHLRGKDFPEETINALDVQF-----DTVIMRGSCVDPMQQLIAGPVY-D
SEQ ID NO:10	GRCFVLGACQCLRKDFPDIPDYL-TDWYDKDPDSIVSOGGSV--ISPLGQVLAGPNF-D
SEQ ID NO:11	AGCFVLSTAWLD---PADYDTITPDRS---LHKAFQGGCH--AISPEGRYLAGPLP-E
SEQ ID NO:12	AGCFVLSTAWLD---PADYDTITPDRS---LHKAFQGGCH--AISPEGRYLAGPLP-E
SEQ ID NO:13	SGCTVINATGWL---AEQKLQITTDEK---MIQALSGGCYTA--ISPEKILCEPIA-E
SEQ ID NO:14	GRCFVLGACQVQ---DSPAALGMEVANWPA---EREPLINGSLVGPLGDLVAGPLL-G
SEQ ID NO:15	GRFVLGACQVMTADAPADYDCHQGNDRP-----ETELIAGGSVIDPMGNILAGPLY-G
SEQ ID NO:16	NQVFCLLSSQ-WT---EQQDKICETEE-QRNFEMKVHGTSK--IAPNME-GNKLAMD
SEQ ID NO:17	NQVYVIMSTNLVG---QDMIDMIGKDEF--SKNFLPLGSGN--ALLSNTG-E-LASIPQD
SEQ ID NO:18	AGCTVISTGTWLIH FDDYASITSESG LHKAFQGGCHCAVTSFEGRYLAGPLP D
SEQ ID NO:19	GRCFVVGACQVQ---ASUEELGLFIANWPA---QRULLAGGSV-VSPMGDLVAGPLV-G
SEQ ID NO:20	GQCTVLSPCAVID---EAGVELFCDTPA--KRELLIPGGTAC--YSPDGRELGTALPPT
SEQ ID NO:21	GQCTTIAASSVVT---QETILDMLEVGEIL--NAPLLKVGSSSM--TAPDGRDIAPIYLPID
SEQ ID NO:22	GQCFVLAPCAVVS---FEMIEMLCDSDA--KRSLLQAGGGHAR--SPDSSDIAATPLGEH
SEQ ID NO:23	GSCFVLAPCAVVS---QAMIDEICDRPD--KIALLIAGGGHAA--TSPDSSALAAQLPPD
SEQ ID NO:24	GQTFVCTTQVVT---PEAHEPFCENE--QRKLIIRGGGZAR--IGPDGRDIAATPLAED
SEQ ID NO:25	GQTFVCTTQVVT---PEAHEPFCENE--QRKLIIRGGGZAR--IGPDGRDIAATPLAED

FIGURE 1E

SEQ ID NO:4	KVENRLEQAAEQRRQASKRLGTLTEQS-----LLAEPVPAK---
SEQ ID NO:5	APEPIVQSTAAAPAVSQ--QSDTLLVQEPS-----
SEQ ID NO:6	HACHLVFQSGALEGVRELNGADEQRALPS-----THSDETDRACTASI
SEQ ID NO:7	RVENYRLIQIADIKKYENALGAATLPLDAPAP-----APEQKSGRAKAEA
SEQ ID NO:8	RVENYRLQIADIKKYENADSATVPELDVTTPEKQSGDVNANGNAKYNTPSAAKAKA
SEQ ID NO:9	P-----
SEQ ID NO:10	KVEKAEDDSNK-----
SEQ ID NO:11	AAALPELPNLEAPAPAEATAEDYLHA-----
SEQ ID NO:12	AAALPELPNLEAPAPAEATAEDYLHA-----
SEQ ID NO:13	VCPNAIPAVSDPELTET--EALPNNPI--SH-----
SEQ ID NO:14	--G-----
SEQ ID NO:15	SRDQATEKKPVSDSE--SQLD-----
SEQ ID NO:16	SAQETVYEE--QYGNKANVYIIS
SEQ ID NO:17	QLNHF--LSYEDLHEDKMDMLILPFRKVA'A-----
SEQ ID NO:18	CASVPLEPACACDALSSMEALNIIV-----
SEQ ID NO:19	--
SEQ ID NO:20	VA--LSVASPAESAD-----DAEPAVR-----
SEQ ID NO:21	APEQGVQSK--ASVAISHPQSDTLLVQEPS-----
SEQ ID NO:22	LPDLFISSES--ELL-----REDIALEV-----
SEQ ID NO:23	ITS--LPVDSAAAAALPGEAAVAREDSI-----
SEQ ID NO:24	HACHCEVPQFGALEGVRELNGADEQRALPS-----THSDETDRACTAL
SEQ ID NO:25	HACHCEVPQFGALEGVRELNGADEQRALPS-----THSDETDRACTAL

FIGURE 1G