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(54) **DETERGENT COMPOSITION**

(57) The need for a hand-dishwashing composition which provides good sudsing and a good suds profile even in the presence of greasy stains comprising higher chain-length saturated and/or unsaturated fatty acid chains, as well as improved removal of such stains, is met by formulating the composition with a non-heme fatty acid decarboxylase and a surfactant system.

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**Description**

## REFERENCE TO A SEQUENCE LISTING

- 5 **[0001]** This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

## FIELD OF THE INVENTION

- 10 **[0002]** The present invention relates to a hand dishwashing detergent composition comprising a surfactant system and at least one non-heme fatty acid decarboxylase. The non-heme fatty acid decarboxylase improve sudsing and grease removal by catalyzing the conversion of at least one fatty acid selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and mixtures thereof.

## 15 BACKGROUND OF THE INVENTION

- [0003]** Hand-dishwashing detergent compositions should have a good suds profile, in particular a long lasting suds profile. Users typically connate the presence of suds with good residual cleaning, a lack of suds can lead to over-use of the detergent composition, especially in the presence of greasy soils. The appearance of the suds, such as its density and whiteness is also often seen as an indicator of the cleaning efficacy of the wash solution. However, greasy soils inhibit suds generation and promote suds collapse, even when sufficient surfactancy is present to ensure good cleaning, including grease removal. It has now been found that greasy soils containing higher chain-length saturated and/or unsaturated fatty acid chains are particularly effective at inhibiting sudsing, especially inhibiting long lasting sudsing. In addition, such greasy soils containing higher chain-length saturated and/or unsaturated fatty acid chains are particularly hard to remove from dishes. Such greasy soils comprise long chain fatty acids, especially long chain unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid, and long chain saturated fatty acids, such as palmitic acid and stearic acid, which can act as a suds suppressors. Conversion of these long chain saturated and/or unsaturated fatty acids into suds neutral or potentially suds boosting compounds is as such desired.

- [0004]** The use of two different classes of fatty acid decarboxylases, OleT-like and UndA-like, to transform these long chain saturated and/or unsaturated fatty acids and as such enhance the sudsing profile of detergent compositions have been previous reported (EP 3,243,896B1). However, OleT-like decarboxylases require H<sub>2</sub>O<sub>2</sub> as a co-substrate, which can be challenging to formulate in hand dish-washing compositions. Several efforts to substitute the use of H<sub>2</sub>O<sub>2</sub> by coupling biological redox systems that utilize O<sub>2</sub> have been done (see for example CN 10,8467,861), but the reduced catalytic efficiency of the systems suggests that the use of peroxide may be necessary for practical applications. Furthermore, UndA-like decarboxylases (US 10,000,775 B2) utilize O<sub>2</sub>, instead of H<sub>2</sub>O<sub>2</sub>, as a co-substrate, but all previously reported UndA-like variants convert exclusively medium chain fatty acids (C10-C14), with no detectable conversion of long chain fatty acids, which are particularly effective at suds inhibition and are particularly challenging to remove. Thus, there is still a need for fatty acid decarboxylases that transform such long chain fatty acids without the need of external co-substrates that are difficult to formulate in hand dish-washing compositions.

- 40 **[0005]** Hence, a need remains for a hand-dishwashing detergent which provides good sudsing and a good suds profile even in the presence of greasy stains comprising higher chain-length saturated and/or unsaturated fatty acid chains, as well as improved removal of such stains.

- [0006]** EP3243896A relates to detergent compositions, especially manual dishwashing detergent compositions and method of washing comprising a surfactant system and a fatty acid decarboxylase enzyme. US 2009/0142821 A1 relates to novel variants of cytochrome P450 oxygenases. These variants have an improved ability to use peroxide as an oxygen donor as compared to the corresponding wild-type enzyme. These variants also have an improved thermostability as compared to the cytochrome P450 BM-3 F87 A mutant. Preferred variants include cytochrome P450 BM-3 heme domain mutants having I58V, F87A, H100R, F107L, A135S, M145A/V, N239H, S274T, L3241, I366V, K434E, E442K, and/or V446I amino acid substitutions. S CHRISTOPHER DAVIS ET AL, "Oxidation of v-Oxo Fatty Acids by Cytochrome P450 BM-3 (CYP102)", ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (19960401), vol. 328, no. 1, pages 35 - 42 discusses the oxidation of aldehydes by cytochrome P450 enzymes either to the corresponding acid or, via a decarboxylation mechanism, to an olefin one carbon shorter than the parent substrate, and explores the factors that control partitioning between these two pathways. The authors have examined the cytochrome P450BM-3 (CYP102)-catalyzed oxidation of fatty acids with a terminal aldehyde group. P450BM-3 has been found to oxidize 18-oxooctadecanoic, 16-oxohexadecanoic, 14-oxotetradecanoic, and 12-oxododecanoic acids exclusively to the corresponding  $\alpha,\omega$ -diacids. The results demonstrated that aldehyde oxidation by cytochrome P450BM-3 is insensitive to changes in substrate structure expected to stabilize the transition state for decarboxylation. Decarboxylation, in contrast to the oxidation of aldehydes to acids, depends on specific substrate-protein interactions and is enzyme-specific. JAMES BELCHER ET AL., "Structure

and Biochemical Properties of the Alkene Producing Cytochrome P450 OleT<sub>JE</sub> (CYP152L1) from the *Jeotgalicoccus* sp. 8456 Bacterium", JOURNAL OF BIOLOGICAL CHEMISTRY, (20140307), vol. 289, no. 10, doi:10.1074/jbc.M113.527325, ISSN 0021-9258, pages 6535 - 6550, presents the biochemical characterization and crystal structures of a cytochrome P450 fatty acid peroxygenase: the terminal alkene forming OleT<sub>JE</sub> (CYP152L1) from *Jeotgalicoccus* sp. 8456. GIRVAN HAZEL M ET AL., "Applications of microbial cytochrome P450 enzymes in biotechnology and synthetic biology", CURRENT OPINION IN CHEMICAL BIOLOGY, (20160322), vol. 31, doi:10.1016/J.CB-PA.2016.02.018, ISSN 1367-5931, pages 136 - 145, XP029536984 [A] 1-15 is a review focusing on the enzymatic properties and reaction mechanisms of P450 enzymes, and on recent studies that highlight their broad applications in the production of oxochemicals.

## SUMMARY OF THE INVENTION

**[0007]** The present invention relates to a hand-dishwashing composition comprising: a surfactant system comprising at least one anionic surfactant; and a non-heme fatty acid decarboxylase; wherein said decarboxylase comprises an amino acid selected from the group consisting of: a) leucine or isoleucine at position 41, b) alanine at position 57, c) glycine, alanine, isoleucine, leucine, valine, serine, or threonine at position 239, and d) combinations thereof; wherein said positions are numbered with reference to SEQ ID NO: 1; and wherein said decarboxylase catalyzes the conversion of at least one fatty acid selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and mixtures thereof.

**[0008]** The present invention further relates to a method of manually washing dishware comprising the steps of delivering a detergent composition of the invention into a volume of water to form a wash solution and immersing the dishware in the solution.

## DETAILED DESCRIPTION OF THE INVENTION

**[0009]** The need for compositions and methods which provide for good sudsing, including a good suds-profile, even in the presence of greasy stains comprising higher chain-length saturated and/or unsaturated fatty acid chains, can be met by formulating the hand-dishwashing composition with a non-heme fatty acid decarboxylase; wherein said decarboxylase comprises an amino acid selected from the group consisting of: a) leucine or isoleucine at position 41, b) alanine at position 57, c) glycine, alanine, isoleucine, leucine, valine, serine, or threonine at position 239, and d) combinations thereof; wherein said positions are numbered with reference to SEQ ID NO: 1. Such compositions are also particularly effective at removing grease stains comprising higher chain-length saturated and/or unsaturated fatty acid chains.

## Definitions

**[0010]** As used herein, "dishware" includes cookware and tableware. (move this sentence to definitions section)

**[0011]** As used herein, the term "non-heme fatty acid decarboxylase" means an enzyme that catalyzes the decarboxylation of fatty acids to alkenes utilizing dioxygen as a co-substrate and non-heme iron or dinuclear iron as a cofactor.

**[0012]** As used herein, the articles "a" and "an" when used in a claim, are understood to mean one or more of what is claimed or described.

**[0013]** As used herein, the term "substantially free of" or "substantially free from" means that the indicated material is present in an amount of no more than about 5 wt%, preferably no more than about 2%, and more preferably no more than about 1 wt% by weight of the composition.

**[0014]** As used therein, the term "essentially free of" or "essentially free from" means that the indicated material is present in an amount of no more than about 0.1 wt% by weight of the composition, or preferably not present at an analytically detectable level in such composition. It may include compositions in which the indicated material is present only as an impurity of one or more of the materials deliberately added to such compositions.

**[0015]** All percentages and ratios used hereinafter are by weight of total composition, unless otherwise indicated. All percentages, ratios, and levels of ingredients referred to herein are based on the actual amount of the ingredient, and do not include solvents, fillers, or other materials with which the ingredient may be combined as a commercially available product, unless otherwise indicated.

**[0016]** As used herein the phrase "detergent composition" refers to compositions and formulations designed for cleaning soiled surfaces. Such compositions include dish-washing compositions.

**[0017]** As used herein the term "improved suds longevity" means an increase in the duration of visible suds in a washing process cleaning soiled articles using the composition comprising one or more enzymes of use in the compositions of the present invention, compared with the suds longevity provided by the same composition and process in the absence of the enzyme.

**[0018]** As used herein, the term "soiled surfaces" refers to soiled dishware.

**[0019]** As used herein, the term "water hardness" or "hardness" means uncomplexed cation ions (*i.e.*,  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ ) present in water that have the potential to precipitate with anionic surfactants or any other anionically charged detergent actives under alkaline conditions, and thereby diminishing the surfactancy and cleaning capacity of surfactants. Further, the terms "high water hardness" and "elevated water hardness" can be used interchangeably and are relative terms for the purposes of the present invention, and are intended to include, but not limited to, a hardness level containing at least 12 grams of calcium ion per gallon water (gpg, "American grain hardness" units).

**[0020]** As used herein, the terms "protein," "polypeptide," and "peptide" are used interchangeably herein to denote a polymer of at least two amino acids covalently linked by an amide bond, regardless of length or post-translational modification (*e.g.*, glycosylation, phosphorylation, lipidation, myristilation, ubiquitination, etc.). Included within this definition are D- and L-amino acids, and mixtures of D- and L-amino acids.

**[0021]** As used herein, "polynucleotide" and "nucleic acid" refer to two or more nucleosides that are covalently linked together. The polynucleotide may be wholly comprised ribonucleosides (*i.e.*, an RNA), wholly comprised of 2' deoxyribonucleotides (*i.e.*, a DNA) or mixtures of ribo- and 2' deoxyribonucleosides. While the nucleosides will typically be linked together via standard phosphodiester linkages, the polynucleotides may include one or more non-standard linkages. The polynucleotide may be single-stranded or double-stranded, or may include both single-stranded regions and double-stranded regions. Moreover, while a polynucleotide will typically be composed of the naturally occurring encoding nucleobases (*i.e.*, adenine, guanine, uracil, thymine, and cytosine), it may include one or more modified and/or synthetic nucleobases (*e.g.*, inosine, xanthine, hypoxanthine, etc.). Such modified or synthetic nucleobases can be encoding nucleobases.

**[0022]** As used herein, "coding sequence" refers to that portion of a nucleic acid (*e.g.*, a gene) that encodes an amino acid sequence of a protein.

**[0023]** As used herein, "naturally occurring," "wild-type," and "WT" refer to the form found in nature. For example, a naturally occurring or wild-type polypeptide or polynucleotide sequence is a sequence present in an organism that can be isolated from a source in nature and which has not been intentionally modified by human manipulation.

**[0024]** As used herein, "non-naturally occurring" or "engineered" or "recombinant" when used in the present invention with reference to (*e.g.*, a cell, nucleic acid, or polypeptide), refers to a material, or a material corresponding to the natural or native form of the material, that has been modified in a manner that would not otherwise exist in nature, or is identical thereto but produced or derived from synthetic materials and/or by manipulation using recombinant techniques. Non-limiting examples include, among others, recombinant cells expressing genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise expressed at a different level.

**[0025]** As used herein the term "identity" means the identity between two or more sequences and is expressed in terms of the identity or similarity between the sequences as calculated over the entire length of a sequence aligned against the entire length of the reference sequence. Sequence identity can be measured in terms of percentage identity; the higher the percentage, the more identical the sequences are. The percentage identity is calculated over the length of comparison. For example, the identity is typically calculated over the entire length of a sequence aligned against the entire length of the reference sequence. Methods of alignment of sequences for comparison are well known in the art and identity can be calculated by many known methods. Various programs and alignment algorithms are described in the art. It should be noted that the terms 'sequence identity' and 'sequence similarity' can be used interchangeably.

**[0026]** As used herein, "percentage of sequence identity," "percent identity," and "percent identical" refer to comparisons between polynucleotide sequences or polypeptide sequences, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which either the identical nucleic acid base or amino acid residue occurs in both sequences or a nucleic acid base or amino acid residue is aligned with a gap to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

**[0027]** As used herein, the term "variant" of non-heme fatty acid decarboxylase enzyme means a modified non-heme fatty acid decarboxylase enzyme amino acid sequence by or at one or more amino acids (for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more amino acid modifications) selected from substitutions, insertions, deletions and combinations thereof. The variant may have "conservative" substitutions, wherein a substituted amino acid has similar structural or chemical properties to the amino acid that replaces it, for example, replacement of leucine with isoleucine. A variant may have "non-conservative" changes, for example, replacement of a glycine with a tryptophan. Variants may also include sequences with amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing the activity of the protein may be found using computer programs well known in the art. Variants may also include truncated forms derived from a wild-type non-heme fatty acid decarboxylase enzyme, such as for example, a protein with a truncated N-terminus. Variants may also include forms

derived by adding an extra amino acid sequence to a wild-type protein, such as for example, an N-terminal tag, a C-terminal tag or an insertion in the middle of the protein sequence.

**[0028]** As used herein, "reference sequence" refers to a defined sequence to which another sequence is compared. A reference sequence may be a subset of a larger sequence, for example, a segment of a full-length gene or polypeptide sequence. Generally, a reference sequence is at least 20 nucleotide or amino acid residues in length, at least 25 residues in length, at least 50 residues in length, or the full length of the nucleic acid or polypeptide. Since two polynucleotides or polypeptides may each (1) comprise a sequence (i.e., a portion of the complete sequence) that is similar between the two sequences, and (2) may further comprise a sequence that is divergent between the two sequences, sequence comparisons between two (or more) polynucleotides or polypeptide are typically performed by comparing sequences of the two polynucleotides over a comparison window to identify and compare local regions of sequence similarity. The term "reference sequence" is not intended to be limited to wild-type sequences, and can include engineered or altered sequences. For example, a "reference sequence" can be a previously engineered or altered amino acid sequence.

**[0029]** As used herein, "comparison window" refers to a conceptual segment of at least about 20 contiguous nucleotide positions or amino acids residues wherein a sequence may be compared to a reference sequence of at least 20 contiguous nucleotides or amino acids and wherein the portion of the sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The comparison window can be longer than 20 contiguous residues, and includes, optionally 30, 40, 50, 100, or longer windows.

**[0030]** As used herein, "corresponding to", "reference to" or "relative to" when used in the context of the numbering of a given amino acid or polynucleotide sequence refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence. In other words, the residue number or residue position of a given polymer is designated with respect to the reference sequence rather than by the actual numerical position of the residue within the given amino acid or polynucleotide sequence. For example, a given amino acid sequence, such as that of an engineered non-heme fatty acid decarboxylase, can be aligned to a reference sequence by introducing gaps to optimize residue matches between the two sequences. In these cases, although the gaps are present, the numbering of the residue in the given amino acid or polynucleotide sequence is made with respect to the reference sequence to which it has been aligned.

**[0031]** As used herein, "increased enzymatic activity" and "increased activity" refer to an improved property of a wild-type or an engineered enzyme, which can be represented by an increase in specific activity (e.g., product produced/time/weight protein) or an increase in percent conversion of the substrate to the product (e.g., percent conversion of starting amount of substrate to product in a specified time period using a specified amount of non-heme fatty acid decarboxylase) as compared to a reference enzyme. Any property relating to enzyme activity may be affected, including the classical enzyme properties of  $K_m$ ,  $V_{max}$  or  $k_{cat}$ , changes of which can lead to increased enzymatic activity. The non-heme fatty acid decarboxylase activity can be measured by any one of standard assays used for measuring non-heme fatty acid decarboxylases, such as change in substrate or product concentration. Comparisons of enzyme activities are made using a defined preparation of enzyme, a defined assay under a set condition, and one or more defined substrates, as further described in detail herein. Generally, when enzymes in cell lysates are compared, the numbers of cells and the amount of protein assayed are determined as well as use of identical expression systems and identical host cells to minimize variations in amount of enzyme produced by the host cells and present in the lysates.

**[0032]** As used herein, "conversion" refers to the enzymatic transformation of a substrate to the corresponding product.

**[0033]** As used herein "percent conversion" refers to the percent of the substrate that is converted to the product within a period of time under specified conditions. Thus, for example, the "enzymatic activity" or "activity" of a non-heme fatty acid decarboxylase polypeptide can be expressed as "percent conversion" of the substrate to the product.

**[0034]** As used herein, "amino acid difference" or "residue difference" refers to a difference in the amino acid residue at a position of a polypeptide sequence relative to the amino acid residue at a corresponding position in a reference sequence. The positions of amino acid differences generally are referred to herein as "X<sub>n</sub>", where n refers to the corresponding position in the reference sequence upon which the residue difference is based. For example, a "residue difference at position X41 as compared to SEQ ID NO: 1" refers to a difference of the amino acid residue at the polypeptide position corresponding to position 41 of SEQ ID NO:1. Thus, if the reference polypeptide of SEQ ID NO:1 has a tyrosine at position 41, then a "residue difference at position X41 as compared to SEQ ID NO:1" refers to an amino acid substitution of any residue other than tyrosine at the position of the polypeptide corresponding to position 41 of SEQ ID NO:1. In most instances herein, the specific amino acid residue difference at a position is indicated as "X<sub>n</sub>Y" where "X<sub>n</sub>" specified the corresponding position as described above, and "Y" is the single letter identifier of the amino acid found in the engineered polypeptide (i.e., the different residue than in the reference polypeptide). In some instances, the present invention also provides specific amino acid differences denoted by the conventional notation "AnB", where A is the single letter identifier of the residue in the reference sequence, "n" is the number of the residue position in the reference sequence, and B is the single letter identifier of the residue substitution in the sequence of the engineered polypeptide. In some instances, a polypeptide of the present invention can include at least one amino acid residue difference relative

to a reference sequence, which is indicated by a list of the specified positions where residue differences are present relative to the reference sequence. Where more than one amino acid can be used in a specific residue position of a polypeptide, the various amino acid residues that can be used are separated by a "/" (e.g., X41(A/G)). The present invention includes engineered polypeptide sequences comprising at least one amino acid differences that include either/or both conservative and non-conservative amino acid substitutions. The amino acid sequences of the specific recombinant non-heme fatty acid decarboxylase polypeptides included in the Sequence Listing of the present invention include an initiating methionine (M) residue (i.e., M represents residue position 1). The skilled artisan, however, understands that this initiating methionine residue can be removed by biological processing machinery, such as in a host cell or in vitro translation system, to generate a mature protein lacking the initiating methionine residue, but otherwise retaining the enzyme's properties. Consequently, the term "amino acid residue difference relative to SEQ ID NO:1 at position Xn" as used herein may refer to position "Xn" or to the corresponding position (e.g., position (X-1)n) in a reference sequence that has been processed so as to lack the starting methionine.

**[0035]** As used herein, the phrase "conservative amino acid substitutions" refers to the interchangeability of residues having similar side chains, and thus typically involves substitution of the amino acid in the polypeptide with amino acids within the same or similar defined class of amino acids. As such, an amino acid with an aliphatic side chain can be substituted with another aliphatic amino acid (e.g., alanine, valine, leucine, and isoleucine); an amino acid with a hydroxyl side chain can be substituted with another amino acid with a hydroxyl side chain (e.g., serine and threonine); an amino acids having aromatic side chains can be substituted with another amino acid having an aromatic side chain (e.g., phenylalanine, tyrosine, tryptophan, and histidine); an amino acid with a basic side chain can be substituted with another amino acid with a basic side chain (e.g., lysine and arginine); an amino acid with an acidic side chain can be substituted with another amino acid with an acidic side chain (e.g., aspartic acid or glutamic acid); and/or a hydrophobic or hydrophilic amino acid can be replaced with another hydrophobic or hydrophilic amino acid, respectively. The appropriate classification of any amino acid or residue will be apparent to those of skill in the art, especially in light of the detailed invention provided herein.

**[0036]** As used herein, the phrase "non-conservative substitution" refers to substitution of an amino acid in the polypeptide with an amino acid with significantly differing side chain properties. Non-conservative substitutions may use amino acids between, rather than within, the defined groups and affects (a) the structure of the peptide backbone in the area of the substitution (e.g., proline for glycine) (b) the charge or hydrophobicity, or (c) the bulk of the side chain. By way of example and not limitation, an exemplary non-conservative substitution can be an acidic amino acid substituted with a basic or aliphatic amino acid; an aromatic amino acid substituted with a small amino acid; and a hydrophilic amino acid substituted with a hydrophobic amino acid.

**[0037]** As used herein, "deletion" refers to modification of the polypeptide by removal of one or more amino acids from the reference polypeptide. Deletions can comprise removal of 1 or more amino acids, 2 or more amino acids, 5 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, or up to 20% of the total number of amino acids making up the polypeptide while retaining enzymatic activity and/or retaining the improved properties of an engineered enzyme. Deletions can be directed to the internal portions and/or terminal portions of the polypeptide. The deletion can comprise a continuous segment or can be discontinuous.

**[0038]** As used herein, "insertion" refers to modification of the polypeptide by addition of one or more amino acids to the reference polypeptide. The improved engineered non-heme fatty acid decarboxylase enzymes can comprise insertions of one or more amino acids to the naturally occurring non-heme fatty acid decarboxylase polypeptide as well as insertions of one or more amino acids to engineered non-heme fatty acid decarboxylase polypeptides. Insertions can be in the internal portions of the polypeptide, or to the carboxy or amino terminus. Insertions as used herein include fusion proteins as is known in the art. The insertion can be a contiguous segment of amino acids or separated by one or more of the amino acids in the naturally occurring polypeptide.

**[0039]** The term "amino acid substitution set" or "substitution set" refers to a group of amino acid substitutions in a polypeptide sequence, as compared to a reference sequence. A substitution set can have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions. A substitution set can refer to the set of amino acid substitutions that is present in any of the variant non-heme fatty acid decarboxylases.

**[0040]** As used herein, "fragment" refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the sequence. Fragments can typically have about 80%, about 90%, about 95%, about 98%, or about 99% of the full-length non-heme fatty acid decarboxylase polypeptide, for example, the polypeptide of SEQ ID NO: 1. The fragment can be "biologically active" (i.e., it exhibits the same enzymatic activity as the full-length sequence).

**[0041]** A "functional fragment", or a "biologically active fragment", used interchangeably, herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion(s) and/or internal deletions, but where the remaining amino acid sequence is identical to the corresponding positions in the sequence to which it is being compared and that retains substantially all of the activity of the full-length polypeptide.

**[0042]** As used herein, "isolated polypeptide" refers to a polypeptide which is substantially separated from other contaminants that naturally accompany it (e.g., protein, lipids, and polynucleotides). The term embraces polypeptides which have been removed or purified from their naturally-occurring environment or expression system (e.g., host cell or in vitro synthesis). The improved non-heme fatty acid decarboxylase enzymes may be present within a cell, present in the cellular medium, or prepared in various forms, such as lysates or isolated preparations. As such, the wild-type or engineered non-heme fatty acid decarboxylase polypeptides of the present invention can be an isolated polypeptide.

**[0043]** As used herein, "substantially pure polypeptide" refers to a composition in which the polypeptide species is the predominant species present (i.e., on a molar or weight basis it is more abundant than any other individual macromolecular species in the composition), and is generally a substantially purified composition when the object species comprises at least about 50 percent of the macromolecular species present by mole or % weight. Generally, a substantially pure wild-type or engineered non-heme fatty acid decarboxylase polypeptide composition will comprise about 60% or more, about 70% or more, about 80% or more, about 90% or more, about 91% or more, about 92% or more, about 93% or more, about 94% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% of all macromolecular species by mole or % weight present in the composition. Solvent species, small molecules (<500 Daltons), and elemental ion species are not considered macromolecular species. The isolated improved non-heme fatty acid decarboxylase polypeptide can be a substantially pure polypeptide composition.

**[0044]** As used herein, when used with reference to a nucleic acid or polypeptide, the term "heterologous" refers to a sequence that is not normally expressed and secreted by an organism (e.g., a wild-type organism). The term can encompass a sequence that comprises two or more subsequences which are not found in the same relationship to each other as normally found in nature, or is recombinantly engineered so that its level of expression, or physical relationship to other nucleic acids or other molecules in a cell, or structure, is not normally found in nature. For instance, a heterologous nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged in a manner not found in nature (e.g., a nucleic acid open reading frame (ORF) of the invention operatively linked to a promoter sequence inserted into an expression cassette, such as a vector). "Heterologous polynucleotide" can refer to any polynucleotide that is introduced into a host cell by laboratory techniques, and includes polynucleotides that are removed from a host cell, subjected to laboratory manipulation, and then reintroduced into a host cell.

**[0045]** As used herein, "codon optimized" refers to changes in the codons of the polynucleotide encoding a protein to those preferentially used in a particular organism such that the encoded protein is efficiently expressed in the organism of interest. The polynucleotides encoding the non-heme fatty acid decarboxylase enzymes may be codon optimized for optimal production from the host organism selected for expression.

**[0046]** As used herein, "suitable reaction conditions" refer to those conditions in the biocatalytic reaction solution (e.g., ranges of enzyme loading, substrate loading, temperature, pH, buffers, cosolvents, etc.) under which a non-heme fatty acid decarboxylase polypeptide of the present invention is capable of converting a substrate compound to a product compound (e.g., conversion of one compound to another compound).

**[0047]** As used herein, "substrate" in the context of a biocatalyst mediated process refers to the compound or molecule acted on by the biocatalyst.

**[0048]** As used herein "product" in the context of a biocatalyst mediated process refers to the compound or molecule resulting from the action of the biocatalyst.

#### Detergent Composition

**[0049]** The hand-dishwashing compositions of the present invention formulate a specific surfactant system with a specific non-heme fatty acid decarboxylase, in order to provide improved sudsing, especially long-lasting sudsing, in the presence of greasy stains comprising higher chain length saturated and/or unsaturated fatty acids, and improved removal of such stains.

**[0050]** The hand-dishwashing composition is preferably in liquid form, more preferably is an aqueous cleaning composition. As such, the composition can comprise from 50% to 90%, preferably from 60% to 75%, by weight of the total composition of water.

**[0051]** Preferably the pH of the detergent composition of the invention, measured as a 10% product concentration in demineralized water at 20°C, is adjusted to between 3 and 14, more preferably between 4 and 13, more preferably between 6 and 12 and most preferably between 8 and 10. The pH of the detergent composition can be adjusted using pH modifying ingredients known in the art.

**[0052]** The composition of the present invention can be Newtonian or non-Newtonian, preferably Newtonian. Preferably, the composition has a viscosity of from 10 mPa·s to 10,000 mPa·s, preferably from 100 mPa·s to 5,000 mPa·s, more preferably from 300 mPa·s to 2,000 mPa·s, or most preferably from 500 mPa·s to 1,500 mPa·s, alternatively combinations thereof. The viscosity is measured at 20°C with a Brookfield RT Viscometer using spindle 31 with the RPM of the viscometer adjusted to achieve a torque of between 40% and 60%.

## Surfactant System

**[0053]** The cleaning composition comprises from 5% to 50%, preferably 8% to 45%, more preferably from 15% to 40%, by weight of the total composition of a surfactant system.

**[0054]** For improved sudsing, the surfactant system comprises anionic surfactant. The surfactant system preferably comprises from 60% to 90%, more preferably from 70% to 80% by weight of the surfactant system of the anionic surfactant. Alkyl sulphated anionic surfactants are preferred, particularly those selected from the group consisting of: alkyl sulphate, alkyl alkoxy sulphate, and mixtures thereof. More preferably, the anionic surfactant consists of alkyl sulphated anionic surfactant selected from the group consisting of: alkyl sulphate, alkyl alkoxy sulphate, and mixtures thereof.

**[0055]** For further improvements in sudsing, the surfactant system can comprise less than 30%, preferably less than 15%, more preferably less than 10% of further anionic surfactant, and most preferably the surfactant system comprises no further anionic surfactant. The alkyl sulphated anionic surfactant preferably has an average alkyl chain length of from 8 to 18, preferably from 10 to 14, more preferably from 12 to 14, most preferably from 12 to 13 carbon atoms. The alkyl sulphated anionic surfactant has an average degree of alkoxylation, of less than 5, preferably less than 3, more preferably from 0.5 to 2.0, most preferably from 0.5 to 0.9. Preferably, the alkyl sulphated anionic surfactant is ethoxylated. That is, the alkyl sulphated anionic surfactant has an average degree of ethoxylation, of less than 5, preferably less than 3, more preferably from 0.5 to 2.0, most preferably from 0.5 to 0.9.

**[0056]** The average degree of alkoxylation is the mol average degree of alkoxylation (*i.e.*, mol average alkoxylation degree) of all the alkyl sulphate anionic surfactant. Hence, when calculating the mol average alkoxylation degree, the mols of non-alkoxylated sulphate anionic surfactant are included:

$$\text{Mol average alkoxylation degree} = (x_1 * \text{alkoxylation degree of surfactant 1} + x_2 * \text{alkoxylation degree of surfactant 2} + \dots) / (x_1 + x_2 + \dots)$$

wherein  $x_1$ ,  $x_2$ , ... are the number of moles of each alkyl (or alkoxy) sulphate anionic surfactant of the mixture and alkoxylation degree is the number of alkoxy groups in each alkyl sulphate anionic surfactant.

**[0057]** The alkyl sulphate anionic surfactant can have a weight average degree of branching of more than 10%, preferably more than 20%, more preferably more than 30%, even more preferably between 30% and 60%, most preferably between 30% and 50%. The alkyl sulphate anionic surfactant can comprise at least 5%, preferably at least 10%, most preferably at least 25%, by weight of the alkyl sulphate anionic surfactant, of branching on the C2 position (as measured counting carbon atoms from the sulphate group for non-alkoxylated alkyl sulphate anionic surfactants, and the counting from the alkoxy-group furthest from the sulphate group for alkoxylated alkyl sulphate anionic surfactants). More preferably, greater than 75%, even more preferably greater than 90%, by weight of the total branched alkyl content consists of C1-C5 alkyl moiety, preferably C1-C2 alkyl moiety. It has been found that formulating the inventive compositions using alkyl sulphate surfactants having the aforementioned degree of branching results in improved low temperature stability. Such compositions require less solvent in order to achieve good physical stability at low temperatures. As such, the compositions can comprise lower levels of organic solvent, of less than 5.0% by weight of the cleaning composition of organic solvent, while still having improved low temperature stability. Higher surfactant branching also provides faster initial suds generation, but typically less suds mileage. The weight average branching, described herein, has been found to provide improved low temperature stability, initial foam generation and suds longevity.

**[0058]** The weight average degree of branching for an anionic surfactant mixture can be calculated using the following formula:

$$\text{Weight average degree of branching (\%)} = [(x_1 * \text{wt\% branched alcohol 1 in alcohol 1} + x_2 * \text{wt\% branched alcohol 2 in alcohol 2} + \dots) / (x_1 + x_2 + \dots)] * 100$$

wherein  $x_1$ ,  $x_2$ , ... are the weight in grams of each alcohol in the total alcohol mixture of the alcohols which were used as starting material before (alkoxylation and) sulphation to produce the alkyl (alkoxy) sulphate anionic surfactant. In the weight average degree of branching calculation, the weight of the alkyl alcohol used to form the alkyl sulphate anionic surfactant which is not branched is included.

**[0059]** The weight average degree of branching and the distribution of branching can typically be obtained from the technical data sheet for the surfactant or constituent alkyl alcohol. Alternatively, the branching can also be determined through analytical methods known in the art, including capillary gas chromatography with flame ionisation detection on



medium polar capillary column, using hexane as the solvent. The weight average degree of branching and the distribution of branching is based on the starting alcohol used to produce the alkyl sulphate anionic surfactant.

**[0060]** The alkyl chain of the alkyl sulphated anionic surfactant preferably has a mol fraction of C12 and C13 chains of at least 50%, preferably at least 65%, more preferably at least 80%, most preferably at least 90%. Suds mileage is particularly improved, especially in the presence of greasy soils, when the C13/C12 mol ratio of the alkyl chain is at least 50/50, preferably at least 57/43, preferably from 60/40 to 90/10, more preferably from 60/40 to 80/20, most preferably from 60/40 to 70/30, while not compromising suds mileage in the presence of particulate soils.

**[0061]** Suitable counterions include alkali metal cation earth alkali metal cation, alkanolammonium or ammonium or substituted ammonium, but preferably sodium.

**[0062]** Suitable examples of commercially available alkyl sulphate anionic surfactants include, those derived from alcohols sold under the Neodol® brand-name by Shell, or the Lial®, Isalchem®, and Safol® brand-names by Sasol, or some of the natural alcohols produced by The Procter & Gamble Chemicals company. The alcohols can be blended in order to achieve the desired mol fraction of C12 and C13 chains and the desired C13/C12 ratio, based on the relative fractions of C13 and C12 within the starting alcohols, as obtained from the technical data sheets from the suppliers or from analysis using methods known in the art.

**[0063]** In order to improve surfactant packing after dilution and hence improve suds mileage, the surfactant system preferably comprises a co-surfactant. Preferred co-surfactants are selected from the group consisting of an amphoteric surfactant, a zwitterionic surfactant, and mixtures thereof. The co-surfactant is preferably an amphoteric surfactant, more preferably an amine oxide surfactant. The co-surfactant is included as part of the surfactant system.

**[0064]** The composition preferably comprises from 0.1% to 20%, more preferably from 0.5% to 15% and especially from 2% to 10% by weight of the cleaning composition of the co-surfactant. The surfactant system of the cleaning composition of the present invention preferably comprises from 10% to 40%, preferably from 15% to 35%, more preferably from 20% to 30%, by weight of the surfactant system of a co-surfactant. The anionic surfactant to the co-surfactant weight ratio can be from 1:1 to 8:1, preferably from 2:1 to 5:1, more preferably from 2.5:1 to 4:1.

**[0065]** As mentioned earlier, amine oxide surfactants are preferred for use as a co-surfactant. The amine oxide surfactant can be linear or branched, though linear are preferred. Suitable linear amine oxides are typically water-soluble, and characterized by the formula  $R1 - N(R2)(R3)O$  wherein R1 is a C8-18 alkyl, and the R2 and R3 moieties are selected from the group consisting of C1-3 alkyl groups, C1-3 hydroxyalkyl groups, and mixtures thereof. For instance, R2 and R3 can be selected from the group consisting of: methyl, ethyl, propyl, isopropyl, 2-hydroxyethyl, 2-hydroxypropyl and 3-hydroxypropyl, and mixtures thereof, though methyl is preferred for one or both of R2 and R3. The linear amine oxide surfactants in particular may include linear C10-C18 alkyl dimethyl amine oxides and linear C8-C12 alkoxy ethyl dihydroxy ethyl amine oxides.

**[0066]** Preferably, the amine oxide surfactant is selected from the group consisting of: alkyl dimethyl amine oxide, alkyl amido propyl dimethyl amine oxide, and mixtures thereof. Alkyl dimethyl amine oxides are preferred, such as C8-18 alkyl dimethyl amine oxides, or C10-16 alkyl dimethyl amine oxides (such as coco dimethyl amine oxide). Suitable alkyl dimethyl amine oxides include C10 alkyl dimethyl amine oxide surfactant, C10-12 alkyl dimethyl amine oxide surfactant, C12-C14 alkyl dimethyl amine oxide surfactant, and mixtures thereof. C12-C14 alkyl dimethyl amine oxide are particularly preferred.

**[0067]** Alternative suitable amine oxide surfactants include mid-branched amine oxide surfactants. As used herein, "mid-branched" means that the amine oxide has one alkyl moiety having  $n_1$  carbon atoms with one alkyl branch on the alkyl moiety having  $n_2$  carbon atoms. The alkyl branch is located on the  $\alpha$  carbon from the nitrogen on the alkyl moiety. This type of branching for the amine oxide is also known in the art as an internal amine oxide. The total sum of  $n_1$  and  $n_2$  can be from 10 to 24 carbon atoms, preferably from 12 to 20, and more preferably from 10 to 16. The number of carbon atoms for the one alkyl moiety ( $n_1$ ) is preferably the same or similar to the number of carbon atoms as the one alkyl branch ( $n_2$ ) such that the one alkyl moiety and the one alkyl branch are symmetric. As used herein "symmetric" means that  $|n_1 - n_2|$  is less than or equal to 5, preferably 4, most preferably from 0 to 4 carbon atoms in at least 50 wt%, more preferably at least 75 wt% to 100 wt% of the mid-branched amine oxides for use herein. The amine oxide further comprises two moieties, independently selected from a C1-3 alkyl, a C1-3 hydroxyalkyl group, or a polyethylene oxide group containing an average of from about 1 to about 3 ethylene oxide groups. Preferably, the two moieties are selected from a C1-3 alkyl, more preferably both are selected as C1 alkyl.

**[0068]** Alternatively, the amine oxide surfactant can be a mixture of amine oxides comprising a mixture of low-cut amine oxide and mid-cut amine oxide. The amine oxide of the composition of the invention can then comprises:

a) from about 10% to about 45% by weight of the amine oxide of low-cut amine oxide of formula  $R1R2R3AO$  wherein R1 and R2 are independently selected from hydrogen, C1-C4 alkyls or mixtures thereof, and R3 is selected from C10 alkyls and mixtures thereof; and

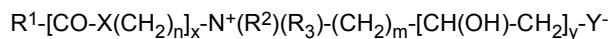
b) from 55% to 90% by weight of the amine oxide of mid-cut amine oxide of formula  $R4R5R6AO$  wherein R4 and R5 are independently selected from hydrogen, C1-C4 alkyls or mixtures thereof, and R6 is selected from C12-C16

alkyls or mixtures thereof

**[0069]** In a preferred low-cut amine oxide for use herein R3 is n-decyl, with preferably both R1 and R2 being methyl. In the mid-cut amine oxide of formula R4R5R6AO, R4 and R5 are preferably both methyl.

**[0070]** Preferably, the amine oxide comprises less than about 5%, more preferably less than 3%, by weight of the amine oxide of an amine oxide of formula R7R8R9AO wherein R7 and R8 are selected from hydrogen, C1-C4 alkyls and mixtures thereof and wherein R9 is selected from C8 alkyls and mixtures thereof. Limiting the amount of amine oxides of formula R7R8R9AO improves both physical stability and suds mileage.

**[0071]** Suitable zwitterionic surfactants include betaine surfactants. Such betaine surfactants includes alkyl betaines, alkylamidobetaine, amidazoliniumbetaine, sulphobetaine (INCI Sultaines) as well as the Phosphobetaine, and preferably meets formula (II):



wherein in formula (II),

R1 is selected from the group consisting of: a saturated or unsaturated C6-22 alkyl residue, preferably C8-18 alkyl residue, more preferably a saturated C10-16 alkyl residue, most preferably a saturated C12-14 alkyl residue;

X is selected from the group consisting of: NH, NR4 wherein R4 is a C1-4 alkyl residue, O, and S,

n is an integer from 1 to 10, preferably 2 to 5, more preferably 3,

x is 0 or 1, preferably 1,

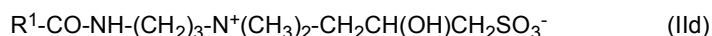
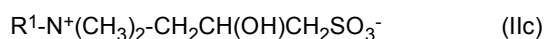
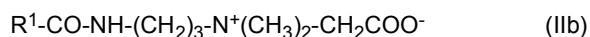
R2 and R3 are independently selected from the group consisting of: a C1-4 alkyl residue, hydroxy substituted such as a hydroxyethyl, and mixtures thereof, preferably both R2 and R3 are methyl,

m is an integer from 1 to 4, preferably 1, 2 or 3,

y is 0 or 1, and

Y is selected from the group consisting of: COO, SO3, OPO(OR5)O or P(O)(OR5)O, wherein R5 is H or a C1-4 alkyl residue.

**[0072]** Preferred betaines are the alkyl betaines of formula (Ia), the alkyl amido propyl betaine of formula (Ib), the sulphobetaines of formula (Ic) and the amido sulphobetaine of formula (Id):



in which R1 has the same meaning as in formula (II). Particularly preferred are the carbobetaines [i.e. wherein Y=COO- in formula (II)] of formulae (Ia) and (Ib), more preferred are the alkylamidobetaine of formula (Ib).

**[0073]** Suitable betaines can be selected from the group consisting or [designated in accordance with INCI]: capryl/capramidopropyl betaine, cetyl betaine, cetyl amidopropyl betaine, cocamidoethyl betaine, cocamidopropyl betaine, cocobetaines, decyl betaine, decyl amidopropyl betaine, hydrogenated tallow betaine / amidopropyl betaine, isostearamidopropyl betaine, lauramidopropyl betaine, lauryl betaine, myristyl amidopropyl betaine, myristyl betaine, oleamidopropyl betaine, oleyl betaine, palmamidopropyl betaine, palmitamidopropyl betaine, palm-kernelamidopropyl betaine, stearamidopropyl betaine, stearyl betaine, tallowamidopropyl betaine, tallow betaine, undecylenamidopropyl betaine, undecyl betaine, and mixtures thereof. Preferred betaines are selected from the group consisting of: cocamidopropyl betaine, cocobetaines, lauramidopropyl betaine, lauryl betaine, myristyl amidopropyl betaine, myristyl betaine, and mixtures thereof. Cocamidopropyl betaine is particularly preferred.

**[0074]** Preferably, the surfactant system of the composition of the present invention further comprises from 1% to 25%, preferably from 1.25% to 20%, more preferably from 1.5% to 15%, most preferably from 1.5% to 5%, by weight of the surfactant system, of a non-ionic surfactant.

**[0075]** Suitable nonionic surfactants can be selected from the group consisting of: alkoxylated non-ionic surfactant, alkyl polyglucoside ("APG") surfactant, and mixtures thereof.

**[0076]** Suitable alkoxylated non-ionic surfactants can be linear or branched, primary or secondary alkyl alkoxylated non-ionic surfactants. Alkyl ethoxylated non-ionic surfactant are preferred. The ethoxylated non-ionic surfactant can comprise on average from 9 to 15, preferably from 10 to 14 carbon atoms in its alkyl chain and on average from 5 to 12,

preferably from 6 to 10, most preferably from 7 to 8, units of ethylene oxide per mole of alcohol. Such alkyl ethoxylated nonionic surfactants can be derived from synthetic alcohols, such as OXO-alcohols and Fisher Tropsh alcohols, or from naturally derived alcohols, or from mixtures thereof. Suitable examples of commercially available alkyl ethoxylate nonionic surfactants include, those derived from synthetic alcohols sold under the Neodol® brand-name by Shell, or the Lial®,

Isalchem®, and Safol® brand-names by Sasol, or some of the natural alcohols produced by The Procter & Gamble Chemicals company.

**[0077]** The compositions of the present invention can comprise alkyl polyglucoside ("APG") surfactant. The addition of alkyl polyglucoside surfactants have been found to improve sudsing beyond that of comparative nonionic surfactants such as alkyl ethoxylated surfactants. Preferably the alkyl polyglucoside surfactant is a C8-C16 alkyl polyglucoside surfactant, preferably a C8-C14 alkyl polyglucoside surfactant. The alkyl polyglucoside preferably has an average degree of polymerization of between 0.1 and 3, more preferably between 0.5 and 2.5, even more preferably between 1 and 2. Most preferably, the alkyl polyglucoside surfactant has an average alkyl carbon chain length between 10 and 16, preferably between 10 and 14, most preferably between 12 and 14, with an average degree of polymerization of between 0.5 and 2.5 preferably between 1 and 2, most preferably between 1.2 and 1.6. C8-C16 alkyl polyglucosides are commercially available from several suppliers (e.g., Simusol® surfactants from Seppic Corporation; and Glucopon® 600 CSUP, Glucopon® 650 EC, Glucopon® 600 CSUP/MB, and Glucopon® 650 EC/MB, from BASF Corporation).

#### Non-Heme fatty acid decarboxylases

**[0078]** Non-heme fatty acid decarboxylases catalyze the decarboxylation of fatty acids to alkenes utilizing dioxygen as a cosubstrate and dinuclear iron as a cofactor. The most well studied member of this family is UndA from *Pseudomonas aeruginosa* Pf-5 (SEQ ID NO: 1), an enzyme with high specificity for C10 to C14 fatty acids. Members from other genera, including *Acinetobacter*, *Myxococcus*, and *Bukholderia*, have also been reported (see for example US 10,000,775 B2; Z. Rui et al., PNAS, (2014), 111, 18237-18242), with more than 1000 homologs identified from public databases.

**[0079]** UndA (SEQ ID NO: 1) is a small enzyme of 261 amino acids with no significant homology to other enzymes of known function. Crystal structures of this protein have been published (PDB ID: 4WWJ, 4WWX, 4WX0), revealing a hydrophobic pocket of limited size that is able to accommodate only medium chain fatty acids (e.g. C10 to C14), while excluding longer chain substrates (e.g. C16 or C18).

**[0080]** Without wishing to be bound by theory, sequence alignment of UndA (SEQ ID NO: 1) and related homologs suggests that several regions of conserved sequence motifs and amino acids may contribute to and/or define the active site of the enzyme. For instance, the residues E101, H104, E159, H194, and H201 are highly conserved and may be important for enzyme catalytic function since they bind the dinuclear iron cofactor. The sequence motif (A/P/Q/L)51-X-X-X-(R/A)55-X-(Y/F/V/A)57-(L/F/M)58-(I/V/A/S)59-(G/N/H/Q/T)60-(G/F/A/V/I/L)61-(W/F/Y)62-(P/L)63-(V/I/L)64-V65-(E/A)66-(Q/S/H)67-F68-(A/S/P)69-(L/V/K/S)70-Y71-M72-(A/S/G)73-X-(N/S/A/T)75-L76-(T/L)77-K78 forms an alpha-helix that contributes to the formation of the substrate binding pocket. The sequence motif G86-(E/V/D)-(D/T/E/A)-(M/E/K/S)-(A/T/I)-R91-(R/N/D)-(W/Y)-L94-(M/I/L)-(R/Q)-N97-(I/L)-(R/K/G)-V100-E101-(L/Q/E/A)-(N/R/K)-H104-(A/L/V)-X-(Y/W/H)-(W/Y/F)-X-(H/N/D)-W111 forms an alpha-helix that includes two amino acids, E101 and H104, that coordinate an iron in the catalytic center of the enzyme. The sequence motif L147-(I/A/P)-(V/I/E/A)-(A/C/S/G)-(I/M/L/I/V)-A152-A153-(T/S)-N155-(Y/L/W)-A157-(I/V)-E159-(G/W/S)-(A/V/I)-T162-G163-(E/D/V)-(W/L)165-(S/T)-(A/I/R) forms an alpha-helix that contributes to the formation of the substrate binding pocket, and contains the amino acid residue E159 that may be involved in iron or oxygen binding and/or serve as a proton donor for the regeneration of the enzyme. The sequence motif W190-L191-(K/R)-(M/L/A/V)-H194-(A/S)-(Q/H/S/R)-Y197-D198-D199-X-H201-P202-(W/Y/E/V)-E204-A205-(L/M)-(E/D)-(I/L)-(I/V) forms an alpha-helix that includes the amino acid residues H194 and H201 that coordinate to the two irons in the catalytic center. The sequence motif (Y/C/M)235-(M/Y/F)-(Y/E/A/T/H)-(L/M/A)-(F/A/S/I/T/L/V/G)-(L/A/G)240-(E/D/S/H)-(R/E/D/C/A)-(C/S/Y)243 forms an alpha-helix that contributes to the formation of the substrate binding pocket. Furthermore, without wishing to be bound by theory, residues 35, 36, 38, 40, 41, 44, 54, 57, 58, 60, 61, 108, 111, 130, 131, 133, 134, 165, 169, 235, 236, 238, 239, 240, and 243 in UndA (SEQ ID NO: 1) may be important for the substrate specificity of the enzyme. Indeed, the variant UndA F239A was recently demonstrated to decarboxylase C16 fatty acids (Knoot, C. J. and H. B. Pakrasi (2019). Sci. Rep. 9(1): 1-12.), but non-heme fatty acid decarboxylases that convert C18 fatty acids have not been reported.

**[0081]** The present invention provides hand dish-washing compositions comprising non-heme fatty acid decarboxylases having increased enzymatic activity for long-chain fatty acid substrates, such as palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid, as compared to the well-known naturally occurring wild-type fatty acid decarboxylases reported previously in the art (e.g. UndA SEQ ID NO: 1), and especially for long chain saturated fatty acids such as stearic acid and palmitic acid, and long chain unsaturated fatty acid substrates such as oleic acid, linoleic acid, and linolenic acid. Surprisingly, Applicant has found that non-heme fatty acid decarboxylases comprising small amino acid residues (e.g. glycine or alanine) at certain positions (e.g. 41, 57, 239) have an increased enzymatic activity towards

long chain fatty acids, such as palmitic acid, stearic acid, oleic acid, linoleic acid, or linolenic acid, and especially for long chain saturated fatty acids such as stearic acid and palmitic acid, and unsaturated fatty acid substrates such as oleic acid, linoleic acid, and linolenic acid, in comparison to the well-known UndA (SEQ ID NO: 1) and that these decarboxylases can provide a benefit when formulated in hand dish-washing compositions.

**[0082]** The hand dish-washing composition can comprise a non-heme fatty acid decarboxylase; wherein said decarboxylase comprises an amino acid selected from the group consisting of: a) leucine or isoleucine at position 41, b) alanine at position 57, c) glycine, alanine, isoleucine, leucine, valine, serine, or threonine at position 239, and d) combinations thereof; wherein said positions are numbered with reference to SEQ ID NO: 1; and wherein said decarboxylase catalyzes the conversion of at least one fatty acid selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and mixtures thereof, preferably stearic acid, oleic acid, and mixtures thereof. The hand dish-washing composition can comprise a non-heme fatty acid decarboxylase; wherein said decarboxylase comprises an amino acid selected from the group consisting of: a) alanine at position 57, b) glycine or alanine at position 239, and c) combinations thereof; wherein said positions are numbered with reference to SEQ ID NO: 1; and wherein said decarboxylase catalyzes the conversion of at least one fatty acid selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and mixtures thereof, preferably stearic acid, oleic acid, and mixtures thereof, more preferably oleic acid. The hand dish-washing composition can comprise a non-heme fatty acid decarboxylase; wherein said decarboxylase comprises an alanine at position 57 and an alanine at position 239; wherein said positions are numbered with reference to SEQ ID NO: 1; and wherein said decarboxylase catalyzes the conversion of at least one fatty acid selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and mixtures thereof, preferably stearic acid, oleic acid, and mixtures thereof.

**[0083]** A suitable non-heme fatty acid decarboxylase comprising an isoleucine at position 41 is SEQ ID NO: 2. A suitable non-heme fatty acid decarboxylase comprising a leucine at position 41 is SEQ ID NO: 3. Suitable non-heme fatty acid decarboxylases comprising an alanine at position 57 are SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, and 57. Suitable non-heme fatty acid decarboxylases comprising a glycine at position 239 are SEQ ID NO: 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, and 70. Suitable non-heme fatty acid decarboxylases comprising an alanine at position 239 are SEQ ID NO: 21, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, and 83. Suitable non-heme fatty acid decarboxylases comprising a valine at position 239 are SEQ ID NO: 84, 85, 86, 87, 88, and 89. Suitable non-heme fatty acid decarboxylases comprising an isoleucine at position 239 are SEQ ID NO: 90 and 91. Suitable non-heme fatty acid decarboxylases comprising a leucine at position 239 are SEQ ID NO: 92, 93, 94, 95, and 96. Suitable non-heme fatty acid decarboxylases comprising a serine at position 239 are SEQ ID NO: 97, 98, 99, 100, and 101. Suitable non-heme fatty acid decarboxylases comprising a threonine at position 239 are SEQ ID NO: 102, 103, 104, 105, and 106. Suitable non-heme fatty acid decarboxylases comprising an alanine at position 57 and an alanine at position 239 are SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, and 57.

**[0084]** The decarboxylases can have an increased enzymatic activity for a substrate selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid, preferably stearic acid and oleic, of at least about 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 150-fold, 500-fold or more relative to the activity of wild-type decarboxylase (SEQ ID NO: 1) under suitable reaction conditions.

**[0085]** The hand dish-washing composition can comprise a non-heme fatty acid decarboxylase having at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, 100% identity to one or more sequences selected from the group consisting of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, and their functional fragments thereof. The hand dish-washing composition may comprise a decarboxylase selected from the group consisting of 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, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, and 83, and their functional fragments. The hand dish-washing composition may comprise a decarboxylase selected from the group consisting of SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, and their functional fragments. The hand dish-washing composition may comprise a decarboxylase with SEQ ID NO: 4.

**[0086]** Identity, or homology, percentages as mentioned herein in respect of the present invention are those that can be calculated, for example, with AlignX obtainable from Thermo Fischer Scientific or with the alignment tool from Uniprot (<https://www.uniprot.org/align/>). Alternatively, a manual alignment can be performed. For enzyme sequence comparison the following settings can be used: Alignment algorithm: Needleman and Wunsch, J. Mol. Biol. 1970, 48: 443-453. As a comparison matrix for amino acid similarity the Blosom62 matrix can be used (Henikoff S. and Henikoff J.G., P.N.A.S.

USA 1992, 89: 10915-10919). The following gap scoring parameters can be used: Gap penalty: 12, gap length penalty: 2, no penalty for end gaps.

**[0087]** A given sequence is typically compared against the full-length sequence or fragments of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, and 106 to obtain a score. Polypeptides of the present disclosure include polypeptides containing an amino acid sequence having at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of any one of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, and 106. Polypeptides of the disclosure also include polypeptides having at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, or at least 80 consecutive amino acids of the amino acid sequence of any one of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, and 106.

**[0088]** The present invention can also include variants of non-heme fatty acid decarboxylases, as discussed previously. Variants of non-heme fatty acid decarboxylases include polypeptide sequences resulting from modification of a wild-type non-heme fatty acid decarboxylase at one or more amino acids. A variant includes a "modified enzyme" or a "mutant enzyme" which encompasses proteins having at least one substitution, insertion, and/or deletion of an amino acid. A modified enzyme may have 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more amino acid modifications (selected from substitutions, insertions, deletions and combinations thereof).

**[0089]** The variants may have "conservative" substitutions. Suitable examples of conservative substitution includes one conservative substitution in the enzyme, such as a conservative substitution in SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, and their functional fragments thereof. Other suitable examples include 10 or fewer conservative substitutions in the protein, such as five or fewer. An enzyme of the invention may therefore include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more conservative substitutions. An enzyme can be produced to contain one or more conservative substitutions by manipulating the nucleotide sequence that encodes that enzyme using, for example, standard procedures such as site-directed mutagenesis or PCR. Examples of amino acids which may be substituted for an original amino acid in an enzyme and which are regarded as conservative substitutions include: Ser for Ala; Lys for Arg; Gln or His for Asn; Glu for Asp; Asn for Gln; Asp for Glu; Pro for Gly; Asn or Gln for His; Leu or Val for Ile; Ile or Val for Leu; Arg or Gln for Lys; Leu or Ile for Met; Met, Leu or Tyr for Phe; Thr for Ser; Ser for Thr; Tyr for Trp; Trp or Phe for Tyr; and Ile or Leu for Val.

**[0090]** The variant of the non-heme fatty acid decarboxylase can comprise a polypeptide sequence comprising at least one amino acid substitution at positions selected from the group consisting of: 35, 36, 38, 40, 41, 44, 54, 57, 58, 60, 61, 108, 111, 130, 131, 133, 134, 165, 169, 235, 236, 238, 239, 240, 243, 247, 40/41, 40/44, 40/57, 40/165, 40/239, 40/240, 41/44, 41/57, 41/131, 41/165, 41/239, 41/240, 41/243, 44/57, 44/131, 44/165, 44/239, 44/240, 44/243, 57/131, 57/165, 57/239, 57/240, 57/243, 133/239, 239/240, 240/243, 40/41/57, 40/41/239, 40/41/240, 40/44/240, 40/57/240, 40/165/240, 40/57/239, 40/57/240, 40/239/240, 41/44/57, 41/44/239, 41/44/240, 41/57/165, 41/57/239, 41/57/240, 41/165/239, 41/165/240, 41/239/240, 44/57/239, 44/57/240, 44/165/240, 44/239/240, 57/165/239, 57/165/240, 57/239/240, 133/238/239, 60/133/239, 133/235/239, 165/239/240, 40/41/57/239, 40/41/57/240, 40/41/239/240, 40/57/239/240, 41/57/165/239, 41/57/165/240, 41/57/239/240, 57/61/239/240, 57/131/239/240, 57/134/239/240, 57/239/240/243, 131/134/240/243, 40/41/57/165, 40/41/57/239/240, 41/57/239/240/165, 57/130/134/239/240, 40/41/44/57/239/240, 40/41/57/165/239/240, and combinations thereof; wherein said positions are numbered with reference to SEQ ID NO: 1. The variant of the non-heme fatty acid decarboxylase can comprise a polypeptide sequence comprising at least one amino acid substitution selected from the group consisting of: alanine, asparagine, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tryptophan, and valine; more preferably alanine, glycine, isoleucine, leucine, methionine, phenylalanine, and valine.

**[0091]** The variant of the non-heme fatty acid decarboxylase can comprise a polypeptide sequence comprising at least one amino acid substitution selected from the group consisting of V35(A/T), V36(A/R/T), L40(A/F/H/M/T/V/W), Y41(A/F/G/I/L/M/N/V/W), M44(A/E/F/I/L/T/V/W), M54(A/G/I/N/Q), Y57(A/F/G/H/I/L/M/V), L58(A/F/G), G60A, G61A, W108(A/F/G/L/M), W111(A/F/G/L/M/S/T/V), L131(A/D/F/G/H/I/L/M/N/T/V), A133G, L134(A/G/T/V), W165(A/F/G/I/L/V),

V169(A/I/L), Y235A, M236(A/G), L238(A/I/Q/Y), F239(A/G/I/S/T/V), L240(A/F/M/Q), C243(A/G/I/L/M/Q), and E247(A/G/I/L/V); wherein said positions are numbered with reference to SEQ ID NO: 1.

**[0092]** It is important that variants of enzymes retain and preferably improve the ability of the wild-type protein to catalyze the conversion of the fatty acids. Some performance drop in a given property of variants may of course be tolerated, but the variants should retain and preferably improve suitable properties for the relevant application for which they are intended. Screening of variants of one of the wild-types can be used to identify whether they retain and preferably improve appropriate properties.

**[0093]** The decarboxylase polypeptides described herein are not restricted to the genetically encoded amino acids. Thus, in addition to the genetically encoded amino acids, the polypeptides described herein may be comprised, either in whole or in part, of naturally-occurring and/or synthetic non-encoded amino acids. Certain commonly encountered non-encoded amino acids of which the polypeptides described herein may be comprised include, but are not limited to: the D-stereoisomers of the genetically-encoded amino acids; 2,3-diaminopropionic acid (Dpr);  $\alpha$ -aminoisobutyric acid (Aib);  $\epsilon$ -aminohexanoic acid (Aha);  $\delta$ -aminovaleric acid (Ava); N-methylglycine or sarcosine (MeGly or Sar); ornithine (Orn); citrulline (Cit); t-butylalanine (Bua); t-butylglycine (Bug); N-methylisoleucine (Melle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); naphthylalanine (Nal); 2-chlorophenylalanine (Oct); 3-chlorophenylalanine (Mcf); 4-chlorophenylalanine (Pcf); 2-fluorophenylalanine (Off); 3-fluorophenylalanine (Mff); 4-fluorophenylalanine (Pff); 2-bromophenylalanine (Obf); 3-bromophenylalanine (Mbf); 4-bromophenylalanine (Pbf); 2-methylphenylalanine (Omf); 3-methylphenylalanine (Mmf); 4-methylphenylalanine (Pmf); 2-nitrophenylalanine (Onf); 3-nitrophenylalanine (Mnf); 4-nitrophenylalanine (Pnf); 2-cyanophenylalanine (Ocf); 3-cyanophenylalanine (Mcf); 4-cyanophenylalanine (Pcf); 2-trifluoromethylphenylalanine (Otf); 3-trifluoromethylphenylalanine (Mtf); 4-trifluoromethylphenylalanine (Ptf); 4-aminophenylalanine (Paf); 4-iodophenylalanine (Pif); 4-aminomethylphenylalanine (Pamf); 2,4-dichlorophenylalanine (Opef); 3,4-dichlorophenylalanine (Mpcf); 2,4-difluorophenylalanine (Opff); 3,4-difluorophenylalanine (Mpff); pyrid-2-ylalanine (2pAla); pyrid-3-ylalanine (3pAla); pyrid-4-ylalanine (4pAla); naphth-1-ylalanine (1nAla); naphth-2-ylalanine (2nAla); thiazolylalanine (taAla); benzothienylalanine (bAla); thienylalanine (tAla); furylalanine (fAla); homophenylalanine (hPhe); homotyrosine (hTyr); homotryptophan (hTrp); pentafluorophenylalanine (5ff); styrylalanine (sAla); aurylalanine (aAla); 3,3-diphenylalanine (Dfa); 3-amino-5-phenylpentanoic acid (Afp); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic);  $\beta$ -2-thienylalanine (Thi); methionine sulfoxide (Mso); N(w)-nitroarginine (nArg); homolysine (hLys); phosphonomethylphenylalanine (pmPhe); phosphoserine (pSer); phosphothreonine (pThr); homoaspartic acid (hAsp); homoglutamic acid (hGlu); 1-aminocyclopent-(2 or 3)-ene-4 carboxylic acid; pipecolic acid (PA), azetidine-3-carboxylic acid (ACA); 1-aminocyclopentane-3-carboxylic acid; allylglycine (aOly); propargylglycine (pgGly); homoalanine (hAla); norvaline (nVal); homoleucine (hLeu), homovaline (hVal); homoisoleucine (hIle); homoarginine (hArg); N-acetyl lysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,3-diaminobutyric acid (Dab); N-methylvaline (MeVal); homocysteine (hCys); homoserine (hSer); hydroxyproline (Hyp) and homoproline (hPro). Additional non-encoded amino acids of which the polypeptides described herein may be comprised will be apparent to those of skill in the art. These amino acids may be in either the L- or D-configuration.

**[0094]** The invention also can include variants in the form of truncated forms or fragments derived from a wild-type enzyme, such as a protein with a truncated N-terminus or a truncated C-terminus. Variants of decarboxylase enzymes can comprise a fragment of any of the decarboxylase polypeptides described herein that retain functional decarboxylase activity and/or an improved property of an engineered decarboxylase polypeptide. Accordingly, the composition can comprise a polypeptide fragment having decarboxylase activity (e.g., capable of converting substrate to product under suitable reaction conditions), wherein the fragment comprises at least about 80%, 90%, 95%, 98%, or 99% of a full-length amino acid sequence of the engineered polypeptide.

**[0095]** The present invention can include a decarboxylase enzyme having an amino acid sequence comprising an insertion as compared to any one of the decarboxylase polypeptide sequences described herein. The insertions can comprise one or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acids, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, where the associated functional activity and/or improved properties of the decarboxylase described herein is maintained. The insertions can be to amino or carboxy terminus, or internal portions of the decarboxylase polypeptide. The invention can also include variants derived by adding an extra amino acid sequence, such as an N-terminal tag or a C-terminal tag. Suitable tags are maltose binding protein (MBP) tag, glutathione S-transferase (GST) tag, thioredoxin (Trx) tag, His-tag, and any other tags known by those skilled in art. Tags can be used to improve solubility and expression levels during fermentation or as a handle for enzyme purification.

**[0096]** Enzymes can also be modified by a variety of chemical techniques to produce derivatives having essentially the same or preferably improved activity as the unmodified enzymes, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified, for example to form a C1-C6 alkyl ester, or converted to an amide, for example of formula CONR1R2 wherein R1 and R2 are each independently H or C1-C6 alkyl, or combined to form a heterocyclic ring, such as a 5- or 6-membered ring. Amino groups of the enzyme, whether amino-terminal or

side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C1-C20 alkyl or dialkyl amino or further converted to an amide. Hydroxyl groups of the protein side chains may be converted to alkoxy or ester groups, for example C1-C20 alkoxy or C1-C20 alkyl ester, using well-recognized techniques. Phenyl and phenolic rings of the protein side chains may be substituted with one or more halogen atoms, such as F, Cl, Br or I, or with C1-C20 alkyl, C1-C20 alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the protein side chains can be extended to homologous C2-C4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the proteins of this disclosure to select and provide conformational constraints to the structure that result in enhanced stability.

**[0097]** The enzymes can be provided on a solid support, such as a membrane, resin, solid carrier, or other solid phase material. A solid support can be composed of organic polymers such as polystyrene, polyethylene, polypropylene, polyfluoroethylene, polyethyleneoxy, and polyacrylamide, as well as co-polymers and grafts thereof. A solid support can also be inorganic, such as glass, silica, controlled pore glass (CPG), reverse phase silica or metal, such as gold or platinum. The configuration of a solid support can be in the form of beads, spheres, particles, granules, a gel, a membrane or a surface. Surfaces can be planar, substantially planar, or nonplanar. Solid supports can be porous or non-porous, and can have swelling or non-swelling characteristics. A solid support can be configured in the form of a well, depression, or other container, vessel, feature, or location.

**[0098]** The polypeptides having decarboxylase activity can be bound or immobilized on the solid support such that they retain at least a portion of their improved properties relative to a reference polypeptide (e.g., SEQ ID NO: 1). Accordingly, it is further contemplated that any of the methods of using the decarboxylase polypeptides of the present invention can be carried out using the same decarboxylase polypeptides bound or immobilized on a solid support.

**[0099]** The decarboxylase polypeptide can be bound non-covalently or covalently. Various methods for conjugation and immobilization of enzymes to solid supports (e.g., resins, membranes, beads, glass, etc.) are well known in the art. Other methods for conjugation and immobilization of enzymes to solid supports (e.g., resins, membranes, beads, glass, etc.) are well known in the art (See, e.g., Yi et al., Proc. Biochem., 42: 895-898 [2007]; Martin et al., Appl. Microbiol. Biotechnol., 76: 843-851 [2007]; Koszelewski et al. J. Mol. Cat. B: Enz., 63: 39-44 [2010]; Truppo et al., Org. Proc. Res. Develop., published online: dx.doi.org/10.1021/op200157c; and Mateo et al., Biotechnol. Prog., 18:629-34 [2002], etc.). Solid supports useful for immobilizing the decarboxylase polypeptides of the present invention include, but are not limited to, beads or resins comprising polymethacrylate with epoxide functional groups, polymethacrylate with amino epoxide functional groups, styrene/DVB copolymer or polymethacrylate with octadecyl functional groups.

**[0100]** The enzymes may be incorporated into the hand dish-washing compositions *via* an additive particle, such as an enzyme granule or in the form of an encapsulate, or may be added in the form of a liquid formulation. Preferably the enzyme is incorporated into the cleaning composition *via* an encapsulate. Encapsulating the enzymes promote the stability of the enzymes in the composition and helps to counteract the effect of any hostile compounds present in the composition, such as bleach, protease, surfactant, chelant, etc. The non-heme fatty acid decarboxylase enzymes may be the only enzymes in the additive particle or may be present in the additive particle in combination with one or more additional co-enzymes.

**[0101]** The hand dish-washing composition can comprise a non-heme fatty acid decarboxylase, wherein said non-heme fatty acid decarboxylase is present in an amount of from 0.0001 wt% to 1 wt%, preferably from 0.001 wt% to 0.2 wt%, by weight of the hand dish-washing composition, based on active protein.

**[0102]** The hand dish-washing composition may further comprise one or more co-enzymes selected from the group consisting of: fatty-acid peroxidases (EC 1.11.1.3), unspecific peroxygenases (EC 1.11.2.1), plant seed peroxygenases (EC 1.11.2.3), fatty acid peroxygenases (EC 1.11.2.4), linoleate diol synthases (EC 1.13.11.44), 5,8-linoleate diol synthases (EC 1.13.11.60 and EC 5.4.4.5), 7,8-linoleate diol synthases (EC 1.13.11.60 and EC 5.4.4.6), 9,14-linoleate diol synthases (EC 1.13.11.B1), 8,11-linoleate diol synthases, oleate diol synthases, other linoleate diol synthases, unspecific monooxygenase (EC 1.14.14.1), alkane 1-monooxygenase (EC 1.14.15.3), oleate 12-hydroxylases (EC 1.14.18.4), fatty acid amide hydrolase (EC 3.5.1.99), oleate hydratases (EC 4.2.1.53), linoleate isomerases (EC 5.2.1.5), linoleate (10E,12Z)-isomerases (EC 5.3.3.B2), fatty acid decarboxylases (OleT-like), alpha-dioxygenases, amylases, lipases, proteases, cellulases, and mixtures thereof; preferably fatty-acid peroxidases (EC 1.11.1.3), unspecific peroxygenases (EC 1.11.2.1), plant seed peroxygenases (EC 1.11.2.3), and fatty acid peroxygenases (EC 1.11.2.4), heme fatty acid decarboxylases (OleT-like), alpha-dioxygenases, and mixtures thereof.

**[0103]** Where necessary, the composition comprises, provides access to, or forms *in situ* any additional substrate necessary for the effective functioning of the enzyme. For example, molecular oxygen can be provided as an additional substrate for non-heme fatty acid decarboxylases. Molecular oxygen can be obtained from the atmosphere or from a precursor that can be transformed to produce oxygen *in situ*. In many applications, oxygen from the atmosphere can be present in sufficient amounts. The hand dish-washing composition may be supplemented with iron (Fe) or a source of iron, preferably a source of iron(II), to enhance or facilitate the conversion of the fatty acids. Non-limiting examples of

sources of iron(II) are such as ammonium iron(II) sulfate, iron(II) sulfate, iron(II) chloride, iron(II) oxide, iron(II) acetate, iron(II) citrate, and iron(II) oxalate. The hand dish-washing composition may also be supplemented with a reducing agent. Non-limiting examples of reducing agents are ascorbic acid and cysteine. The hand dish-washing composition may be supplemented with combinations of various compounds and/or reagents, such as, for example, a source of iron, ascorbic acid, and/or cysteine.

#### Methods of Producing Decarboxylase Polypeptides

**[0104]** Standard methods of culturing organisms such as, for example, bacteria and yeast, for production of enzymes are well-known in the art and are described herein. For example, host cells may be cultured in a standard growth media under standard temperature and pressure conditions, and in an aerobic environment. Standard growth media for various host cells are commercially available and well-known in the art, as are standard conditions for growing various host cells.

**[0105]** Decarboxylase enzymes expressed in a host cell can be recovered from the cells and/or the culture medium using any one or more of the well-known techniques for protein purification, including, among others, lysozyme treatment, sonication, filtration, salting-out, ultra-centrifugation, and chromatography. Suitable solutions for lysing and the high efficiency extraction of proteins from bacteria, such as *E. coli*, are commercially available under the trade name CellLytic B (Sigma-Aldrich). Chromatographic techniques for isolation of the decarboxylase polypeptide include, among others, reverse phase chromatography, high performance liquid chromatography (HPLC), ion exchange chromatography, gel electrophoresis, and affinity chromatography. Conditions for purifying a particular enzyme will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity, molecular weight, molecular shape, etc., and will be apparent to those having skill in the art.

**[0106]** The decarboxylases may also be prepared and used in the form of cells expressing the enzymes, as crude extracts, or as isolated or purified preparations. The decarboxylases may be prepared as lyophilizates, in powder form (e.g., acetone powders), or prepared as enzyme solutions. The decarboxylases can be in the form of substantially pure preparations.

#### Adjunct Ingredients

**[0107]** The cleaning composition herein may optionally comprise a number of other adjunct ingredients such as additional enzymes, enzyme stabilisers, organic solvents, polymers, cleaning amines, chelants, builders (e.g., preferably citrate), structurants, emollients, humectants, skin rejuvenating actives, scrubbing particles, bleach and bleach activators, perfumes, malodor control agents, pigments, dyes, opacifiers, beads, pearlescent particles, capsules, inorganic cations such as alkaline earth metals such as Ca/Mg-ions, antibacterial agents, preservatives, viscosity adjusters (e.g., salt such as NaCl, and other mono-, di- and trivalent salts) and pH adjusters and buffering means (e.g., carboxylic acids such as citric acid, HCl, NaOH, KOH, alkanolamines, phosphoric and sulfonic acids, carbonates such as sodium carbonates, bicarbonates, sesquicarbonates, borates, silicates, phosphates, imidazole and alike).

#### Additional Enzymes

**[0108]** Preferred compositions of the invention comprise one or more enzymes selected from lipases, proteases, cellulases, amylases and any combination thereof.

**[0109]** Each additional enzyme is typically present in an amount from 0.0001 wt% to 1 wt% (weight of active protein) more preferably from 0.0005 wt% to 0.5 wt%, most preferably 0.005-0.1%. It may be particularly preferred for the compositions of the present invention to additionally comprise a lipase enzyme. Lipases break down fatty ester soils into fatty acids which are then acted upon by the saturated and/or unsaturated fatty acid-transforming enzyme according to the invention into suds neutral or suds boosting agents.

**[0110]** It may be particularly preferred for the compositions of the present invention to additionally comprise a protease enzyme. Since oleic acid and other foam suppressing saturated and/or unsaturated fatty acids are present in body soils or even human skin, as protease enzyme acts as a skin care agent, or breaks down proteinaceous soils, fatty acids released are broken down, preventing suds suppression.

**[0111]** It may be particularly preferred for the compositions of the present invention to additionally comprise an amylase enzyme. Since oily soils are commonly entrapped in starchy soils, the amylase and saturated and/or unsaturated fatty acid transforming enzymes work synergistically together: fatty acid soils are released by breakdown of starchy soils with amylase, thus, the saturated and/or unsaturated fatty acid transforming enzyme of use in the invention is particularly effective in ensuring there is no negative impact on suds in the wash liquor.



Enzyme Stabiliser

**[0112]** Preferably the composition of the invention comprises an enzyme stabilizer. Suitable enzyme stabilizers may be selected from the group consisting of (a) univalent, bivalent and/or trivalent cations preferably selected from the group of inorganic or organic salts of alkaline earth metals, alkali metals, aluminum, iron, copper and zinc, preferably alkali metals and alkaline earth metals, preferably alkali metal and alkaline earth metal salts with halides, sulfates, sulfites, carbonates, hydrogencarbonates, nitrates, nitrites, phosphates, formates, acetates, propionates, citrates, maleates, tartrates, succinates, oxalates, lactates, and mixtures thereof. The salt can be selected from the group consisting of sodium chloride, calcium chloride, potassium chloride, sodium sulfate, potassium sulfate, sodium acetate, potassium acetate, sodium formate, potassium formate, calcium lactate, calcium nitrate and mixtures thereof. Most preferred are salts selected from the group consisting of calcium chloride, potassium chloride, potassium sulfate, sodium acetate, potassium acetate, sodium formate, potassium formate, calcium lactate, calcium nitrate, and mixtures thereof, and in particular potassium salts selected from the group of potassium chloride, potassium sulfate, potassium acetate, potassium formate, potassium propionate, potassium lactate and mixtures thereof. Most preferred are potassium acetate and potassium chloride. Preferred calcium salts are calcium formate, calcium lactate and calcium nitrate including calcium nitrate tetrahydrate. Calcium and sodium formate salts may be preferred. These cations are present at at least 0.01 wt%, preferably at least 0.03 wt%, more preferably at least 0.05 wt%, most preferably at least 0.25 wt% up to 2 wt% or even up to 1 wt% by weight of the total composition. These salts are formulated from 0.1 wt% to 5 wt%, preferably from 0.2 wt% to 4 wt%, more preferably from 0.3 wt% to 3 wt%, most preferably from 0.5 wt% to 2 wt% relative to the total weight of the composition. Further enzyme stabilizers can be selected from the group (b) carbohydrates selected from the group consisting of oligosaccharides, polysaccharides and mixtures thereof, such as a monosaccharide glycerate as described in WO2012/19844; (c) mass efficient reversible protease inhibitors selected from the group consisting of phenyl boronic acid and derivatives thereof, preferably 4-formyl phenylboronic acid; (d) alcohols such as 1,2-propane diol, propylene glycol; (e) peptide aldehyde stabilizers such as tripeptide aldehydes such as Cbz-Gly-Ala-Tyr-H, or disubstituted alaninamide; (f) carboxylic acids such as phenyl alkyl dicarboxylic acid as described in WO2012/19849 or multiply substituted benzyl carboxylic acid comprising a carboxyl group on at least two carbon atoms of the benzyl radical such as described in WO2012/19848, phthaloyl glutamine acid, phthaloyl asparagine acid, aminophthalic acid and/or an oligoamino-biphenyl-oligocarboxylic acid; and (g) mixtures thereof.

**[0113]** The composition of the present invention may optionally comprise from 0.01% to 3%, preferably from 0.05% to 2%, more preferably from 0.2% to 1.5%, or most preferably 0.5% to 1%, by weight of the total composition of a salt, preferably a monovalent, divalent inorganic salt or a mixture thereof, preferably sodium chloride. Most preferably the composition alternatively or further comprises a multivalent metal cation in the amount of from 0.01 wt% to 3 wt%, preferably from 0.05% to 2%, more preferably from 0.2% to 1.5%, or most preferably 0.5% to 1% by weight of said composition, preferably said multivalent metal cation is magnesium, aluminium, copper, calcium or iron, more preferably magnesium, most preferably said multivalent salt is magnesium chloride. Without wishing to be bound by theory, it is believed that use of a multivalent cation helps with the formation of protein/ protein, surfactant/ surfactant or hybrid protein/ surfactant network at the oil water and air water interface that is strengthening the suds.

**[0114]** Preferably the composition of the present invention comprises one or more carbohydrates selected from the group comprising O-glycan, N-glycan, and mixtures thereof. Preferably the cleaning composition further comprises one or more carbohydrates selected from the group comprising derivatives of glucose, mannose, lactose, galactose, allose, altrose, gulose, idose, talose, fucose, fructose, sorbose, tagatose, psicose, arabinose, ribose, xylose, lyxose, ribulose, and xylulose. More preferably the cleaning composition comprises one or more carbohydrates selected from the group of  $\alpha$ -glucans and  $\beta$ -glucans. Glucans are polysaccharides of D-glucose monomers, linked by glycosidic bonds. Suitable  $\alpha$ -glucans are dextran, starch, floridean starch, glycogen, pullulan, and their derivatives. Suitable  $\beta$ -glucans are cellulose, chrysolaminarin, curdlan, laminarin, lentinan, lichenin, oat beta-glucan, pleuran, zymosan, and their derivatives.

Hydrotrope

**[0115]** The composition of the present invention may optionally comprise from 1% to 10%, or preferably from 0.5% to 10%, more preferably from 1% to 6%, or most preferably from 0.1% to 3%, or combinations thereof, by weight of the total composition of a hydrotrope, preferably sodium cumene sulfonate. Other suitable hydrotropes for use herein include anionic-type hydrotropes, particularly sodium, potassium, and ammonium xylene sulfonate, sodium, potassium and ammonium toluene sulfonate, sodium potassium and ammonium cumene sulfonate, and mixtures thereof, as disclosed in U.S. Patent 3,915,903. Preferably the composition of the present invention is isotropic. An isotropic composition is distinguished from oil-in-water emulsions and lamellar phase compositions. Polarized light microscopy can assess whether the composition is isotropic. See e.g., *The Aqueous Phase Behaviour of Surfactants*, Robert Laughlin, Academic Press, 1994, pp. 538-542. Preferably an isotropic composition is provided. Preferably the composition comprises 0.1% to 3% by weight of the total composition of a hydrotrope, preferably wherein the hydrotrope is selected from sodium,

potassium, and ammonium xylene sulfonate, sodium, potassium and ammonium toluene sulfonate, sodium potassium and ammonium cumene sulfonate, and mixtures thereof.

#### Organic solvent

**[0116]** The composition of the present invention may optionally comprise an organic solvent. Suitable organic solvents include C4-14 ethers and diethers, polyols, glycols, alkoxyated glycols, C6-C16 glycol ethers, alkoxyated aromatic alcohols, aromatic alcohols, aliphatic linear or branched alcohols, alkoxyated aliphatic linear or branched alcohols, alkoxyated C1-C5 alcohols, C8-C14 alkyl and cycloalkyl hydrocarbons and halo hydrocarbons, and mixtures thereof. Preferably the organic solvents include alcohols, glycols, and glycol ethers, alternatively alcohols and glycols. The composition comprises from 0% to less than 50%, preferably from 0.01% to 25%, more preferably from 0.1% to 10%, or most preferably from 0.5% to 5%, by weight of the total composition of an organic solvent, preferably an alcohol, more preferably an ethanol, a polyalkyleneglycol, more preferably polypropyleneglycol, and mixtures thereof.

#### Polymer:

**[0117]** The composition can comprise a polymer, preferably at a level of from 0.1% to 5%, more preferably from 0.2% to 3%, even more preferably from 0.3% to 2% by weight of the liquid composition. Suitable polymers can be selected from triblock copolymers, amphiphilic alkoxyated polyalkyleneimine, ethoxyated polyalkyleneimine, polyester soil release polymers, and mixtures thereof, preferably triblock copolymers, amphiphilic alkoxyated polyalkyleneimine, and mixtures thereof.

**[0118]** Suitable triblock copolymers comprise alkylene oxide moieties according to Formula (I):  $(EO)_x(PO)_y(EO)_x$ , wherein EO represents ethylene oxide, and each x represents the number of EO units within the EO block. Each x is independently a number average between 3 and 50, preferably between 5 and 25, more preferably between 10 and 15. Preferably x is the same for both EO blocks, wherein the "same" means that the x between the two EO blocks varies within a maximum 2 units, preferably within a maximum of 1 unit, more preferably both x's are the same number of units. PO represents propylene oxide, and y represents the number of PO units in the PO block. Each y is a number average between 5 and 60, preferably between 10 and 40, more preferably between 25 and 35.

**[0119]** The triblock co-polymer can have a ratio of y to each x of from 0.8:1 to 5:1, preferably from 1:1 to 3:1, more preferably from 1.5:1 to 2.5:1. The triblock co-polymer can have an average weight percentage of total EO of between 30% and 50% by weight of the triblock co-polymer. As such, the triblock co-polymer can have an average weight percentage of total PO of between 50% and 70% by weight of the triblock copolymer. It is understood that the average total weight % of EO and PO for the triblock co-polymer adds up to 100%, excluding the end-caps. The end-caps are preferably hydrogen, hydroxyl, methyl, and mixtures thereof, more preferably hydrogen, methyl, and mixtures thereof, and most preferably hydrogen. The triblock co-polymer has a number average molecular weight of between 550 and 8000, preferably between 1000 and 4500, more preferably between 2000 and 3100. Number average molecular weight and compositional analysis of the co-polymer is determined using a  $^1H$  NMR spectroscopy (see Thermo scientific application note No. AN52907). It is an established tool for polymer characterization, including number-average molecular weight determination and co-polymer composition analysis.

**[0120]** EO-PO-EO triblock co-polymers are commercially available from BASF such as the Pluronic® PE series, and from the Dow Chemical Company such as Tergitol™ L series. Particularly preferred triblock co-polymer from BASF are sold under the tradenames Pluronic® L44 (MW ca 2200, ca 40wt% EO), Pluronic® PE6400 (MW ca 2900, ca 40wt% EO), Pluronic® PE4300 (MW ca 1600, ca 30wt% EO), and Pluronic® PE 9400 (MW ca 4600, 40 wt% EO). Particularly preferred triblock co-polymer from the Dow Chemical Company is sold under the tradename of Tergitol™ L64 (MW ca 2900, ca 40 wt% EO). The preparation method for such triblock co-polymers is well known to polymer manufacturers.

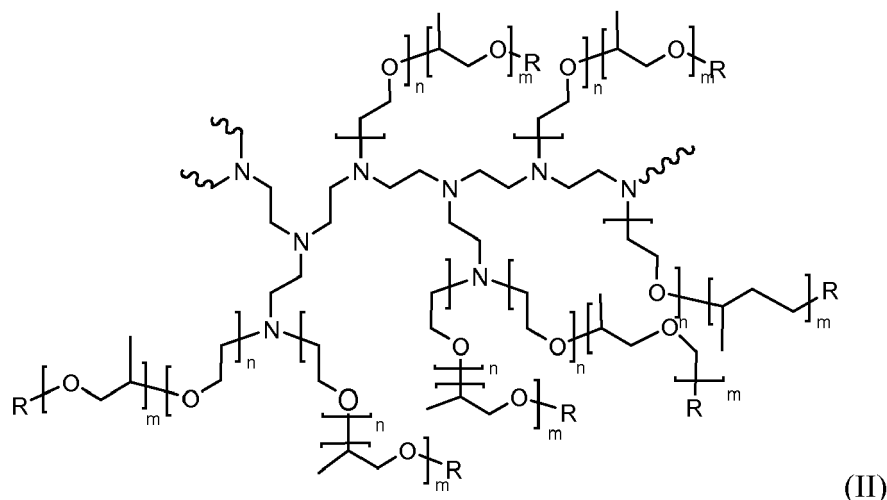
**[0121]** Suitable amphiphilic polymers can be selected from the group consisting of: amphiphilic alkoxyated polyalkyleneimine and mixtures thereof. Preferably, the amphiphilic alkoxyated polyalkyleneimine is an alkoxyated polyethyleneimine polymer comprising a polyethyleneimine backbone having a weight average molecular weight range of from 100 to 5,000, preferably from 400 to 2,000, more preferably from 400 to 1,000 Daltons. The polyethyleneimine backbone comprises the following modifications:

(i) one or two alkoxylation modifications per nitrogen atom, dependent on whether the modification occurs at an internal nitrogen atom or at a terminal nitrogen atom, in the polyethyleneimine backbone, the alkoxylation modification consisting of the replacement of a hydrogen atom on by a polyalkoxyene chain having an average of about 1 to about 50 alkoxy moieties per modification, wherein the terminal alkoxy moiety of the alkoxylation modification is capped with hydrogen, a C1-C4 alkyl or mixtures thereof;

(ii) a substitution of one C1-C4 alkyl moiety and one or two alkoxylation modifications per nitrogen atom, dependent on whether the substitution occurs at a internal nitrogen atom or at a terminal nitrogen atom, in the polyethyleneimine

backbone, the alkoxylation modification consisting of the replacement of a hydrogen atom by a polyalkoxy chain having an average of about 1 to about 50 alkoxy moieties per modification wherein the terminal alkoxy moiety is capped with hydrogen, a C1-C4 alkyl or mixtures thereof; or  
(iii) a combination thereof.

**[0122]** A preferred amphiphilic alkoxyated polyethyleneimine polymer has the general structure of formula (II):



wherein the polyethyleneimine backbone has a weight average molecular weight of about 600,  $n$  of formula (II) has an average of about 10,  $m$  of formula (II) has an average of about 7 and  $R$  of formula (II) is selected from hydrogen, a C<sub>1</sub>-C<sub>4</sub> alkyl and mixtures thereof, preferably hydrogen. The degree of permanent quaternization of formula (II) may be from 0% to about 22% of the polyethyleneimine backbone nitrogen atoms. The molecular weight of this amphiphilic alkoxyated polyethyleneimine polymer preferably is between 10,000 and 15,000 Da.

**[0123]** More preferably, the amphiphilic alkoxyated polyethyleneimine polymer has the general structure of formula (II) but wherein the polyethyleneimine backbone has a weight average molecular weight of about 600 Da,  $n$  of Formula (II) has an average of about 24,  $m$  of Formula (II) has an average of about 16 and  $R$  of Formula (II) is selected from hydrogen, a C<sub>1</sub>-C<sub>4</sub> alkyl and mixtures thereof, preferably hydrogen. The degree of permanent quaternization of Formula (II) may be from 0% to about 22% of the polyethyleneimine backbone nitrogen atoms, and is preferably 0%. The molecular weight of this amphiphilic alkoxyated polyethyleneimine polymer preferably is between 25,000 and 30,000, most preferably 28,000 Da.

**[0124]** The amphiphilic alkoxyated polyethyleneimine polymers can be made by the methods described in more detail in PCT Publication No. WO 2007/135645.

**[0125]** Alternatively, the alkoxyated polyalkyleneimine polymer can be an ethoxylated polyalkyleneimine which comprises no further alkoxylation, and as such, is hydrophilic rather than amphiphilic. That is, the ethoxylated polyalkyleneimine comprises no further alkoxylation such as propoxylation or butoxylation. Preferred ethoxylated polyalkyleneimines consist of alkyleneimine monomer units and ethoxylation (-EO-) monomer units, with the exception of any end-caps, which are typically hydrogen. Ethyleneimine monomer units are highly preferred alkyleneimine monomer units. More preferably, the hydrophilic ethoxylated polyethyleneimine polymer has the general structure of formula (II) but wherein the polyethyleneimine backbone has a weight average molecular weight of about 600 Da,  $n$  of Formula (II) has an average of about 20,  $m$  of Formula (II) is zero and  $R$  of Formula (II) is selected from hydrogen, a C<sub>1</sub>-C<sub>4</sub> alkyl and mixtures thereof, preferably hydrogen. The degree of permanent quaternization of Formula (II) may be from 0% to about 22% of the polyethyleneimine backbone nitrogen atoms, and is preferably 0%. The molecular weight of this ethoxylated polyethyleneimine polymer preferably is between 10,000 and 15,000, most preferably 12,600 Da.

**[0126]** Polyester soil release agents are also suitable polymers. Soil release agents are polymers having soil release properties, i.e. having the property to enhance the cleaning efficacy of the detergent composition by improving release of greasy and oil during the laundry process. See soil release agents' definition, p.278-279, "Liquid Detergents" by Kuo-Yann Lai.

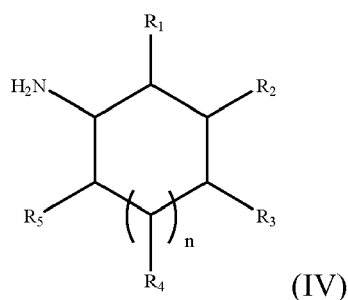
**[0127]** Suitable polyester soil release agents can encompass simple copolymeric blocks of ethylene terephthalate or propylene terephthalate with polyethylene oxide or polypropylene oxide terephthalate (see US 3,959,230 and US 3,893,929). Other suitable polyester soil release agents can be polyesters with repeat units containing 10-15% by weight of ethylene terephthalate together with 90-80% by weight of polyoxyethylene terephthalate, derived from a polyoxyeth-

ylene glycol of average molecular weight 300-5,000. Commercial examples include ZELCON® 5126 from Dupont and MILEASE®T from ICI. Suitable polymeric soil release agents can be prepared by art-recognized methods. US 4, 702, 857 and US 4,711,730 describe the preferred method of synthesis for the block polyesters of use.

## Cyclic Polyamine

**[0128]** The composition can comprise a cyclic polyamine having amine functionalities that helps cleaning. The composition of the invention preferably comprises from about 0.1% to about 3%, more preferably from about 0.2% to about 2%, and especially from about 0.5% to about 1%, by weight of the composition, of the cyclic polyamine.

**[0129]** The amine can be subjected to protonation depending on the pH of the cleaning medium in which it is used. Preferred cyclic polyamines have the following Formula (IV):



wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  are independently selected from the group consisting of  $NH_2$ ,  $-H$ , linear or branched alkyl having from about 1 to about 10 carbon atoms, and linear or branched alkenyl having from about 1 to about 10 carbon atoms,  $n$  is from about 1 to about 3, preferably  $n$  is 1, and wherein at least one of the  $R_s$  is  $NH_2$  and the remaining " $R_s$ " are independently selected from the group consisting of  $NH_2$ ,  $-H$ , linear or branched alkyl having about 1 to about 10 carbon atoms, and linear or branched alkenyl having from about 1 to about 10 carbon atoms. Preferably, the cyclic polyamine is a diamine, wherein  $n$  is 1,  $R_2$  is  $NH_2$ , and at least one of  $R_1$ ,  $R_3$ ,  $R_4$  and  $R_5$  is  $CH_3$  and the remaining  $R_s$  are  $H$ .

**[0130]** The cyclic polyamine has at least two primary amine functionalities. The primary amines can be in any position in the cyclic amine but it has been found that in terms of grease cleaning, better performance is obtained when the primary amines are in positions 1,3. It has also been found that cyclic amines in which one of the substituents is  $-CH_3$  and the rest are  $H$  provided for improved grease cleaning performance.

**[0131]** Accordingly, the most preferred cyclic polyamine for use with the detergent composition of the present invention are cyclic polyamine selected from the group consisting of: 2-methylcyclohexane-1,3-diamine, 4-methylcyclohexane-1,3-diamine and mixtures thereof. These specific cyclic polyamines work to improve suds and grease cleaning profile through-out the dishwashing process when formulated together with the surfactant system of the composition of the present invention.

## Chelant

**[0132]** The detergent composition herein can comprise a chelant at a level of from 0.1% to 20%, preferably from 0.2% to 5%, more preferably from 0.2% to 3% by weight of total composition.

**[0133]** As commonly understood in the detergent field, chelation herein means the binding or complexation of a bi- or multidentate ligand. These ligands, which are often organic compounds, are called chelants, chelators, chelating agents, and/or sequestering agent. Chelating agents form multiple bonds with a single metal ion. Chelants, are chemicals that form soluble, complex molecules with certain metal ions, inactivating the ions so that they cannot normally react with other elements or ions to produce precipitates or scale, or forming encrustations on soils turning them harder to be removed. The ligand forms a chelate complex with the substrate. The term is reserved for complexes in which the metal ion is bound to two or more atoms of the chelant.

**[0134]** Preferably, the composition of the present invention comprises one or more chelant, preferably selected from the group comprising carboxylate chelants, amino carboxylate chelants, amino phosphonate chelants such as MGDA (methylglycine-N,N-diacetic acid), GLDA (glutamic-N,N-diacetic acid), and mixtures thereof.

**[0135]** Suitable chelating agents can be selected from the group consisting of amino carboxylates, amino phosphonates, polycarboxylate chelating agents and mixtures thereof.

**[0136]** Other chelants include homopolymers and copolymers of polycarboxylic acids and their partially or completely neutralized salts, monomeric polycarboxylic acids and hydroxycarboxylic acids and their salts. Suitable polycarboxylic acids are acyclic, alicyclic, heterocyclic and aromatic carboxylic acids, in which case they contain at least two carboxyl

groups which are in each case separated from one another by, preferably, no more than two carbon atoms. A suitable hydroxycarboxylic acid is, for example, citric acid. Another suitable polycarboxylic acid is the homopolymer of acrylic acid. Preferred are the polycarboxylates end capped with sulfonates.

## Method of washing

**[0137]** Other aspects of the invention are directed to methods of washing ware especially dishware with a composition of the present invention. Accordingly, there is provided a method of manually washing dishware comprising the steps of delivering a hand-dishwashing composition of the invention into a volume of water to form a wash solution and immersing the dishware in the solution. Preferably the non-heme fatty acid decarboxylase is present at a concentration from 0.005 ppm to 15 ppm, preferably from 0.02 ppm to 0.5 ppm, in an aqueous wash liquor during the washing process. As such, the composition herein will be applied in its diluted form to the dishware. Soiled surfaces e.g. dishes are contacted with an effective amount, typically from 0.5 mL to 20 mL (per 25 dishes being treated), preferably from 3mL to 10 mL, of the detergent composition of the present invention, preferably in liquid form, diluted in water. The actual amount of detergent composition used will be based on the judgment of user, and will typically depend upon factors such as the particular product formulation of the composition, including the concentration of active ingredients in the composition, the number of soiled dishes to be cleaned, the degree of soiling on the dishes, and the like. Generally, from 0.01 mL to 150 mL, preferably from 3 mL to 40 mL of a liquid detergent composition of the invention is combined with from 2,000 mL to 20,000 mL, more typically from 5,000 mL to 15,000 mL of water in a sink having a volumetric capacity in the range of from 1,000 mL to 20,000 mL, more typically from 5,000 mL to 15,000 mL. The soiled dishes are immersed in the sink containing the diluted compositions then obtained, where contacting the soiled surface of the dish with a cloth, sponge, or similar article cleans them. The cloth, sponge, or similar article may be immersed in the detergent composition and water mixture prior to being contacted with the dish surface, and is typically contacted with the dish surface for a period of time ranged from 1 to 10 seconds, although the actual time will vary with each application and user. The contacting of cloth, sponge, or similar article to the surface is preferably accompanied by a concurrent scrubbing of the surface.

**[0138]** Alternatively, the dishwashing composition can be applied directly onto a cleaning implement or the dishes to be cleaned without any pre-dilution step, or with slight dissolutions as is the case when applied using a damp sponge or other implement.

## TEST METHODS

**[0139]** The following assays set forth must be used in order that the invention described and claimed herein may be more fully understood.

### Test Method 1 - Enzyme activity assay for non-heme fatty acid decarboxylases

**[0140]** Enzymatic reactions with non-heme fatty acid decarboxylases can be performed as follows. Aliquots of sodium salts of fatty acids (e.g. sodium palmitate, sodium stearate, sodium oleate, sodium linoleate, or sodium linolenate; final concentration 100  $\mu$ M), ammonium iron(II) sulfate (final concentration 100  $\mu$ M), and ascorbic acid (final concentration 1 mM) are resuspended in a suitable reaction buffer (pH 6 to pH 8). The reaction is started by addition of the enzyme (final concentration 10  $\mu$ M) and the solutions are incubated for up to 240 minutes at a suitable temperature. Aliquots of 100  $\mu$ L of the reaction solutions are collected at different time points and mixed with 900  $\mu$ L of isopropyl alcohol to stop the reaction. Analysis of the samples is performed by reversed-phase LC/MS/MS or GC/MS using standard procedures known in the art to determine the concentrations of salts of fatty acid remaining in the solutions and the percent conversion is calculated. As used herein, a non-heme fatty acid decarboxylase catalyzes the conversion of a fatty acid when the percent conversion of said fatty acid is at least 5% under optimal reaction conditions in 240 minutes or less time.

## EXAMPLES

**[0141]** Hereinafter, the present invention is described in more detail based on examples. All percentages are by weight unless otherwise specified.

### Example 1 - Production of non-heme fatty acid decarboxylases

**[0142]** A codon optimized gene encoding for a non-heme fatty acid decarboxylase (SEQ ID NO: 4), including an N-terminal amino acid sequence containing a His-tag, is designed and synthesized. After gene synthesis, the complete synthetic gene sequence is subcloned into a pET30a vector for heterologous expression. Then, *Escherichia coli* BL21

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(DE3) cells are transformed with the recombinant plasmid and a single colony is inoculated into TB medium containing kanamycin. Pre-starter cultures are then inoculated into a bioreactor containing the same media and cultivation is performed at 25 °C. At an OD<sub>600nm</sub> = 4, isopropyl β-D-1-thiogalactopyranoside (IPTG; final concentration 0.5 mM) and iron(III) chloride (FeCl<sub>3</sub>; final concentration 50 μM) are added to induce protein expression. Cells are harvested by centrifugation and the pellets are lysed by sonication. After centrifugation, the supernatant is collected and the protein is purified by one-step purification using a nickel affinity column and standard protocols known in the art. The protein is stored in a buffer containing 50 mM Tris-HCl, 150 mM NaCl, and 10% Glycerol at pH 8.0.

### Example 2. Exemplary Manual Dish-Washing Detergent Composition

#### [0143]

Level (as 100% active)	
Sodium alkyl ethoxy sulfate (C1213EO0.6S)	22.91%
n-C12-14 Di Methyl Amine Oxide	7.64%
Lutensol XP80 (non-ionic surfactant supplied by BASF)	0.45%
Sodium Chloride	1.2%
Poly Propylene Glycol (weight average molecular wt. 2000)	1%
Ethanol	2%
Sodium Hydroxide	0.24%
Non-heme fatty acid decarboxylase (SEQ ID NO: 4)	0.1%
Minors (perfume, preservative, dye) + water	To 100 %
pH (@ 10% solution)	9

**[0144]** All percentages and ratios given for enzymes are based on active protein. All percentages and ratios herein are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated.

**[0145]** It should be understood that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

**[0146]** The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as "40 mm" is intended to mean "about 40 mm."

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## SEQUENCE LISTING

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<120> Detergent composition

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<170> PatentIn version 3.5

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35 40 45

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

Phe Ala Arg His Pro Gly Glu Asp Met Ala Arg Arg Trp Leu Met Arg  
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Asn Ile Arg Val Glu Leu Asn His Ala Asp Tyr Trp Val His Trp Ser  
100 105 110

Arg Ala His Gly Val Thr Leu Glu Asp Leu Gln Ala Gln Gln Val Pro  
115 120 125

Pro Glu Leu His Ala Leu Ser His Trp Cys Trp His Thr Ser Ser Ala  
130 135 140

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

Ala Thr Gly Glu Trp Ser Ala Leu Val Cys Ser Asn Gly Ile Tyr Ala  
165 170 175

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5	Met	His	Ala	Gln	Tyr	Asp	Asp	Ala	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
	Ile	Val	Thr	Leu	Ala	Gly	Leu	Asn	Pro	Thr	Lys	Ala	Leu	Gln	Ala	Glu	
10		210					215					220					
	Leu	Arg	Gln	Ala	Ile	Cys	Lys	Ser	Tyr	Asp	Tyr	Met	Tyr	Leu	Phe	Leu	
	225					230					235					240	
15	Glu	Arg	Cys	Met	Gln	Gln	Glu	Lys	Thr	Ala	Val	Thr	Arg	Glu	Arg	Leu	
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	Ile	Asn	Gly	Trp	Pro	Val	Val	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Met	
	50						55					60					
45	Asn	Leu	Gln	Lys	Leu	Arg	Tyr	Gly	Gly	Ser	Arg	Gly	His	Glu	Leu	Ala	
	65					70					75					80	
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				100					105					110			
55	Met	Met	Gln	Ser	Arg	Pro	Ser	Ala	Ala	Tyr	Ser	Leu	Ser	His	Trp	Cys	



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	115		120		125
5	Trp Lys Ser Ser Asn Ala Asp Pro Leu Ala Val Ser Ile Ala Ala Thr				
	130		135		140
10	Asn Tyr Ala Ile Glu Gly Val Thr Gly Glu Trp Thr Ser Leu Val Cys				
	145		150		155
15	Gly Ala Gly Lys Tyr Thr Ala Ser Phe Pro Glu Ser Val Arg Gln Lys				
		165		170	175
20	Ala Thr Tyr Trp Leu Arg Leu His Ala His Tyr Asp Asp Glu His Pro				
		180		185	190
25	Trp Glu Ala Leu Glu Ile Val Ala Thr Leu Leu Gly Arg Asp Pro Gln				
		195		200	205
30	Arg Ser Glu Ile Glu Ala Val Arg Gln Ala Ile Gln Met Ser Phe Glu				
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		20		25	30
60	Arg Gln Val Thr Glu His Glu Ile Leu Trp Arg Met Thr Thr Leu Gln				
		35		40	45
65	Leu Asp Ala Ala Gly Ser His Asp Phe Phe Ala Gly Leu Trp Pro Phe				
		50		55	60
70	Ile Glu Arg Phe Pro Ser Phe Met Ala Leu Ser Leu Leu Lys Thr Arg				
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75	Tyr Gly Arg Ser Glu Gly Asp Asp Met Ala Arg Arg Trp Leu Val Arg				
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80	Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asn Trp Ala				

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10	Ala Gly Thr Asp Gly Leu Tyr Arg Trp Gly Glu Glu Ile Ser Thr Asp		140
	130	135	
15	Gly Ser Leu Ala Ala Gly Leu Ala Ala Ala Asn Tyr Ala Ile Glu Gly		160
	145	150	155
20	Ala Thr Gly Asp Trp Ala Arg Thr Ile Asn Asp Ser Ala Val Tyr Cys		175
	165	170	
25	Asp Ser Phe Ala Pro Glu Thr Arg Ala Ala Ser Leu Arg Trp Leu Lys		190
	180	185	
30	Met His Ala Ala Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Glu Ile		205
	195	200	
35	Val Cys Ala Leu Leu Gly Met Asn Pro Ser Val Ala Glu Val Ala His		220
	210	215	
40	Leu Gly Glu Cys Ile Glu Arg Ser Tyr Arg Cys Met Ala Leu Phe Gly		240
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	20	25	30
70	Phe Trp Asp Glu Leu Ile Pro Ala Lys Asp Arg Val Gly Gln His Pro		
	35	40	45
75	Leu Phe Gln Asp Met Ala Asn Gly Arg Leu Asn Leu Lys Cys Phe Arg		

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

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Val Val Arg His Pro Leu Phe Asp Glu Met Ala Ala Gly Ser Leu Ser  
35 40 45

25 Leu Pro Arg Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val Glu  
50 55 60

30 Asn Phe Pro Lys Tyr Met Gly Leu Asn Leu Ala Lys Thr Arg Pro Gly  
65 70 75 80

Arg His Ala Gly His Glu Glu Ala Lys Asn Trp Leu Ile Gly Asn Ile  
85 90 95

Lys Ile Glu Gln Arg His Ala Tyr Trp Tyr Gln Asp Trp Ala Met Gly  
100 105 110

40 Phe Gly Ile Thr Leu Arg Asp Leu Glu Phe Val Glu Pro Pro Ala Ala  
115 120 125

45 Met Asp Ala Val Asn His Phe Leu Trp Asn Met Gly His Gln Gly Thr  
130 135 140

Leu Glu Glu Gly Ile Ala Ser Thr Asn Leu Ala Ile Glu Trp Ala Thr  
145 150 155 160

Gly Glu Trp Ser Gln Ser Val Val Lys Gly Met Lys Ala Tyr Gln Glu  
165 170 175

55 Asn Gly Val Ala Thr Ile Asn Arg His Ser Met Ala Trp Leu Arg Ala  
180 185 190

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His Ala Ser Tyr Asp Asp Asp His Pro His Glu Ala Met Glu Leu Ile  
 195 200 205  
 5 Lys Leu Val Cys Val Asp Glu Ala Gln Lys Glu Arg Ala Phe Ala Ala  
 210 215 220  
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 Asp Gln Ala Gly  
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 30 Val Val Glu His Pro Val Phe Val Glu Met Gly Ala Gly Thr Leu Ser  
 35 40 45  
 Leu Pro Arg Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val Glu  
 50 55 60  
 35 Asn Phe Pro Lys Tyr Met Gly Leu Asn Leu Ala Lys Thr Gln Pro Gly  
 65 70 75 80  
 40 Arg Tyr Pro Gly His Glu Glu Ser Lys Asn Trp Leu Ile Ser Asn Ile  
 85 90 95  
 45 Lys Val Glu Gln Arg His Ala Tyr Trp Tyr Gln Asp Trp Ala Met Gly  
 100 105 110  
 Phe Gly Ile Ser Leu Glu Glu Leu Glu Phe Val Thr Pro Pro Ala Ala  
 115 120 125  
 50 Met Asp Ala Val Asn His Tyr Leu Trp His Met Gly Arg Gln Ala Ser  
 130 135 140  
 55 Leu Glu Glu Gly Leu Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr  
 145 150 155 160

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					165					170					175		
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				180					185					190			
10	His	Ala	Ser	Tyr	Asp	Asp	Ala	His	Pro	His	Glu	Ala	Met	Glu	Leu	Ile	
			195					200					205				
15	Lys	Leu	Thr	Cys	Ala	Asp	Glu	Ala	Ser	Arg	Glu	Arg	Ala	Phe	Ala	Ala	
		210					215					220					
20	Ala	Ala	Lys	Gly	Leu	Glu	Tyr	Tyr	Ile	Leu	Ala	Leu	Asp	His	Cys	Tyr	
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	1				5					10					15		
35	Pro	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Asp	Leu	Val	Pro	Leu	Lys	Asp	Arg	
				20					25					30			
40	Val	Val	Arg	His	Pro	Leu	Phe	Glu	Glu	Met	Ala	Ala	Gly	Ser	Leu	Ser	
			35					40					45				
45	Leu	Pro	Arg	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Glu	
		50					55					60					
50	Asn	Phe	Pro	Lys	Tyr	Met	Gly	Leu	Asn	Leu	Ala	Lys	Thr	Arg	Pro	Gly	
	65					70					75					80	
55	Arg	His	Ala	Gly	His	Glu	Glu	Ala	Lys	Asn	Trp	Leu	Ile	Gly	Asn	Ile	
					85					90					95		
60	Lys	Ile	Glu	Gln	Arg	His	Ala	Tyr	Trp	Tyr	Gln	Asp	Trp	Ala	Met	Gly	
				100					105					110			
65	Phe	Gly	Ile	Thr	Leu	Arg	Asp	Leu	Glu	Phe	Val	Glu	Pro	Pro	Ala	Ala	
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	Met	Asp	Ala	Val	Asn	His	Phe	Leu	Trp	Asn	Met	Gly	His	Gln	Gly	Thr	
	130						135					140					
5	Leu	Glu	Glu	Gly	Ile	Ala	Ser	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	Thr	
	145					150					155					160	
10	Gly	Glu	Trp	Ser	Gln	Ser	Val	Val	Lys	Gly	Met	Lys	Ala	Tyr	Gln	Glu	
					165					170					175		
15	Asn	Gly	Val	Ala	Thr	Ile	Asn	Arg	His	Ser	Met	Ala	Trp	Leu	Arg	Ala	
				180					185					190			
20	His	Ala	Ser	Tyr	Asp	Asp	Asp	His	Pro	His	Glu	Ala	Met	Glu	Leu	Ile	
			195					200					205				
25	Lys	Leu	Val	Cys	Val	Asp	Glu	Ala	Gln	Lys	Glu	Arg	Ala	Phe	Ala	Ala	
		210					215					220					
30	Ala	Ser	Arg	Gly	Leu	Glu	Tyr	Tyr	Ile	Gln	Ala	Leu	Asp	Tyr	Cys	Tyr	
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	1				5					10					15		
55	Gly	Trp	Ala	Gln	Ala	Phe	Trp	Asp	Asp	Leu	Val	Pro	Val	Lys	Glu	Arg	
				20					25					30			
60	Val	Ser	His	His	Pro	Leu	Phe	Ile	Asp	Met	Ala	Asn	Gly	Lys	Leu	Ser	
			35					40					45				
65	Leu	Ala	Cys	Phe	Arg	Leu	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Ala	
	50						55					60					
70	His	Phe	Pro	Ser	Tyr	Met	Ala	Leu	Ala	Leu	Ser	Lys	Ala	Thr	Asp	Phe	
	65					70					75					80	
75	Thr	Gln	Pro	Gly	Val	Thr	Glu	Ser	Arg	Asp	Trp	Leu	Ile	Gln	Asn	Ile	
					85					90					95		

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	Lys	Ile	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Arg	Glu	Trp	Ala	Val	Gly	
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5	Phe	Gly	Leu	Thr	Val	Asp	Glu	Leu	Asp	Arg	Val	Thr	Pro	Pro	Val	Glu	
			115					120					125				
10	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asn	Met	Asn	Tyr	Arg	Ser	Ser	
		130					135					140					
15	Leu	Ala	Glu	Cys	Leu	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	Thr	
	145					150					155					160	
20	Gly	Asp	Trp	Ser	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Asn	Ala	Tyr	Ile	Asp	
					165					170					175		
25	His	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	Ala	
				180					185					190			
30	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	Ile	
			195					200					205				
35	Lys	Arg	Leu	Cys	Asp	His	Gln	Pro	Glu	Leu	Gln	Thr	Lys	Ala	Phe	Lys	
		210					215					220					
40	Ala	Ala	Arg	Glu	Gly	Leu	Glu	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Tyr	Cys	
	225					230					235					240	
45	Tyr	Lys	Gln	His	Ala												
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	1				5					10					15		
60	Gly	Trp	Ala	Gln	Ala	Phe	Trp	Asp	Asp	Leu	Val	Pro	Val	Lys	Glu	Arg	
				20				25						30			
65	Val	Ser	His	His	Pro	Leu	Phe	Ile	Asp	Met	Ala	Asn	Gly	Lys	Leu	Ser	
			35				40						45				
70	Leu	Ala	Cys	Phe	Arg	Leu	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Ala	
		50					55					60					

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

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

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20 Val Val Asn His Pro Val Phe Ala Glu Met Ala Ser Gly Gln Leu Ser  
 35 40 45

25 Leu Pro Arg Phe Arg Cys Ala Leu Leu Asn Phe Tyr Pro Leu Val Glu  
 50 55 60

30 Asn Phe Pro Lys Tyr Met Gly Leu Asn Leu Ala Lys Thr Arg Pro Gly  
 65 70 75 80

35 Arg Phe Pro Gly His Glu Gln Ala Lys Asn Trp Leu Ile Gly Asn Ile  
 85 90 95

40 Lys Ile Glu Gln Arg His Ala Tyr Trp Tyr Gln Asp Trp Ala Met Gly  
 100 105 110

45 Phe Gly Leu Thr Leu Glu Asp Leu Glu Phe Val Glu Pro Pro Ala Ala  
 115 120 125

50 Met Asp Ala Val Asn Asn Phe Leu Trp Thr Met Gly Arg Gln Gly Ser  
 130 135 140

55 Leu Glu Glu Gly Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr  
 145 150 155 160

Gly Glu Trp Ser Gln Ser Val Val Lys Gly Met Lys Ala Tyr Gln Glu  
 165 170 175

Asp Gly Val Ala Thr Ile Asn Arg His Ser Met Ala Trp Leu Arg Ala  
 180 185 190

His Ala Ser Tyr Asp Asp Asp His Pro His Glu Ala Met Glu Leu Val  
 195 200 205

Lys Leu Leu Cys Val Glu Gln Glu Ser Arg Asp Arg Ala Phe Lys Ala

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	210		215		220
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20	Pro Trp Ala Gln Gln Phe Trp Asp Glu Leu Val Pro Leu Lys Asp Lys				
			20		25 30
25	Val Val His His Pro Leu Phe Val Glu Met Ala Ser Gly Gly Leu Ser				
			35		40 45
	Leu Pro Arg Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val Glu				
			50		55 60
30	Asn Phe Pro Lys Tyr Met Gly Leu Asn Leu Ala Lys Thr Arg Pro Gly				
			65		70 75 80
35	Arg His Pro Gly His Glu Glu Ala Lys Asn Trp Leu Ile Ser Asn Ile				
			85		90 95
	Lys Ile Glu Gln Arg His Ala Tyr Trp Tyr Gln Asp Trp Ala Met Gly				
			100		105 110
40	Phe Gly Leu Thr Met His Asp Leu Glu Phe Val Asp Pro Pro Ala Ala				
			115		120 125
45	Met Asp Ala Val Asn His Phe Leu Trp Ser Ile Gly Arg Gln Ser Ser				
			130		135 140
50	Leu Glu Glu Gly Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr				
			145		150 155 160
	Gly Glu Trp Ser Gln Ser Val Val Lys Gly Met Lys Ala Tyr Glu Asp				
			165		170 175
55	Gln Gly Val Ala Thr Ile Asn Arg His Ser Met Ala Trp Leu Arg Ala				

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[illegible]



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10	Arg	Gly	Ile	Ala	Thr	Ile	Asn	Arg	His	Ser	Met	Ala	Trp	Leu	Arg	Ala
			180						185					190		
15	His	Ala	Ser	Tyr	Asp	Asp	Glu	His	Pro	His	Glu	Ala	Met	Glu	Leu	Val
			195					200					205			
20	Lys	Arg	Leu	Ala	Val	Asp	Asp	Glu	Arg	Arg	Asp	Lys	Ala	Phe	Lys	Ala
		210					215					220				
25	Ala	Lys	Arg	Gly	Leu	Glu	Tyr	Tyr	Ile	Val	Ala	Leu	Asp	His	Cys	Tyr
	225					230					235					240
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45	Glu	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Asp	Leu	Val	Pro	Leu	Lys	Asn	Lys
			20					25						30		
50	Val	Ala	Gln	His	Arg	Leu	Phe	Arg	Glu	Met	Ala	Asp	Gly	Ser	Leu	Ser
			35					40					45			
55	Leu	Glu	Arg	Phe	Arg	Arg	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Glu
	50						55					60				
60	Asn	Phe	Pro	Lys	Tyr	Met	Gly	Gln	His	Leu	Ala	Lys	Thr	Arg	Pro	Gly
	65					70					75					80
65	Ile	Met	Pro	Gly	His	Glu	Glu	Ala	Lys	Ile	Trp	Leu	Ile	Asp	Asn	Ile
				85						90					95	
70	Arg	Val	Glu	Gln	Arg	His	Ala	Phe	Trp	Tyr	Gln	Asp	Trp	Ala	Glu	Gly
			100						105					110		
75	Phe	Gly	Ile	Glu	Ile	Glu	Ala	Leu	Glu	Arg	Cys	Glu	Pro	Pro	Ala	Ala

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	115		120		125											
5	Met 130	Asp	Ala	Ile	Asn	His	Phe	Leu	Trp	Asn	Met	Gly 140	Ala	His	Gly	Ser
10	Leu 145	Glu	Glu	Gly	Leu	Ala	Ala	Thr	Asn	Leu	Ala	Val	Glu	Trp	Ala	Thr 160
15	Gly	Glu	Trp	Ser	Gln 165	Gln	Val	Ala	Lys	Gly 170	Ile	Arg	Arg	Tyr	Thr 175	Glu
20	His	Asp	Thr	Ile 180	Thr	Ile	Asn	Arg	Arg	Thr	Glu	Ala	Trp	Leu	Arg	Ala
25	His	Ala	Ala	Tyr 195	Asp	Asp	Glu	His	Pro	Tyr	Glu	Ala	Met 205	Glu	Leu	Ile
30	Lys	Arg	Thr	Ala	Thr	Thr	Asp	Asp	Ala	Arg	Thr	Arg	Ala	Phe	Lys	Ala
35	Ala	Gln	Arg	Gly	Leu	Glu	Tyr	Tyr	Ile	Leu	Ala	Leu	Asp	Asp	Cys	Tyr 240
40	Asp	Pro	Leu	Leu	Gly 245											
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75	1				5					10					15	
80	Ser	Glu	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Asp	Leu	Leu	Pro	Ser	Lys	Glu
85				20					25					30		
90	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Thr	Asp	Met	Ala	Asn	Gly	Ser	Leu
95				35				40					45			
100	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val
105		50					55					60				
110	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ser	Leu	Ala	Lys	Ala	Thr	Ser
115	65					70					75					80
120	Phe	Glu	Leu	Asp	Gly	Val	Thr	Glu	Thr	Arg	Asp	Trp	Leu	Ile	Gln	Asn

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

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	50	55	60
5	Ala His Phe Pro Ser Tyr Met	Ala Gly Ser Leu Ala Lys Ala Thr Ser	65 70 75 80
10	Phe Glu Leu Asp Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln Asn	85 90 95	
15	Ile Lys Val Glu Glu Arg His Leu Asn Trp Tyr Gln Asp Trp Ala Gly	100 105 110	
20	Gly Phe Gly Leu Thr Val Glu Met Leu Asn Asn Val Lys Pro Pro Val	115 120 125	
25	Ala Met Asn Ala Val Asn His Phe Leu Trp Asp Val Asn Phe Arg Gly	130 135 140	
30	Thr Leu Ala Glu Ser Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala	145 150 155 160	
35	Thr Gly Asp Trp Thr Ile Gln Val Tyr Lys Gly Ile Gln Val Tyr Thr	165 170 175	
40	Gln His Pro Glu Val Asn Ile Asn Lys Arg Ser Leu Ala Trp Leu Arg	180 185 190	
45	Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu	195 200 205	
50	Ile Lys Arg Leu Cys Asp Lys Asp Pro Val Leu Gln Gln Lys Ala Phe	210 215 220	
55	Leu Ala Ala Gln Glu Gly Leu Ala Tyr Tyr Glu Leu Ala Leu Asp Glu	225 230 235 240	
	Cys Tyr Lys Leu Gln His Lys Asn	245	
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	<213> Acinetobacter sp.		
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	Met Thr Ala Met Asn Gln Tyr Gly Thr Lys Leu Glu Ile Thr Ala His	1 5 10 15	
	Ser Glu Trp Ser Gln Lys Phe Trp Asp Glu Leu Phe Pro Ala Lys Glu		

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20

25

30

5

Arg Val Ser Lys His Pro Leu Phe Leu Asp Met Ala Asn Gly Ser Leu  
35 40 45

10

Ser Leu Glu Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val  
50 55 60

15

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

20

Phe Ser Leu Glu Gly Val Ile Asp Thr Arg Asn Trp Leu Ile Gln Asn  
85 90 95

Ile Lys Val Glu Glu Ser His Leu Arg Trp Tyr Gln Asp Trp Ala Arg  
100 105 110

Gly Phe Gly Leu Thr Ala Glu Met Leu Asn Glu Val Arg Pro Pro Ala  
115 120 125

25

Ala Met Asn Ala Val Asn His Phe Leu Trp Asp Val Asn Phe Arg Gly  
130 135 140

30

Thr Leu Ala Glu Ser Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala  
145 150 155 160

Thr Gly Asp Trp Thr Ile Gln Val Tyr Lys Gly Ile Gln Ala Tyr Thr  
165 170 175

35

Glu His Pro Lys Val Ser Ile Asn Lys Arg Ser Leu Ala Trp Leu Arg  
180 185 190

40

Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu  
195 200 205

45

Ile Lys Arg Leu Cys Asp Lys Asp Pro Val Leu Gln Lys Lys Ala Phe  
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Leu Ala Ala Gln Glu Gly Leu Ala Tyr Tyr Glu Leu Ala Leu Asp Ala  
225 230 235 240

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

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Cys Tyr Lys Leu Arg  
245

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20 Asp Arg Ile Ser Gln His Pro Phe Phe Lys Ala Met Ala Asp Gly Ser  
35 40 45

Leu Ser Val Glu Ser Phe Arg Tyr Ala Leu Thr Asn Phe Tyr Pro Leu  
50 55 60

25 Val Ala His Phe Pro Ser Tyr Met Gly Leu Ala Leu Ser Lys Ala Thr  
65 70 75 80

30 Ala Phe His Leu Pro Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln  
85 90 95

35 Asn Ile Lys Val Glu Glu Arg His Leu Asp Trp Tyr Arg Asp Trp Ala  
100 105 110

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

40 Pro Ala Met Asn Ala Val Asn His Phe Leu Trp Asn Ile Asn Thr Arg  
130 135 140

45 Gly Ser Leu Val Glu Cys Leu Ala Ala Thr Asn Leu Ala Ile Glu Trp  
145 150 155 160

Ala Thr Gly Asp Trp Ser Cys Gln Val Tyr Ala Gly Ile Glu Lys Tyr  
165 170 175

50 Lys Leu His Pro Glu Val Asn Val Asp Lys Arg Thr Leu Ala Trp Leu  
180 185 190

55 Arg Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu  
195 200 205

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	Leu	Ile	Lys	Arg	Leu	Cys	Gly	Asp	Asn	Thr	Glu	Leu	Gln	Lys	Lys	Ala	
	210						215					220					
5	Phe	His	Ala	Ala	Lys	Thr	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	
	225					230					235					240	
	Glu	Cys	Leu	Lys	Met	Gln	Ser	Asn	Tyr	Lys	Lys	Ala	Ile				
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20	1				5					10					15		
	Pro	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Glu	Leu	Ile	Pro	Tyr	Lys	Asp	Arg	
				20					25					30			
25	Val	Ser	Gln	His	Pro	Leu	Phe	Gln	Asn	Met	Ala	Ser	Gly	Gln	Leu	Ser	
			35					40					45				
	Leu	Asp	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Ala	
30		50					55					60					
	His	Phe	Pro	Ser	Tyr	Met	Ala	Leu	Gly	Leu	Ser	Lys	Ala	Ile	Asp	Phe	
35	65					70					75					80	
	Ser	Ala	Gln	Gly	Val	Thr	Glu	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn	Ile	
					85					90					95		
40	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly	Gly	
				100					105					110			
	Phe	Gly	Leu	Ser	Ile	Asp	Gln	Leu	Asn	Gln	Val	Arg	Pro	Pro	Val	Ala	
45			115					120					125				
	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	His	Thr	Asn	Thr	Thr	Gly	Ser	
50		130					135					140					
	Leu	Ala	Glu	Cys	Leu	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	Thr	
	145					150					155					160	
55	Gly	Asp	Trp	Ser	Val	Gln	Val	Tyr	Lys	Gly	Ile	His	Ala	Tyr	Ile	Asp	
					165					170					175		



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	His	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	Ala	
				180					185					190			
5	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	Ile	
			195					200					205				
10	Lys	Arg	Leu	Cys	Ala	Asp	Gln	Pro	Glu	Leu	Gln	Gln	Lys	Ala	Phe	Leu	
		210					215					220					
15	Ala	Ala	Lys	Glu	Gly	Leu	Glu	Tyr	Tyr	Ala	Leu	Ala	Leu	Asp	Glu	Cys	
	225					230					235					240	
20	Tyr	Lys	Leu	Gln	Ser	Arg	Thr	Ala									
					245												
25	<210>	24															
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30	<400>	24															
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	1				5					10					15		
35	Pro	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Glu	Leu	Ile	Pro	Tyr	Lys	Asp	Arg	
				20					25					30			
40	Val	Ser	Gln	His	Pro	Leu	Phe	Gln	Asn	Met	Ala	Ser	Gly	Gln	Leu	Ser	
			35					40					45				
45	Leu	Asp	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Ala	
		50					55					60					
50	His	Phe	Pro	Ser	Tyr	Met	Ala	Leu	Gly	Leu	Ser	Lys	Ala	Ile	Asp	Phe	
	65					70					75					80	
55	Ser	Ala	Gln	Gly	Val	Thr	Glu	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn	Ile	
					85					90					95		
60	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly	Gly	
				100					105					110			
65	Phe	Gly	Leu	Ser	Ile	Asp	Gln	Leu	Asn	Gln	Val	Arg	Pro	Pro	Val	Ala	
			115					120					125				
70	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	His	Thr	Asn	Thr	Thr	Gly	Ser	
		130					135					140					

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	Leu	Ala	Glu	Cys	Leu	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	Thr	145	150	155	160
5	Gly	Asp	Trp	Ser	Val	Gln	Val	Tyr	Lys	Gly	Ile	His	Ala	Tyr	Leu	Asp	165	170	175	
10	Gln	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	Ala	180	185	190	
15	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	Ile	195	200	205	
20	Lys	Arg	Leu	Cys	Ala	Asp	Gln	Pro	Glu	Leu	Gln	Gln	Lys	Ala	Phe	Leu	210	215	220	
25	Ala	Ala	Lys	Glu	Gly	Leu	Glu	Tyr	Tyr	Ala	Leu	Ala	Leu	Asp	Glu	Cys	225	230	235	240
30	Tyr	Lys	Leu	Gln	Ser	Lys	Thr	Ala									245			
	<210>	25																		
	<211>	248																		
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	<213>	Acinetobacter soli																		
	<400>	25																		
35	Met	Ser	Ala	Tyr	Thr	Lys	Gly	Thr	Ala	Leu	Glu	Ile	Thr	Ser	His	Ser	1	5	10	15
40	Pro	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Glu	Leu	Ile	Pro	Tyr	Lys	Asp	Arg	20	25	30	
45	Val	Ser	Gln	His	Pro	Leu	Phe	Gln	Asn	Met	Ala	Ser	Gly	Gln	Leu	Ser	35	40	45	
50	Leu	Asp	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Ala	50	55	60	
55	His	Phe	Pro	Ser	Tyr	Met	Ala	Leu	Gly	Leu	Ser	Lys	Ala	Ile	Asp	Phe	65	70	75	80
	Ser	Ala	Gln	Gly	Val	Thr	Glu	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn	Ile	85	90	95	
	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly	Gly	100	105	110	

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	Phe Gly Leu Ser Ile Asp Gln Leu Asn Gln Val Arg Pro Pro Val Ala	
	115	120 125
5	Met Asn Ala Val Asn His Phe Leu Trp His Thr Asn Thr Thr Gly Ser	
	130	135 140
10	Leu Ala Glu Cys Leu Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr	
	145	150 155 160
15	Gly Asp Trp Ser Val Gln Val Tyr Lys Gly Ile His Ala Tyr Ile Asp	
		165 170 175
20	His Pro Glu Val Asn Ile Asn Lys Arg Ser Leu Ala Trp Leu Arg Ala	
		180 185 190
25	His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu Ile	
		195 200 205
30	Lys Arg Leu Cys Ala Asp Gln Pro Glu Leu Gln Gln Lys Ala Phe Leu	
		210 215 220
35	Ala Ala Lys Asp Gly Leu Glu Tyr Tyr Ala Leu Ala Leu Asp Glu Cys	
		225 230 235 240
40	Tyr Lys Leu Gln Ser Lys Thr Ala	
		245
45	<210> 26	
	<211> 248	
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	<213> Acinetobacter sp.	
50	<400> 26	
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	1	5 10 15
60	Pro Trp Ala Gln Gln Phe Trp Asp Glu Leu Ile Pro Tyr Lys Asp Arg	
		20 25 30
65	Val Ser Gln His Pro Leu Phe Gln Asn Met Ala Ser Gly Gln Leu Ser	
		35 40 45
70	Leu Asp Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val Ala	
		50 55 60
75	His Phe Pro Ser Tyr Met Ala Leu Gly Leu Ser Lys Ala Ile Asp Phe	
		65 70 75 80

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	Ser	Ala	Gln	Gly	Val	Thr	Glu	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn	Ile	
					85					90					95		
5	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly	Gly	
				100					105					110			
10	Phe	Gly	Leu	Ser	Ile	Asp	Gln	Leu	Asn	Gln	Val	Arg	Pro	Pro	Val	Ala	
			115					120					125				
15	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	His	Thr	Asn	Thr	Thr	Gly	Ser	
		130					135					140					
20	Leu	Ala	Glu	Cys	Leu	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	Thr	
	145					150					155					160	
25	Gly	Asp	Trp	Ser	Val	Gln	Val	Tyr	Lys	Gly	Ile	His	Ala	Tyr	Ile	Asp	
					165					170					175		
30	His	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	Ala	
				180					185					190			
35	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	Ile	
			195					200					205				
40	Lys	Arg	Leu	Cys	Ala	Asp	Gln	Pro	Glu	Leu	Gln	Gln	Lys	Ala	Phe	Leu	
		210					215					220					
45	Ala	Ala	Lys	Glu	Gly	Leu	Glu	Tyr	Tyr	Ala	Leu	Ala	Leu	Asp	Glu	Cys	
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50	Tyr	Lys	Leu	Gln	Ser	Lys	Thr	Ala									
					245												
55	<210>	27															
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	1				5					10					15		
65	Pro	Trp	Ala	Gln	Arg	Phe	Trp	Asp	Ala	Leu	Val	Pro	Leu	Lys	Asp	Arg	
				20					25					30			
70	Val	Ala	Gly	His	Pro	Leu	Phe	Gln	Glu	Met	Gly	Glu	Gly	Ala	Leu	Ser	
			35					40					45				

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	Leu	Pro	Arg	Phe	Arg	Asp	Ala	Leu	Leu	His	Phe	Tyr	Pro	Leu	Val	Glu	
	50						55					60					
5	His	Phe	Pro	Lys	Tyr	Met	Gly	Leu	Ala	Leu	Ala	Lys	Thr	Arg	Pro	Gly	
	65					70					75					80	
	Leu	Tyr	Pro	Gly	His	Glu	Glu	Thr	Arg	Asn	Trp	Leu	Ile	Gly	Asn	Ile	
10					85					90					95		
	Lys	Val	Glu	Gln	Arg	His	Ala	Tyr	Trp	Tyr	Gln	Asp	Trp	Ala	Ala	Gly	
				100					105					110			
15																	
	Phe	Gly	Leu	Ala	Leu	Ala	Glu	Leu	Glu	Gln	Val	Arg	Pro	Pro	Ala	Ala	
			115					120					125				
20	Met	Asn	Ala	Ile	Asn	His	Tyr	Leu	Trp	Asp	Val	Gly	His	Gln	Gly	Ser	
		130					135					140					
	Leu	Asp	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Val	Glu	Trp	Ala	Thr	
25	145					150					155					160	
	Gly	Glu	Trp	Ser	Gln	His	Val	Val	Ser	Gly	Met	Gln	His	Tyr	Ala	Glu	
					165					170					175		
30																	
	Gln	Gly	Gln	Ala	Ser	Ile	Thr	Arg	His	Thr	Leu	Ala	Trp	Leu	Arg	Ala	
				180					185					190			
	His	Ala	Ser	Tyr	Asp	Asp	Ala	His	Pro	His	Glu	Ala	Met	Glu	Leu	Val	
35			195					200					205				
	Lys	Arg	Leu	Ala	Thr	Asp	Glu	Pro	Ala	Arg	Gln	Arg	Ala	Phe	Ala	Ala	
40		210					215					220					
	Ala	Gln	Arg	Gly	Leu	Glu	Tyr	Tyr	Leu	Leu	Ala	Leu	Asp	Asp	Cys	Tyr	
	225					230					235					240	
45																	
	Gln	Gln	Gly	Glu	Gln	Arg	Thr	Ala	Gln	Thr	Ala	Pro	Asp	Leu	Gly	Glu	
					245					250					255		
50	<210>	28															
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	<212>	PRT															
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	<400>	28															
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	1				5					10					15		

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	Pro	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Asp	Leu	Val	Pro	Leu	Lys	Asn	Lys
				20					25					30		
5	Val	Val	Asn	His	Pro	Val	Phe	Ala	Glu	Met	Ala	Ser	Gly	Arg	Leu	Ser
			35					40					45			
	Leu	Pro	Arg	Phe	Arg	Cys	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	Glu
10		50					55					60				
	Asn	Phe	Pro	Lys	Tyr	Met	Gly	Leu	Asn	Leu	Ala	Lys	Thr	Leu	Pro	Gly
	65					70					75					80
15	Arg	Phe	Pro	Gly	His	Glu	Gln	Ala	Lys	Asn	Trp	Leu	Ile	Ser	Asn	Ile
				85						90					95	
	Lys	Ile	Glu	Gln	Arg	His	Ala	Tyr	Trp	Tyr	Gln	Asp	Trp	Ala	Met	Gly
20				100					105					110		
	Phe	Gly	Leu	Thr	Leu	Glu	Asp	Leu	Glu	Phe	Val	Glu	Pro	Pro	Ala	Ala
25			115					120					125			
	Met	Asp	Ala	Val	Asn	Asn	Phe	Leu	Trp	Thr	Met	Gly	Arg	Gln	Gly	Ser
	130						135					140				
30	Leu	Glu	Glu	Gly	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	Thr
	145					150					155					160
	Gly	Glu	Trp	Ser	Gln	Ser	Val	Val	Lys	Gly	Met	Lys	Ala	Tyr	Gln	Glu
35					165					170					175	
	Asp	Gly	Val	Ala	Thr	Ile	Asn	Arg	His	Ser	Met	Ala	Trp	Leu	Arg	Ala
			180						185					190		
40	His	Ala	Ser	Tyr	Asp	Asp	Asp	His	Pro	His	Glu	Ala	Met	Glu	Leu	Val
			195					200					205			
	Lys	Leu	Leu	Cys	Val	Glu	Gln	Glu	Ser	Arg	Asp	Arg	Ala	Phe	Lys	Ala
45		210					215					220				
	Ala	Gln	Arg	Gly	Leu	Glu	Tyr	Tyr	Ile	Gln	Ala	Leu	Asp	Tyr	Cys	Tyr
	225					230					235					240
50	Gln	Thr	Pro	Glu	Ala											
					245											
55	<210>	29														
	<211>	245														
	<212>	PRT														

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<213> Alcanivorax sp.

<400> 29

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

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Gln Thr Pro Glu Ala  
245

5 <210> 30  
<211> 226  
<212> PRT  
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10 <400> 30

Gln Gln Phe Trp Asp Asp Leu Val Pro Leu Lys Asn Lys Val Val Asn  
1 5 10 15

15 His Pro Val Phe Ala Glu Met Ala Ser Gly Gln Leu Ser Leu Pro Arg  
20 25 30

20 Phe Arg Cys Ala Leu Leu Asn Phe Tyr Pro Leu Val Glu Asn Phe Pro  
35 40 45

Lys Tyr Met Gly Leu Asn Leu Ala Lys Thr Arg Pro Gly Arg Phe Pro  
50 55 60

25 Gly His Glu Gln Ala Lys Asn Trp Leu Ile Gly Asn Ile Lys Ile Glu  
65 70 75 80

30 Gln Arg His Ala Tyr Trp Tyr Gln Asp Trp Ala Met Gly Phe Gly Leu  
85 90 95

35 Thr Leu Glu Asp Leu Glu Phe Val Glu Pro Pro Ala Ala Met Asp Ala  
100 105 110

Val Asn Asn Phe Leu Trp Thr Met Gly Arg Gln Gly Ser Leu Glu Glu  
115 120 125

40 Gly Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr Gly Glu Trp  
130 135 140

45 Ser Gln Ser Val Val Lys Gly Met Lys Ala Tyr Gln Glu Asp Gly Val  
145 150 155 160

Ala Thr Ile Asn Arg His Ser Met Ala Trp Leu Arg Ala His Ala Ser  
165 170 175

50 Tyr Asp Asp Asp His Pro His Glu Ala Met Glu Leu Val Lys Leu Leu  
180 185 190

55 Cys Val Glu Gln Glu Ser Arg Asp Arg Ala Phe Lys Ala Ala Gln Arg  
195 200 205



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Gly Leu Glu Tyr Tyr Ile Gln Ala Leu Asp Tyr Cys Tyr Gln Thr Pro  
210 215 220

5 Glu Ala  
225

10 <210> 31  
<211> 258  
<212> PRT  
<213> Alcanivorax gelatiniphagus  
  
<400> 31

15 Met Gly Val Ala Arg Tyr Lys Asp Ala Leu Thr Leu Thr Glu His Pro  
1 5 10 15

20 Ser Trp Ala Gln Arg Phe Trp Asp Ala Leu Val Pro Leu Lys Asp Arg  
20 25 30

25 Val Val Asp His Pro Leu Phe Val Glu Met Gly Glu Gly His Leu Ser  
35 40 45

Leu Glu Arg Phe Gln Asp Ala Leu Leu His Phe Tyr Pro Leu Val Glu  
50 55 60

30 Asn Phe Pro Lys Tyr Met Gly Leu Ala Leu Ala Lys Thr Arg Ala Gly  
65 70 75 80

35 Arg Ala Pro Gly His Glu Asp Thr Lys Asn Trp Leu Ile Gly Asn Ile  
85 90 95

Arg Ile Glu Gln Arg His Ala Tyr Trp Tyr Gln Asp Trp Ala Ala Gly  
100 105 110

40 Phe Gly Leu Ser Arg Glu Arg Leu Glu Thr Thr Thr Pro Pro Pro Ala  
115 120 125

45 Met Asp Ala Val Asn Gln Phe Leu Trp Thr Thr Gly His Gln Gly Thr  
130 135 140

50 Leu Glu Glu Ser Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr  
145 150 155 160

Gly Glu Trp Ser Gln Arg Val Val Ala Gly Val Lys His Tyr Ala Glu  
165 170 175

55 Gln Gly Arg Ala Glu Ile Asn Arg His Thr Met Ala Trp Leu Arg Ala  
180 185 190

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His Ala Ser Tyr Asp Asp Ala His Pro His Glu Ala Met Glu Leu Ile  
 195 200 205  
 5 Lys Leu Leu Ala Val Asp Glu Pro Ser Arg Arg Arg Ala Phe Thr Ala  
 210 215 220  
 10 Ala Arg Arg Gly Leu Glu Tyr Tyr Leu Leu Ala Leu Asp Asp Cys Tyr  
 225 230 235 240  
 Arg His Ser Gly Glu Arg Ala Pro Thr Glu Leu Pro Gly Leu Leu Asp  
 245 250 255  
 15 Ser Val  
 20 <210> 32  
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 25 <400> 32  
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 30 Ser Glu Trp Ser Gln Arg Phe Trp Asp Asp Leu Val Pro Ala Lys Glu  
 20 25 30  
 Arg Val Ser Lys His Pro Leu Phe Leu Asp Met Ala Asn Gly Thr Leu  
 35 35 40 45  
 Ser Leu Glu Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val  
 50 55 60  
 40 Ala His Phe Pro Ser Tyr Met Ala Gly Ala Leu Ala Lys Ala Thr Ala  
 65 70 75 80  
 45 Phe Ser Leu Asp Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln Asn  
 85 90 95  
 Ile Lys Val Glu Glu Arg His Leu Asn Trp Tyr Gln Asp Trp Ala Gly  
 100 105 110  
 50 Gly Phe Gly Leu Thr Leu Glu Met Leu Asn Glu Val Arg Pro Pro Ala  
 115 120 125  
 55 Ala Met Asn Ala Val Asn His Phe Leu Trp Asp Val Asn Phe Arg Gly  
 130 135 140

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	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	145	150	155	160
5	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Thr	Tyr	Thr		165	170	175
10	Glu	His	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg		180	185	190
15	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu		195	200	205
20	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Val	Leu	Gln	Lys	Lys	Ala	Phe		210	215	220
25	Arg	Ala	Ala	Gln	Asp	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Glu		225	230	235
30	Cys	Tyr	Lys	Leu	Gln	His	Lys	Asn										245		
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	<211>	248																		
	<212>	PRT																		
	<213>	Acinetobacter sp.																		
	<400>	33																		
35	Met	Thr	Ala	Met	Asn	Gln	Tyr	Gly	Thr	Lys	Leu	Glu	Ile	Thr	Pro	His	1	5	10	15
40	Ser	Gly	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Glu	Leu	Leu	Pro	Ser	Lys	Glu		20	25	30
45	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Leu	Asp	Met	Ala	Asn	Gly	Ser	Leu		35	40	45
50	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val		50	55	60
55	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Thr	Leu	Ala	Lys	Ala	Thr	Ser	65	70	75	80
	Phe	Glu	Leu	Asp	Gly	Val	Thr	Glu	Thr	Arg	Asp	Trp	Leu	Ile	Gln	Asn		85	90	95
	Ile	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly		100	105	110

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	Gly	Phe	Gly	Leu	Thr	Val	Glu	Met	Leu	Asn	Asn	Ala	Arg	Pro	Pro	Val	
			115					120					125				
5	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Ile	Asn	Phe	Arg	Gly	
		130					135					140					
10	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	
	145					150					155					160	
15	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Ala	Tyr	Thr	
					165					170					175		
20	Gln	His	Pro	Glu	Val	His	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	
				180					185					190			
25	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	
			195					200					205				
30	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Gly	Leu	Gln	Arg	Lys	Ala	Phe	
	210						215					220					
35	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Glu	
	225					230					235					240	
40	Cys	Tyr	Lys	Leu	Gln	His	Lys	Asn									
					245												
45	<210>	34															
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50	<400>	34															
	Met	Thr	Ala	Met	Asn	Gln	Tyr	Gly	Thr	Lys	Leu	Glu	Ile	Thr	Pro	His	
	1				5					10					15		
55	Ser	Glu	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Asp	Leu	Leu	Pro	Ala	Lys	Glu	
				20					25					30			
60	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Thr	Asp	Met	Ala	Asn	Gly	Ser	Leu	
			35					40					45				
65	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	
	50						55					60					
70	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ser	Leu	Ala	Lys	Ala	Thr	Ser	
	65					70					75					80	

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Phe Glu Leu Asp Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln Asn  
85 90 95

5 Ile Lys Val Glu Glu Arg His Leu Asn Trp Tyr Gln Asp Trp Ala Gly  
100 105 110

10 Gly Phe Gly Leu Thr Val Glu Met Leu Asn Gln Val Lys Pro Pro Val  
115 120 125

Ala Met Asn Ala Val Asn His Phe Leu Trp Asp Val Asn Phe Arg Gly  
130 135 140

15 Thr Leu Ala Glu Ser Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala  
145 150 155 160

20 Thr Gly Asp Trp Thr Ile Gln Val Tyr Lys Gly Ile Gln Val Tyr Thr  
165 170 175

25 Gln His Pro Glu Val Asn Ile Asn Lys Arg Ser Leu Ala Trp Leu Arg  
180 185 190

Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu  
195 200 205

30 Ile Lys Arg Leu Cys Asp Lys Asp Pro Val Leu Gln Gln Lys Ala Phe  
210 215 220

35 Leu Ala Ala Gln Glu Gly Leu Ala Tyr Tyr Glu Leu Ala Leu Asp Glu  
225 230 235 240

Cys Tyr Lys Leu Gln His Lys Asn  
245

40

<210> 35  
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<212> PRT  
<213> Acinetobacter sp.

45

<400> 35

Met Thr Ala Met Asn Gln Tyr Gly Thr Lys Leu Glu Ile Thr Pro His  
1 5 10 15

50 Ser Gly Trp Ala Gln Arg Phe Trp Asp Glu Leu Leu Pro Ser Lys Glu  
20 25 30

55 Arg Val Ser Lys His Pro Leu Phe Leu Asp Met Ala Asn Gly Ser Leu  
35 40 45

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	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	
	50						55					60					
5	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ser	Leu	Ala	Lys	Ala	Thr	Ala	
	65					70					75					80	
10	Phe	Ser	Leu	Asp	Gly	Val	Thr	Glu	Thr	Arg	Asp	Trp	Leu	Ile	Gln	Asn	
					85					90					95		
15	Ile	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly	
				100					105					110			
20	Gly	Phe	Gly	Leu	Thr	Val	Glu	Met	Leu	Asn	Asn	Val	Lys	Pro	Pro	Val	
			115					120					125				
25	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Val	Asn	Phe	Arg	Gly	
		130					135					140					
30	Ser	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	
	145					150				155					160		
35	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Asn	Tyr	Thr	
					165					170					175		
40	Gln	His	Pro	Asp	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	
				180					185					190			
45	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	
			195					200					205				
50	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Ile	Leu	Gln	Arg	Lys	Ala	Phe	
		210					215					220					
55	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Glu	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Ala	
	225				230					235					240		
60	Cys	Tyr	Lys	Leu	Gln	His	Lys	Ser									
				245													
65	<210>	36															
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	<213>	Acinetobacter sp.															
70	<400>	36															
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	1				5					10					15		

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	Ser	Glu	Trp	Ala	Gln	Gln	Phe	Trp	Asp	Asp	Leu	Leu	Pro	Ala	Lys	Glu	
				20					25					30			
5	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Thr	Asp	Met	Ala	Asn	Gly	Ser	Leu	
			35					40					45				
	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	
10		50					55					60					
	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ser	Leu	Ala	Lys	Ala	Thr	Ser	
	65					70					75					80	
15																	
	Phe	Glu	Leu	Asp	Gly	Val	Thr	Glu	Thr	Arg	Asp	Trp	Leu	Ile	Gln	Asn	
					85					90					95		
	Ile	Lys	Val	Glu	Glu	Arg	His	Leu	Asn	Trp	Tyr	Gln	Asp	Trp	Ala	Gly	
20				100					105					110			
	Gly	Phe	Gly	Leu	Thr	Val	Glu	Met	Leu	Asn	Gln	Val	Lys	Pro	Pro	Val	
25			115					120					125				
	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Val	Asn	Phe	Arg	Gly	
		130					135					140					
30																	
	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	
	145					150					155					160	
35	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Ala	Tyr	Thr	
					165					170					175		
	Gln	His	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	
				180					185					190			
40																	
	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	
			195					200					205				
45	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Ser	Val	Leu	Gln	Gln	Lys	Ala	Phe	
		210					215					220					
	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Glu	
50		225				230					235					240	
	Cys	Tyr	Lys	Leu	Gln	His	Lys	Asn									
					245												
55																	
	<210>	37															
	<211>	248															

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<212> PRT  
<213> Acinetobacter sp.

<400> 37

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



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Cys Tyr Lys Leu Gln His Lys Asn  
245

5 <210> 38  
<211> 248  
<212> PRT  
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10 <400> 38

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15 Ser Gly Trp Ala Gln Arg Phe Trp Asp Glu Leu Leu Pro Ser Lys Glu  
20 25 30

Arg Val Ser Lys His Pro Leu Phe Leu Asp Met Ala Asn Gly Cys Leu  
35 40 45

Ser Leu Glu Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val  
50 55 60

25 Ala His Phe Pro Ser Tyr Met Ala Gly Ser Leu Ala Lys Ala Thr Asp  
65 70 75 80

30 Phe Ser Leu Asp Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln Asn  
85 90 95

Ile Lys Val Glu Glu Arg His Leu Asn Trp Tyr Gln Asp Trp Ala Gly  
100 105 110

35 Gly Phe Gly Leu Thr Val Asp Met Leu Asn Asn Val Lys Pro Pro Val  
115 120 125

40 Ala Met Asn Ala Val Asn His Phe Leu Trp Asp Val Asn Phe Arg Gly  
130 135 140

45 Ser Leu Ala Glu Ser Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala  
145 150 155 160

Thr Gly Asp Trp Thr Ile Gln Val Tyr Lys Gly Ile Gln Thr Tyr Thr  
165 170 175

50 Gln His Pro Glu Val Asn Ile Asn Lys Arg Ser Leu Ala Trp Leu Arg  
180 185 190

55 Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu  
195 200 205

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Ile Lys Arg Leu Cys Asp Lys Asp Pro Ala Leu Gln Lys Lys Ala Phe  
210 215 220

5 Leu Ala Ala Lys Glu Gly Leu Glu Tyr Tyr Glu Leu Ala Leu Asp Glu  
225 230 235 240

10 Cys Tyr Lys Leu Gln His Lys Asn  
245

<210> 39

<211> 248

<212> PRT

15 <213> Acinetobacter sp.

<400> 39

20 Met Thr Ala Met Asn Gln Tyr Ala Thr Lys Leu Glu Ile Thr Pro His  
1 5 10 15

Thr Glu Trp Ala Gln Arg Phe Trp Asp Gly Leu Leu Pro Ala Lys Glu  
20 25 30

25 Arg Val Ser Lys His Pro Leu Phe Leu Asp Met Ala Ser Gly Ser Leu  
35 40 45

30 Ser Leu Glu Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val  
50 55 60

35 Ala His Phe Pro Ser Tyr Met Ala Gly Ala Leu Ala Lys Ala Thr Ser  
65 70 75 80

Phe Ser Leu Asp Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln Asn  
85 90 95

40 Ile Lys Val Glu Glu Arg His Leu Asn Trp Tyr Gln Asp Trp Ala Met  
100 105 110

45 Gly Phe Gly Leu Thr Leu Glu Met Leu Asn Glu Val Thr Pro Pro Ala  
115 120 125

Ala Met Asn Ala Val Asn His Phe Leu Trp Thr Thr Asn Tyr Arg Gly  
130 135 140

50 Ser Leu Ala Glu Ser Ile Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala  
145 150 155 160

55 Thr Gly Asp Trp Thr Ile Gln Val Tyr Lys Gly Ile Gln Thr Tyr Thr  
165 170 175

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

5 Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu  
195 200 205

10 Ile Lys Arg Leu Cys Asp Lys Asp Pro Val Leu Gln Arg Lys Ala Phe  
210 215 220

15 Leu Ala Ala Lys Glu Gly Leu Ala Tyr Tyr Glu Leu Ala Leu Asp Glu  
225 230 235 240

Cys Tyr Lys Leu Gln His Lys Asn  
245

20 <210> 40  
<211> 248  
<212> PRT  
<213> Acinetobacter parvus

25 <400> 40

Met Thr Ala Met Asn Gln Tyr Ala Thr Lys Leu Glu Ile Thr Pro His  
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30 Asn Glu Trp Ala Gln Arg Phe Trp Asp Gly Leu Leu Pro Ala Lys Glu  
20 25 30

35 Arg Val Ser Lys His Pro Leu Phe Leu Asp Met Ala Ser Gly Ser Leu  
35 40 45

Ser Leu Glu Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val  
50 55 60

40 Ala His Phe Pro Ser Tyr Met Ala Gly Ala Leu Ala Lys Ala Thr Ser  
65 70 75 80

45 Phe Ser Leu Asp Gly Val Thr Glu Thr Arg Asp Trp Leu Ile Gln Asn  
85 90 95

Ile Lys Val Glu Glu Arg His Leu Asn Trp Tyr Gln Asp Trp Ala Met  
100 105 110

50 Gly Phe Gly Leu Thr Leu Glu Met Leu Asn Glu Val Thr Pro Pro Ala  
115 120 125

55 Ala Met Asn Ala Val Asn His Phe Leu Trp Thr Thr Asn Tyr Arg Gly  
130 135 140

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	Ser	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	145	150	155	160
5	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Thr	Tyr	Thr		165	170	175
10	Gln	His	Pro	Glu	Val	Asn	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg		180	185	190
15	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu		195	200	205
20	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Val	Leu	Gln	Arg	Lys	Ala	Phe		210	215	220
25	Leu	Ala	Ala	Lys	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Glu		225	230	235
30	Cys	Tyr	Lys	Leu	Gln	His	Lys	Asn										245		
	<210>	41																		
	<211>	249																		
	<212>	PRT																		
	<213>	Acinetobacter sp.																		
	<400>	41																		
35	Met	Thr	Ile	Ile	Lys	Gln	Gln	Tyr	Gly	Thr	Lys	Leu	Glu	Ile	Thr	Thr	1	5	10	15
40	His	Ser	Glu	Trp	Ser	Gln	Gln	Phe	Trp	Asp	Gln	Leu	Val	Pro	Ala	Lys		20	25	30
45	Glu	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Leu	Asn	Met	Ala	Asp	Gly	Thr		35	40	45
50	Leu	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu		50	55	60
55	Val	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ala	Leu	Ala	Lys	Ala	Thr		65	70	75
	Ala	Phe	Ser	Leu	Asp	Gly	Val	Thr	Glu	Thr	Arg	Asp	Trp	Leu	Ile	Gln		85	90	95
	Asn	Ile	Lys	Val	Glu	Glu	Arg	His	Leu	His	Trp	Tyr	Gln	Asp	Trp	Ala		100	105	110

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	Lys	Gly	Phe	Gly	Leu	Thr	Thr	Glu	Met	Leu	Asn	Asp	Val	Arg	Pro	Pro	
			115					120					125				
5	Ala	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Val	Asn	Phe	Arg	
			130				135					140					
10	Gly	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	
	145					150					155					160	
15	Ala	Thr	Gly	Asp	Trp	Ser	Ile	His	Val	Tyr	Lys	Gly	Ile	Gln	Ser	Tyr	
				165						170					175		
20	Thr	Gln	His	Pro	Glu	Val	Thr	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	
				180					185						190		
25	Arg	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	
			195				200						205				
30	Leu	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Ile	Leu	Gln	Arg	Lys	Ala	
	210						215					220					
35	Phe	Leu	Ala	Ala	Lys	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	
	225					230					235					240	
40	Glu	Cys	Tyr	Lys	Leu	Gln	His	Lys	Asn								
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45	<210>	42															
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50	<400>	42															
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	1				5					10					15		
60	Ser	Glu	Trp	Ser	Gln	Lys	Phe	Trp	Asp	Glu	Leu	Phe	Pro	Ala	Lys	Glu	
				20					25					30			
65	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Leu	Asp	Met	Ala	Asn	Gly	Ser	Leu	
			35					40					45				
70	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	
		50					55					60					
75	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ala	Leu	Ala	Lys	Ala	Thr	Ser	
	65					70					75					80	

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	Phe	Ser	Leu	Glu	Gly	Val	Ile	Asp	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn	
					85					90					95		
5	Ile	Lys	Val	Glu	Glu	Ser	His	Leu	Arg	Trp	Tyr	Gln	Asp	Trp	Ala	Arg	
				100					105					110			
10	Gly	Phe	Gly	Leu	Thr	Ala	Glu	Met	Leu	Asn	Glu	Val	Arg	Pro	Pro	Ala	
			115					120					125				
15	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Val	Asn	Phe	Arg	Gly	
		130					135					140					
20	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	
	145					150					155					160	
25	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Ala	Tyr	Thr	
					165					170					175		
30	Glu	His	Pro	Lys	Val	Ser	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	
				180					185					190			
35	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	
			195					200					205				
40	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Val	Leu	Gln	Lys	Lys	Ala	Phe	
	210						215					220					
45	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Ala	
	225					230					235					240	
50	Cys	Tyr	Lys	Leu	Arg												
					245												
55	<210>	43															
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	<400>	43															
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	1				5					10					15		
65	Ser	Glu	Trp	Ser	Gln	Lys	Phe	Trp	Asp	Glu	Leu	Phe	Pro	Ala	Lys	Glu	
				20					25					30			
70	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Leu	Asp	Met	Ala	Asn	Gly	Ser	Leu	
			35					40					45				

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	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val		
	50						55				60							
5	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Thr	Leu	Ala	Lys	Ala	Thr	Ser		
	65					70					75					80		
	Leu	Ser	Leu	Glu	Gly	Val	Ile	Asp	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn		
10					85					90					95			
	Ile	Lys	Val	Glu	Glu	Arg	His	Leu	His	Trp	Tyr	Gln	Asp	Trp	Ala	Arg		
				100					105					110				
15	Gly	Phe	Gly	Leu	Thr	Val	Glu	Met	Leu	Asn	Glu	Val	Arg	Pro	Pro	Val		
			115					120					125					
	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Ile	Asn	Phe	Arg	Gly		
20		130					135					140						
	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala		
25		145				150				155					160			
	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Thr	Tyr	Ile		
					165					170					175			
30	Glu	His	Pro	Glu	Val	Ser	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg		
				180					185				190					
	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu		
35			195					200					205					
	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Val	Leu	Gln	Lys	Lys	Ala	Phe		
40		210					215					220						
	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Thr		
		225				230					235					240		
45	Cys	Tyr	Lys	Leu	Arg	245												
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50	<211>	245																
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	<213>	Acinetobacter sp.																
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	1				5					10					15			

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	Ser	Glu	Trp	Ser	Gln	Lys	Phe	Trp	Asp	Glu	Leu	Phe	Pro	Ala	Lys	Glu	
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5	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Leu	Asp	Met	Ala	Asn	Gly	Ser	Leu	
			35					40					45				
10	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Ala	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	
		50					55					60					
15	Ala	His	Phe	Pro	Ala	Tyr	Met	Ala	Gly	Ala	Leu	Ala	Lys	Ala	Thr	Ser	
	65					70					75					80	
20	Phe	Ser	Leu	Glu	Gly	Val	Ile	Asp	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn	
					85					90					95		
25	Ile	Lys	Val	Glu	Glu	Ser	His	Leu	Arg	Trp	Tyr	Gln	Asp	Trp	Ala	Arg	
				100					105					110			
30	Gly	Phe	Gly	Leu	Thr	Ala	Glu	Met	Leu	Asn	Glu	Val	Arg	Pro	Pro	Ala	
			115					120					125				
35	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Val	Asn	Phe	Arg	Gly	
		130					135					140					
40	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala	
	145					150					155					160	
45	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Ala	Tyr	Thr	
					165					170					175		
50	Glu	His	Pro	Lys	Val	Ser	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	
				180					185					190			
55	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	
			195					200					205				
60	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Val	Leu	Gln	Lys	Lys	Ala	Phe	
		210					215					220					
65	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Ala	
	225					230					235					240	
70	Cys	Tyr	Lys	Leu	Arg												
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<211> 244  
 <212> PRT  
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5 <400> 45

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10 Pro Trp Ala Gln Gln Phe Trp Asp Glu Leu Ile Pro Ala Lys Asp Arg  
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15 Val Gly Gln His Pro Leu Phe Gln Asp Met Ala Asn Gly Arg Leu Asn  
 35 40 45

Leu Lys Cys Phe Arg Ser Ala Leu Leu Asn Phe Tyr Pro Leu Val Ala  
 50 55 60

20 His Phe Pro Ser Tyr Met Ala Leu Ala Leu Ser Lys Ala Thr Asp Phe  
 65 70 75 80

25 Thr Glu Ala Gly Val Thr Glu Thr Arg Asn Trp Leu Ile Gln Asn Ile  
 85 90 95

Lys Val Glu Glu Arg His Leu Asn Trp Tyr Arg Asp Trp Ala Gly Gly  
 100 105 110

30 Phe Gly Leu Thr Val Glu Glu Leu Asp Arg Val Arg Pro Pro Val Ala  
 115 120 125

35 Met Asp Ala Val Asn His Phe Leu Trp Asn Ile Asn Thr Lys Gly Ser  
 130 135 140

40 Leu Ala Glu Cys Leu Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr  
 145 150 155 160

Gly Asp Trp Ser Ile Gln Val Tyr Lys Gly Ile Asn Ala Tyr Ile Asp  
 165 170 175

45 His Pro Glu Val Ser Ile Asn Lys Arg Ser Leu Ala Trp Leu Arg Ala  
 180 185 190

50 His Ala His Tyr Asp Asp Ile His Pro Tyr Glu Ala Met Glu Leu Ile  
 195 200 205

Lys Arg Leu Gly Glu Gly Lys Pro Glu Ile Gln Glu Lys Ala Phe Gln  
 210 215 220

55 Ala Ala Gln Asp Gly Leu Ala Tyr Tyr Glu Leu Ala Leu Asp Glu Cys

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225 230 235 240

Tyr Lys His Gln

5

<210> 46  
<211> 250  
<212> PRT  
<213> Acinetobacter pittii  
  
<400> 46

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15

Asn Gly Trp Ser Gln Arg Phe Trp Asp Asp Leu Leu Pro Ala Lys Glu  
20 25 30

20

Arg Val Ser Lys His Pro Phe Phe Thr Glu Met Ala Asn Gly Gly Leu  
35 40 45

25

Ser Leu Asp Ser Phe Arg Tyr Ala Leu Leu Asn Phe Tyr Pro Leu Val  
50 55 60

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

30

Phe Ser Glu Pro Gly Val Thr Glu Ala Arg Asp Trp Leu Ile Gln Asn  
85 90 95

35

Ile Lys Val Glu Glu Arg His Leu Lys Trp Tyr Arg Asp Trp Ala Arg  
100 105 110

Gly Phe Gly Leu Thr Val Glu Glu Leu Asp His Val Arg Pro Pro Ala  
115 120 125

40

Ala Met Asn Ala Val Asn His Phe Leu Trp Asn Met Ser His Arg Gly  
130 135 140

45

Asn Leu Ala Glu Cys Leu Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala  
145 150 155 160

Thr Gly Asp Trp Ser Ile Gln Val Tyr Lys Gly Ile His Thr Tyr Thr  
165 170 175

50

Asn His Pro Glu Val Thr Ile Asp Lys Arg Ser Leu Ala Trp Leu Arg  
180 185 190

55

Ala His Ala His Tyr Asp Asp Leu His Pro Tyr Glu Ala Met Glu Leu

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	195		200		205											
5	Ile	Lys	Arg	Leu	Cys	Asn	Asp	Arg	Pro	Asp	Trp	Gln	Gln	Lys	Ala	Phe
	210						215					220				
10	His	Ala	Ala	Glu	Glu	Gly	Leu	Arg	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Asp
	225					230					235					240
15	Cys	Tyr	Arg	Val	Gln	Leu	Gln	Ala	Ser	Ala						
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25	Asp His Pro Asp Val Thr Ile Asp Lys Arg Ser Leu Ala Trp Leu Arg 180 185 190		
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Phe Gly Leu Ser Ile Asp Gln Leu Asp Thr Val Thr Pro Pro Pro Ala  
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Tyr Lys Leu Gln Ala  
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Val Ser Gln His Pro Val Phe Ile Gly Met Ala Asn Gly Thr Leu Ser  
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Leu Glu Cys Phe Arg Ala Ala Leu Leu Asn Phe Tyr Pro Leu Val Ala  
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Asn Glu Ala Gly Val Val Pro Thr Arg Asp Trp Leu Ile Gln Asn Ile  
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Lys Val Glu Glu Arg His Leu Thr Trp Tyr Arg Asp Trp Ala Ser Gly  
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Phe Gly Leu Thr Val Asp Gln Leu Asp His Val Thr Pro Pro Pro Ala  
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Leu Ala Glu Cys Leu Ala Ala Thr Asn Leu Ala Ile Glu Trp Ala Thr  
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Gly Asp Trp Ser Ser His Val Tyr Lys Gly Ile His Asn Tyr Gln Asn  
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His Pro Asp Val Lys Ile Asp Lys Arg Ser Leu Ala Trp Leu Arg Ala  
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	145					150					155					160	
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					165					170					175		
25	Asn	His	Pro	Asp	Val	Lys	Ile	Asp	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg	
				180					185					190			
30	Ala	His	Ala	His	Tyr	Asp	Asp	Ile	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu	
			195					200					205				
35	Ile	Lys	Arg	Leu	Cys	Glu	Gly	Lys	Pro	Glu	Leu	Gln	Gln	Lys	Ala	Phe	
	210						215					220					
40	His	Ala	Ala	Arg	Glu	Gly	Leu	Glu	Tyr	Tyr	Ala	Leu	Ala	Leu	Asp	Glu	
	225					230					235					240	
45	Cys	Tyr	Lys	Leu	Gln	Gly											
					245												
50	<210>	58															
	<211>	257															
	<212>	PRT															
	<213>	Dyella sp.															
55	<400>	58															
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	1				5					10					15		
65	Tyr	Pro	Gln	Trp	Ala	Gln	Asp	Leu	Val	Ala	Ala	Cys	Glu	Pro	Ala	Arg	
				20					25					30			
70	Arg	Arg	Val	Arg	Asp	His	Leu	Met	Trp	Asp	Leu	Met	Gly	Thr	Gly	Arg	
			35					40					45				
75	Ile	Asp	His	Ala	Thr	Met	Arg	Asn	Phe	Met	Val	Gly	Thr	Trp	Ser	Leu	
	50						55					60					

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	Ile	Glu	Arg	Phe	Pro	Ser	Phe	Met	Ala	Gln	Asn	Leu	Leu	Lys	Thr	Gln	
	65					70					75					80	
5	Tyr	Gly	Arg	Ser	Ala	Gly	Asp	Asn	Leu	Ala	Arg	Arg	Trp	Leu	Val	Arg	
					85					90					95		
	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Asp	Trp	Ala	
10				100					105					110			
	Glu	Gly	Ala	Gly	Val	Ala	Arg	Glu	Glu	Val	Leu	Lys	Gly	Arg	Pro	Pro	
			115					120					125				
15	Arg	Gly	Thr	Gln	Ala	Ala	Ala	Glu	Trp	Cys	His	Glu	Val	Cys	Gly	Arg	
		130					135					140					
	Asp	Thr	Leu	Ala	Ala	Gly	Ile	Ala	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
20						150					155					160	
	Val	Thr	Gly	Asp	Trp	Ser	Gln	Lys	Val	Tyr	Asp	Ser	Val	Ala	Tyr	Ala	
25					165					170					175		
	Gln	Gly	Leu	Pro	Gln	Ala	Gly	Arg	Lys	Ala	Thr	Leu	Arg	Trp	Leu	Gln	
				180					185					190			
30	Leu	His	Ala	Ala	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
	Val	Cys	Thr	Leu	Met	Gly	Asn	His	Pro	Ala	Pro	Glu	Glu	Val	Ala	His	
35		210					215					220					
	Leu	Gln	Glu	Cys	Ile	Glu	Arg	Ser	Tyr	Val	Ser	Leu	His	Tyr	Gly	Leu	
	225					230					235					240	
40	Glu	Arg	Cys	Leu	Val	Gln	Glu	Val	Ala	Glu	Gln	Val	Glu	Glu	Gln	Ala	
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45	Ala																
	<210>	59															
50	<211>	257															
	<212>	PRT															
	<213>	Dyella soli															
	<400>	59															
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	1				5					10					15		



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

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<210> 60  
 <211> 257  
 <212> PRT  
 <213> Dyella sp.

<400> 60

Met	Asn	Thr	Asn	Phe	Glu	Arg	Thr	Gly	Pro	Leu	Gly	Glu	Leu	Ser	Ser	1	5	10	15
Tyr	Pro	Gln	Trp	Ala	Gln	Asp	Met	Ile	Ala	Ala	Cys	Glu	Pro	Ala	Arg	20	25	30	
Arg	Arg	Val	Arg	Asp	His	Leu	Met	Trp	Asp	Leu	Met	Gly	Glu	Gly	Arg	35	40	45	
Ile	Asp	Pro	Val	Thr	Met	Ala	Asn	Phe	Met	Ile	Gly	Thr	Trp	Ser	Leu	50	55	60	
Ile	Glu	Arg	Phe	Pro	Ser	Phe	Met	Ala	Leu	Asn	Leu	Met	Lys	Thr	Gln	65	70	75	80
Tyr	Gly	Arg	Ser	Val	Gly	Asp	Asn	Met	Ala	Arg	Arg	Trp	Leu	Val	Arg	85	90	95	
Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Asp	Trp	Ala	100	105	110	
Asp	Gly	Ala	Gly	Val	Pro	Arg	Gln	Gln	Val	Phe	Asp	Gly	Leu	Pro	Pro	115	120	125	
Arg	Gly	Thr	Gln	Ala	Ala	Thr	Glu	Trp	Cys	Leu	Glu	Val	Ser	Gly	Gln	130	135	140	
Asp	Thr	Leu	Ala	Ala	Gly	Met	Leu	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	145	150	155	160
Val	Thr	Gly	Glu	Trp	Ser	Gln	Lys	Val	Tyr	Asp	Ser	Val	Ala	Tyr	Ala	165	170	175	
Asp	Ser	Leu	Pro	Thr	Ala	Gly	Arg	Lys	Ala	Thr	Leu	Arg	Trp	Leu	Gln	180	185	190	
Leu	His	Ala	Ala	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	195	200	205	
Val	Cys	Thr	Leu	Met	Gly	Asn	Asn	Pro	Ala	Pro	Glu	Glu	Val	Asp	His	210	215	220	

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

Ala

10

<210> 61  
<211> 258  
<212> PRT  
15 <213> Dyella sp.

<400> 61

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Met Ala Asp Lys Phe Lys Leu Asn Gly Ser Leu Thr Lys Leu Ser Ser  
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Tyr Pro Gln Trp Ala Gln Thr Met Val Glu Ser Cys Glu Pro Ser Arg  
20 25 30

25

Lys Arg Val Arg Asp His Ala Val Trp Asp Met Met Cys Asp Ala Thr  
35 40 45

30

Ile Asp Asn His Thr Met Arg Asn Phe Met Leu Gly Thr Trp Pro Leu  
50 55 60

35

Ile Glu Arg Phe Pro Ser Tyr Met Ala Asn Ser Leu Met Lys Thr Arg  
65 70 75 80

Tyr Gly Arg Ser Pro Gly Asp Asp Leu Ala Arg Arg Trp Leu Val Arg  
85 90 95

40

Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asp Trp Ala  
100 105 110

45

Asp Gly Ala Gly Val Asp Arg Gln Asp Val Leu Thr Ser Thr Pro Pro  
115 120 125

Gln Gly Thr Gln Leu Ala Ala Asp Trp Cys Glu Glu Val Cys Glu Ser  
130 135 140

50

Asp Ser Leu Val Ala Gly Met Ala Ala Thr Asn Tyr Ala Ile Glu Gly  
145 150 155 160

55

Val Thr Gly Glu Trp Ser Gln Lys Val Tyr Glu Ser Ala Ala Tyr Ala  
165 170 175

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	Ala	Ser	Val	Ser	Pro	Ser	Gly	Arg	Lys	Ala	Thr	Leu	Arg	Trp	Leu	Gln
				180					185					190		
5	Met	His	Ala	Ala	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile
			195					200					205			
10	Ile	Cys	Ala	Leu	Met	Gly	Asn	Ala	Pro	Asp	Pro	Gly	Glu	Ile	Glu	His
		210					215					220				
	Val	Arg	Glu	Cys	Ile	Glu	Arg	Ser	Tyr	Val	Ser	Leu	Tyr	Tyr	Gly	Leu
	225					230					235					240
15																
	Glu	Arg	Cys	Leu	Val	His	Ser	Arg	Glu	His	Glu	Pro	Arg	Asp	Ala	Ala
					245					250					255	
20																
	Cys	Met														
25																
	<210>	62														
	<211>	257														
	<212>	PRT														
	<213>	Dyella jiangningensis														
	<400>	62														
30	Met	Asn	Thr	His	Phe	Glu	Arg	Thr	Gly	Pro	Leu	Gly	Asp	Leu	Ser	Ser
	1				5					10					15	
35																
	Tyr	Pro	Gln	Trp	Ala	Gln	Asp	Leu	Val	Ala	Ala	Cys	Glu	Pro	Ala	Arg
				20					25					30		
40																
	Arg	Arg	Val	Arg	Asp	His	Ala	Met	Trp	Asp	Leu	Met	Gly	Glu	Gly	Arg
			35					40					45			
45																
	Ile	Asp	Ala	Val	Thr	Met	Arg	Asn	Phe	Met	Val	Gly	Thr	Trp	Ser	Leu
		50					55					60				
50																
	Ile	Glu	Arg	Phe	Pro	Ser	Phe	Met	Ala	Leu	Asn	Leu	Leu	Lys	Thr	Gln
	65					70					75					80
55																
	Tyr	Gly	Arg	Ser	Ala	Gly	Asp	Asn	Met	Ala	Arg	Arg	Trp	Leu	Val	Arg
					85					90					95	
60																
	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Asp	Trp	Ala
				100					105					110		
65																
	Asp	Gly	Ser	Gly	Val	Pro	Arg	Ala	Glu	Val	Leu	Asp	Gly	Arg	Pro	Pro
			115					120					125			

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	Arg	Gly	Thr	Gln	Ala	Ala	Thr	Glu	Trp	Cys	Leu	Glu	Val	Cys	Gly	Gln	
	130						135					140					
5	Asp	Ser	Leu	Ala	Ala	Gly	Met	Leu	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
	145					150					155					160	
	Val	Thr	Gly	Glu	Trp	Ser	Gln	Lys	Val	Tyr	Asp	Ser	Val	Ala	Tyr	Ala	
10					165					170					175		
	Glu	Ser	Leu	Pro	Ser	Ala	Gly	Arg	Lys	Ala	Thr	Leu	Arg	Trp	Leu	Gln	
				180					185					190			
15	Leu	His	Ala	Ala	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
	Val	Cys	Thr	Leu	Met	Gly	Ser	Arg	Pro	Ala	Pro	Glu	Ala	Val	Glu	His	
20			210				215					220					
	Leu	Arg	Glu	Cys	Val	Glu	Arg	Ser	Tyr	Leu	Ser	Leu	His	Tyr	Gly	Leu	
25						230					235					240	
	Glu	Arg	Cys	Leu	Leu	Gln	Pro	Gln	Met	Lys	Glu	Val	Glu	Glu	Gln	Ala	
				245						250					255		
30	Ala																
	<210>	63															
35	<211>	257															
	<212>	PRT															
	<213>	Dyella sp.															
	<400>	63															
40	Met	Asn	Thr	Asn	Thr	Val	Arg	Thr	Gly	Ser	Leu	Thr	Glu	Leu	Ser	Ser	
	1				5					10					15		
	Tyr	Pro	Lys	Trp	Ala	Arg	Asp	Met	Ile	Glu	Ala	Cys	Glu	Pro	Ala	Arg	
45				20					25					30			
	Arg	Ser	Val	Arg	Asp	His	Val	Met	Trp	Asp	Met	Met	Ala	Asp	Gly	Ser	
50			35					40					45				
	Ile	Asp	Leu	Ser	Thr	Met	Arg	Asn	Phe	Met	Val	Gly	Thr	Trp	Ser	Leu	
		50					55					60					
55	Ile	Glu	Gln	Phe	Pro	Ser	Phe	Met	Ala	Leu	Asn	Leu	Leu	Lys	Thr	Arg	
	65					70					75					80	

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Tyr Gly Arg Ser Ala Gly Asp Asn Met Ala Arg Arg Trp Leu Val Arg  
 85 90 95  
 5 Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asp Trp Ala  
 100 105 110  
 10 Glu Gly Ala Gly Val Ser Arg Glu Glu Ile Ile Asp Gly Met Pro Pro  
 115 120 125  
 Arg Gly Thr Gln Thr Ala Ala Glu Trp Cys His Asp Val Cys Gly Arg  
 130 135 140  
 15 Asp Thr Leu Ala Ala Gly Ile Ala Ala Thr Asn Tyr Ala Ile Glu Gly  
 145 150 155 160  
 20 Val Thr Gly Glu Trp Ser Gln Lys Val Phe Asp Ser Val Ala Tyr Ala  
 165 170 175  
 Asn Ser Leu Pro Ala Thr Gly Arg Lys Ser Thr Leu Arg Trp Leu Gln  
 180 185 190  
 25 Leu His Ala Ala Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Glu Ile  
 195 200 205  
 30 Val Cys Thr Leu Met Gly Ser Gln Pro Ala Pro Glu Glu Val Glu His  
 210 215 220  
 35 Val Arg Glu Cys Ile Glu Arg Ser Tyr Val Ser Leu His Tyr Gly Leu  
 225 230 235 240  
 Glu Arg Cys Leu Met Arg His Pro Val Gly Glu Met Glu Glu Gln Ala  
 245 250 255  
 40 Ala  
 45 <210> 64  
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 <212> PRT  
 <213> Dyella jiangningensis  
 50 <400> 64  
 Met Asn Thr Gln Phe Glu Arg Thr Gly Pro Leu Gly Asp Leu Ser Ser  
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 55 Tyr Pro Gln Trp Ala Gln Asp Leu Val Ala Ala Cys Glu Pro Ala Arg  
 20 25 30

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	Arg	Arg	Val	Arg	Asp	His	Val	Met	Trp	Asp	Leu	Met	Gly	Asp	Gly	Arg	
			35					40					45				
5	Ile	Asp	Pro	Leu	Thr	Met	Arg	Asn	Phe	Met	Val	Gly	Thr	Trp	Ser	Leu	
		50					55					60					
	Ile	Glu	Arg	Phe	Pro	Ser	Phe	Met	Ala	Leu	Asn	Leu	Leu	Lys	Thr	Gln	
10		65				70					75					80	
	Tyr	Gly	Arg	Ser	Val	Gly	Asp	Asn	Met	Ala	Arg	Arg	Trp	Leu	Val	Arg	
					85					90					95		
15	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Asp	Trp	Ala	
				100					105					110			
	Asp	Gly	Ser	Gly	Val	Pro	Arg	Glu	Glu	Val	Ile	Asp	Gly	Arg	Pro	Pro	
20			115					120					125				
	Arg	Gly	Thr	Gln	Ala	Ala	Thr	Glu	Trp	Cys	Leu	Glu	Val	Cys	Gly	Gln	
25		130					135					140					
	Asp	Asn	Leu	Ala	Ala	Gly	Met	Leu	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
		145				150					155					160	
30	Val	Thr	Gly	Glu	Trp	Ser	Gln	Lys	Val	Tyr	Asp	Ser	Val	Ala	Tyr	Ala	
				165						170					175		
	Asp	Ser	Leu	Pro	Thr	Ala	Gly	Arg	Lys	Ala	Thr	Leu	Arg	Trp	Leu	Gln	
35				180					185					190			
	Leu	His	Ala	Ala	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
40	Val	Cys	Thr	Leu	Met	Gly	Asn	Arg	Pro	Ala	Pro	Glu	Ala	Val	Glu	His	
		210					215					220					
	Leu	Arg	Glu	Cys	Val	Glu	Arg	Ser	Tyr	Leu	Ser	Leu	His	Tyr	Gly	Leu	
45		225				230					235					240	
	Glu	Arg	Cys	Leu	Leu	Gln	Pro	Gln	Val	Lys	Glu	Val	Glu	Glu	Gln	Ala	
50				245						250					255		
	Ala																
55	<210>	65															
	<211>	257															

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<212> PRT  
<213> Dyella sp.

<400> 65

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



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Glu Arg Cys Leu Met Arg His Ala Val Gly Glu Val Glu Glu Gln Ala  
245 250 255

5 Ala

10 <210> 66  
<211> 257  
<212> PRT  
<213> Dyella sp.

<400> 66

15 Met Asn Thr Asn Thr Val Arg Thr Gly Ser Leu Thr Glu Leu Ser Ser  
1 5 10 15

20 Tyr Pro Lys Trp Ala Arg Glu Met Val Glu Ala Cys Glu Pro Ala Arg  
20 25 30

Arg Ser Val Arg Asp His Val Met Trp Asp Met Met Arg Asp Gly Ser  
35 40 45

25 Ile Asp Pro Gln Thr Met His Asn Phe Met Val Gly Thr Trp Ser Leu  
50 55 60

30 Ile Glu Arg Phe Pro Ser Phe Met Ala Leu Ser Leu Leu Lys Thr Arg  
65 70 75 80

35 Tyr Gly Arg Ser Ala Gly Asp Asn Met Ala Arg Arg Trp Leu Val Arg  
85 90 95

Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asp Trp Ala  
100 105 110

40 Glu Gly Ala Gly Val Pro Arg Glu Asp Val Leu Asp Gly Thr Pro Pro  
115 120 125

45 Arg Gly Thr Gln Thr Ala Ala Asp Trp Cys His Asp Val Cys Gly Arg  
130 135 140

50 Asp Thr Leu Ala Ala Gly Ile Ala Ala Thr Asn Tyr Ala Ile Glu Gly  
145 150 155 160

Val Thr Gly Glu Trp Ser Ala Lys Val Tyr Asp Ser Val Ala Tyr Ala  
165 170 175

55 Asn Ser Leu Pro Glu Ala Gly Arg Lys Ala Thr Leu Arg Trp Leu Gln  
180 185 190

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Leu His Ala Ala Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Glu Ile  
 195 200 205  
 5 Val Cys Thr Leu Met Gly Ser Gln Pro Ala Pro Glu Glu Val Glu His  
 210 215 220  
 10 Val Arg Glu Cys Ile Glu Arg Ser Tyr Val Ser Leu His Tyr Gly Leu  
 225 230 235 240  
 Glu Arg Cys Leu Val Gln His Ala Val Gly Glu Val Glu Glu Gln Ala  
 245 250 255  
 15 Ala  
 20 <210> 67  
 <211> 257  
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 25 <400> 67  
 Met Asn Thr His Phe Glu Arg Thr Gly Thr Leu Thr Glu Leu Thr Ser  
 1 5 10 15  
 30 Tyr Pro Arg Trp Val Gln Glu Leu Val Glu Ala Cys Glu Pro Ala Arg  
 20 25 30  
 Arg Ser Val Arg Asp His Val Met Trp Asp Met Met Ser Glu Gly His  
 35 35 40 45  
 Ile Asp Gln Ala Thr Met Arg Asn Phe Met Val Gly Thr Trp Ser Leu  
 50 55 60  
 40 Ile Glu Arg Phe Pro Ser Phe Met Ala Gln Asn Leu Leu Lys Thr Gln  
 65 70 75 80  
 45 Tyr Gly Arg Ser Val Gly Asp Asn Leu Ala Arg Arg Trp Leu Val Arg  
 85 90 95  
 Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asp Trp Ala  
 100 105 110  
 50 Glu Gly Ala Gly Ile Ala Arg Asp Val Val Leu Asn Gly Arg Pro Pro  
 115 120 125  
 55 Arg Gly Thr Gln Ala Ala Ala Glu Trp Cys His Glu Val Cys Gly Asn  
 130 135 140

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

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Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asp Trp Ala  
100 105 110

5            Glu Gly Ala Gly Val Pro Arg Glu Asp Val Leu Asp Gly Met Pro Pro  
               115                          120                          125

Arg Gly Thr Gln Thr Ala Ala Asp Trp Cys His Asp Val Cys Gly Arg  
130 135 140

Asp Ser Leu Ala Ala Gly Ile Ala Ala Thr Asn Tyr Ala Ile Glu Gly  
145 150 155 160

Val	Thr	Gly	Glu	Trp	Ser	Gln	Lys	Val	Tyr	Asp	Ser	Val	Ala	Tyr	Ala
				165					170					175	

20 Asn Ser Leu Pro Glu Ala Gly Arg Lys Ala Thr Leu Arg Trp Leu Gln  
180 185 190

Leu His Ala Ala Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Glu Ile  
195 200 205

Val	Cys	Thr	Leu	Met	Gly	Ser	Gln	Pro	Ala	Pro	Glu	Glu	Val	Glu	His
210						215					220				

Val	Arg	Glu	Cys	Ile	Glu	Arg	Ser	Tyr	Val	Ser	Leu	His	Tyr	Gly	Leu
225					230					235					240

Glu Arg Cys Leu Met Gln His Thr Val Gly Ala Val Glu Glu Gln Ala  
 35                   245                   250                   255

Ala

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          <211>    257
          <212>    PRT
          <213>    Dyella sp.
45          <400>    69

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Met	Asn	Thr	Asn	Thr	Val	Arg	Thr	Gly	Ser	Leu	Thr	Glu	Leu	Ser	Ser
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Tyr Pro Lys Trp Ala Arg Glu Met Ile Glu Ala Cys Glu Pro Ala Arg  
20 25 30

Arg Ser Val Arg Asp His Val Met Trp Asp Met Met Ser Glu Gly Ser  
35 40 45

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	Ile	Asp	Leu	Ala	Thr	Val	Arg	Asn	Phe	Met	Val	Gly	Thr	Trp	Ser	Leu	
	50						55					60					
5	Ile	Glu	Arg	Phe	Pro	Gly	Phe	Met	Ala	Leu	Ser	Leu	Leu	Lys	Thr	Arg	
	65					70					75					80	
10	Tyr	Gly	Arg	Ser	Ala	Gly	Asp	Asn	Met	Ala	Arg	Arg	Trp	Leu	Val	Arg	
					85					90					95		
15	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Asp	Trp	Ala	
				100					105					110			
20	Glu	Gly	Ser	Gly	Val	Ser	Arg	Glu	Glu	Val	Leu	Asp	Gly	Met	Pro	Pro	
			115					120					125				
25	Arg	Gly	Thr	Gln	Thr	Ala	Ala	Glu	Trp	Cys	His	Asp	Val	Cys	Ala	Arg	
		130				135					140						
30	Asp	Thr	Leu	Ala	Ala	Gly	Ile	Ala	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
	145				150					155					160		
35	Val	Thr	Gly	Glu	Trp	Ser	Gln	Lys	Val	Phe	Asp	Ser	Val	Ala	Tyr	Ala	
				165					170					175			
40	Asn	Ser	Leu	Pro	Ala	Ala	Gly	Arg	Lys	Ser	Thr	Leu	Arg	Trp	Leu	Gln	
			180					185					190				
45	Leu	His	Ala	Ala	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
		195				200					205						
50	Val	Cys	Thr	Leu	Met	Gly	Ser	Gln	Pro	Ala	Ala	Glu	Glu	Val	Glu	His	
	210				215					220							
55	Leu	Arg	Glu	Cys	Ile	Glu	Arg	Ser	Tyr	Val	Ser	Leu	His	Tyr	Gly	Leu	
	225				230					235					240		
60	Glu	Arg	Cys	Leu	Val	Gln	His	Thr	Val	Asn	Glu	Val	Ala	Glu	Gln	Ala	
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<400> 70

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

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245

250

255

Ala

5

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 <213> *Vogesella* sp.

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Tyr Pro Glu Trp Ala Gln Gln Leu Leu Ala Asp Cys Ala Ala Arg  
 20 25 30

20

Gln Arg Val Val Arg His Glu Phe Tyr Gln Arg Leu Arg Asp Ala Glu  
 35 40 45

25

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

30

Val Glu Gln Phe Pro Leu Tyr Met Ala Gln Asn Leu Leu Lys Thr Arg  
 65 70 75 80

Tyr Gly Arg His Arg Gly Glu Asp Met Ala Arg Arg Phe Leu Val Arg  
 85 90 95

35

Asn Ile Arg Val Glu Gln Asn His Ala Asp Tyr Trp Leu Ala Trp Ala  
 100 105 110

40

Ala Ala Cys Gly Ile Ala Ala Thr Glu Leu Gln Ala Gln Arg Val Pro  
 115 120 125

Glu Pro Leu Gln Gln Leu Gly His Trp Cys Arg His Asn Ser Arg His  
 130 135 140

45

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

50

Ala Thr Gly Glu Trp Thr Gln Leu Val Cys Ala Pro Gly Ile Tyr Glu  
 165 170 175

Ala Ser Leu Pro Ala Met Gln Arg Lys Ser Ala Met Arg Trp Leu Lys  
 180 185 190

55

Leu His Ala Arg Tyr Asp Asp Asp His Pro Trp Glu Ala Leu Asp Ile

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



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165170175

Asn Gly Phe Ser Asn Glu Ala Arg Lys Gln Ala Met Lys Trp Leu Arg  
                180                    185               190

Val His Ala His Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Glu Ile  
                195                    200               205

Ile Ala Thr Leu Leu Gly Gln Lys Pro Glu Ala Arg Asp Ile Ala Asn  
                210                    215               220

Val Arg Ser Ala Ile Val Lys Ser Tyr His Tyr Met Ser Ala Ala Phe  
225                    230                    235               240

Asp Asp Cys Phe Ala Ala Glu Gln Arg Asn Ala Pro Arg Arg Gln Cys  
                245                    250               255

Arg Pro Gly Val Pro Thr Leu Leu Ala Ser  
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Met Lys Glu Phe Gln Arg Thr Gly Glu Leu Lys Asp Ile Gly Ser Tyr  
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Pro Leu Trp Leu Gln Gln Val Val Arg Asp Thr Gln Arg Asp Lys Leu  
                20                    25               30

Arg Val Val Asp His Glu Leu Phe Ala Leu Met Arg Asp Ala Lys Leu  
                35                    40               45

Pro Leu Ala Ala Met Gln Arg Phe Leu Val Gly Val Trp Pro Thr Ile  
50                    55                    60

Glu Arg Phe Pro Arg Phe Met Ser Met Thr Leu Lys Lys Val Ser Tyr  
65                    70                    75               80

Gly Arg Ser Pro Gly Glu Asp Met Ala Arg Arg Tyr Leu Met His Asn  
                85                    90               95

Ile Arg Val Glu Gln Lys His Ala Glu Tyr Trp Val Glu Trp Ala Arg  
                100                    105              110

Ser Ala Gly Leu Thr Ala Arg Asp Leu Ala Glu Ser Glu Glu Thr Glu

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	115		120		125
5	Gly 130	Leu Lys	Ala Leu	Ala His 135	Trp Cys Trp Phe Val 140
10	Ser 145	Leu Ser	Val Ala	Ile Ala 150	Ala Thr Asn Tyr 155
15	Thr 165	Gly Glu	Trp Ser	Cys Val 170	Val Cys Ser Lys Ala Asp Tyr 175
20	Ser 180	Leu Pro	Ala Glu	Val Arg 185	Gly Pro Ala Met Arg Trp Leu Arg Val 190
25	His 195	Ala Glu	Tyr Asp	Asp Thr 200	His Pro Trp Glu Ala Leu Asp Ile Val 205
30	Ala 210	Thr Leu	Leu Gly	His Ala 215	Pro Ser Gln Arg Glu Ile Asp Asp Val 220
35	Arg 225	Arg Ala	Ile Arg	Ala Ser 230	Tyr Ala Tyr Met Ala Met Ala Leu Asp 240
40	His 245	Ala Met	Ala Ala	Ile His 250	Gly Ser Phe Asp Glu Thr Ala Ser 255
45	Asn 260	Ala Ser	Thr Leu	Gly Met 265	Leu Asp Ala Ala
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	<213> Metallibacterium scheffleri				
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70	Tyr 20	Pro Leu	Trp Ala	Gln Glu 25	Val Met Glu Ser Cys Glu Pro Ala Lys 30
75	Arg 35	Ala Val	Leu Glu	His Pro 40	Ile Trp Ala Met Met Arg Glu Gly Ser 45
80	Leu 50	Ser Asp	Ala Ala	Met Gln 55	Arg Phe Ile Leu Ser Ala Trp Pro Val 60
85	Ile 55	Glu Gln	Phe Pro	Gln Tyr	Met Ala Met Asn Leu Leu Lys Ala Arg

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

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

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Tyr Pro Gln Trp Leu Gln Glu Ile Val Ser Glu Cys Asp Asp Ala Arg  
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His Thr Val Ile Ala His Glu Ile Phe Gln Gln Met His Ala Gly Val  
35 40 45

Leu Pro Ala Thr Ala Met Arg Cys Phe Leu Ala Ser Phe Trp Pro Val  
50 55 60

Ile Glu Gln Phe Pro Gln Tyr Met Ala Met Asn Leu Leu Lys Val Gln  
65 70 75 80

Tyr Gly Leu Gly Ala Gly His Ala Met Ala Arg Lys Tyr Leu Ile Arg  
85 90 95

Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Val	Glu	Tyr	Trp	Ile	Asp	Trp	Ser
			100					105					110		

Gln Gly His Gly Leu Thr Arg Asp Asn Leu Leu Ser Gly Trp Arg Ser  
115 120 125

Asp Ser Ala Asp Ala Leu Ser His Trp Cys Trp His Thr Cys Glu Arg  
130 135 140

Asp Pro Leu Ala Ile Ala Met Ala Ala Thr Asn Tyr Ala Ile Glu Gly  
145 150 155 160

Ala Thr Gly Glu Trp Ala Ala Phe Val Cys Gly Ser Ser Ala Tyr Glu  
165 170 175

Asn Gly Phe Pro Asp Asp Val Arg Lys Gln Ala Met Lys Trp Leu Arg  
180 185 190

Val His Ala His Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Glu Ile  
195 200 205

Ile Ala Thr Leu Leu Gly His Gln Pro Gln Ala Arg Asp Ile Ala Asn  
210 215 220

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	Val	Arg	Ser	Ala	Ile	Val	Lys	Ser	Tyr	His	Tyr	Met	Ser	Ala	Ala	Phe
	225					230					235					240
5	Asp	Asp	Cys	Leu	Thr	Glu	Asp	Lys	Arg	Arg	Ala	Pro	Arg	Arg	Gln	His
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10	Arg	Pro	Gly	Val	Pro	Ala	Leu	Gln	Leu	Ala	Glu	Asp	Leu	Ser		
				260					265					270		
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15	<212>	PRT														
	<213>	Steroidobacter sp.														
	<400>	77														
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	Tyr	Pro	Glu	Trp	Leu	Gln	Asp	Ile	Val	Ala	Glu	Cys	Asp	Asp	Ala	Arg
				20					25					30		
25	His	Thr	Val	Val	Ala	His	Glu	Ile	Phe	Gln	Gln	Met	His	Ala	Gly	Val
			35					40					45			
30	Leu	Ser	Pro	Ala	Ala	Met	Arg	Cys	Phe	Leu	Ala	Ser	Phe	Trp	Pro	Val
		50					55					60				
35	Ile	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Met	Asn	Leu	Leu	Lys	Val	Gln
	65					70					75					80
	Tyr	Gly	Leu	Gly	Ala	Gly	His	Thr	Met	Ala	Arg	Lys	Tyr	Leu	Ile	Arg
					85					90					95	
40	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Val	Glu	Tyr	Trp	Ile	Asp	Trp	Ser
				100					105					110		
45	Gln	Gly	His	Gly	Leu	Ser	Arg	Asp	Ala	Leu	Leu	Ser	Gly	Trp	Arg	Ser
			115					120					125			
	Asn	Ser	Ala	Asp	Ala	Leu	Ser	His	Trp	Cys	Trp	His	Thr	Cys	Glu	Arg
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	Asp	Pro	Leu	Ala	Val	Ala	Met	Ala	Ala	Thr	Asn	Phe	Ala	Ile	Glu	Gly
	145					150					155					160
55	Thr	Thr	Gly	Glu	Trp	Ala	Ala	Phe	Val	Cys	Ser	Ser	Ser	Thr	Tyr	Glu
					165					170					175	

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	Asn	Gly	Phe	Ala	Asn	Asp	Val	Arg	Lys	Gln	Ala	Met	Lys	Trp	Leu	Arg	
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5	Val	His	Ala	His	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
10	Ile	Ala	Thr	Leu	Leu	Gly	His	Gln	Pro	Gln	Ala	Arg	Glu	Ile	Ala	Asn	
		210					215					220					
15	Val	Arg	Ser	Ala	Ile	Val	Lys	Ser	Tyr	Gln	Tyr	Met	Ser	Ser	Ala	Phe	
	225					230					235					240	
20	Asp	Asp	Cys	Leu	Ala	Glu	Glu	Lys	Arg	Gln	Ala	Pro	Arg	Arg	Gln	His	
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25	Arg	Pro	Gly	Val	Pro	Ala	Leu	Leu	Ala	Ser							
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35	Tyr	Pro	Val	Trp	Val	Gln	Arg	Val	Val	Arg	Glu	Thr	Ala	Pro	Tyr	Lys	
				20					25					30			
40	Gln	Arg	Val	Val	Glu	His	Glu	Leu	Phe	Ala	Leu	Met	Arg	Glu	Gly	Lys	
			35					40					45				
45	Leu	Pro	Met	Ser	Ala	Met	Arg	Arg	Phe	Leu	Val	Gly	Val	Trp	Pro	Thr	
		50					55					60					
50	Ile	Glu	Gln	Phe	Pro	Arg	Phe	Met	Ser	Met	Asn	Leu	Lys	Lys	Ile	Gly	
	65					70					75					80	
55	Tyr	Gly	Asp	Ser	Val	Gly	Glu	Asp	Met	Ala	Arg	Arg	Tyr	Leu	Ile	Gln	
					85					90					95		
60	Asn	Ile	Arg	Val	Glu	Gln	Lys	His	Ser	Glu	His	Trp	Ala	Thr	Trp	Ala	
				100					105					110			
65	Ser	Ser	Ala	Gly	Leu	Thr	Leu	His	Asp	Leu	Arg	Thr	Gly	Gln	Asp	Val	
			115					120					125				

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	Glu	Glu	Met	Ala	Ala	Leu	Ala	His	Trp	Cys	Trp	Phe	Ile	Ser	Asp	Gln	
	130						135					140					
5	Ala	Lys	Leu	Ala	Val	Ala	Ile	Ala	Ala	Thr	Asn	Tyr	Ala	Val	Glu	Gly	
	145					150					155				160		
10	Ala	Thr	Gly	Glu	Trp	Ser	Cys	Val	Val	Cys	Ser	Lys	Asn	Thr	Tyr	Ala	
					165					170					175		
15	Gln	Ser	Leu	Pro	Glu	Asp	Ile	Arg	Val	Pro	Ala	Met	Arg	Trp	Leu	Lys	
				180					185					190			
20	Val	His	Ala	Glu	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Asp	Ile	
			195					200					205				
25	Val	Ala	Thr	Leu	Leu	Gly	His	Ala	Pro	Ser	Gln	Gln	Asp	Ile	Thr	Glu	
		210					215					220					
30	Val	Arg	Arg	Ala	Ile	Gln	Ser	Ser	Tyr	Thr	Tyr	Met	Lys	Met	Ala	Leu	
	225					230					235					240	
35	Asp	Ser	Ala	Leu	Met	Ala	Ser	Ile	His	Gly	Ser	Phe	Asp	Glu	Thr	Ala	
				245						250					255		
40	Ser	Asn	Ser	Ala	Thr	Leu	Gly	Leu	Glu	Gly	Leu	His	Ala	Ala			
				260					265					270			
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	<211>	220															
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	<213>	Cytophagaceae bacterium															
50	<400>	79															
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	1				5					10					15		
60	Phe	Pro	Arg	Phe	Met	Ala	Met	Asn	Leu	Lys	Lys	Val	Gly	Tyr	Ala	Asp	
				20					25					30			
65	Ser	Leu	Gly	Glu	Asp	Met	Ala	Arg	Arg	Tyr	Leu	Ile	Gln	Asn	Ile	Arg	
			35					40					45				
70	Val	Glu	Gln	Lys	His	Ala	Glu	His	Trp	Ala	Ala	Trp	Ala	Arg	Ser	Ala	
		50					55					60					
75	Asp	Val	Asn	Leu	Ser	Asp	Leu	Arg	Ser	Gly	Glu	Ile	Ser	Gly	Glu	Ser	
	65					70					75					80	



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	Thr	Glu	Glu	Leu	His	Ser	Leu	Ala	His	Trp	Cys	Trp	Tyr	Ile	Cys	Asp	
					85					90					95		
5	Gln	Pro	Ser	Leu	Ala	Val	Ala	Val	Ala	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	
				100					105					110			
10	Gly	Ala	Thr	Gly	Glu	Trp	Ala	Cys	Leu	Val	Cys	Ser	Thr	Asp	Lys	Tyr	
			115					120					125				
15	Ser	Gln	Ser	Ile	Pro	Asp	Asp	Ile	Arg	Ser	Ser	Ser	Met	Arg	Trp	Leu	
		130					135					140					
20	Lys	Val	His	Ala	Glu	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Asp	
	145					150					155					160	
25	Ile	Ile	Ser	Thr	Leu	Leu	Gly	His	Ala	Pro	Ser	Thr	Ala	Glu	Val	Ala	
					165					170					175		
30	Lys	Ile	Arg	Lys	Ser	Ile	Lys	Thr	Ser	Tyr	Arg	Tyr	Met	Glu	Leu	Ala	
				180					185					190			
35	Leu	Asp	Ala	Ala	Val	Ala	Ala	Ala	His	Val	Ser	Phe	Asp	Glu	Thr	Ala	
			195					200					205				
40	Ser	Asn	Ser	Ser	Thr	Leu	Gly	Asp	Leu	Tyr	Ala	Ala					
		210					215					220					
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	1				5					10					15		
60	Ser	Glu	Trp	Ser	Gln	Lys	Phe	Trp	Asp	Glu	Leu	Phe	Pro	Ala	Lys	Glu	
			20						25					30			
65	Arg	Val	Ser	Lys	His	Pro	Leu	Phe	Leu	Asp	Met	Ala	Asn	Gly	Ser	Leu	
			35					40					45				
70	Ser	Leu	Glu	Cys	Phe	Arg	Ser	Thr	Leu	Leu	Asn	Phe	Tyr	Pro	Leu	Val	
		50					55					60					
75	Ala	His	Phe	Pro	Ser	Tyr	Met	Ala	Gly	Ala	Leu	Ala	Lys	Ala	Thr	Ser	
	65					70					75					80	

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	Phe	Ser	Ser	Asp	Gly	Val	Ile	Asp	Thr	Arg	Asn	Trp	Leu	Ile	Gln	Asn
					85					90					95	
5	Ile	Lys	Val	Glu	Glu	Arg	His	Leu	Tyr	Trp	Tyr	Gln	Asp	Trp	Ala	Arg
				100					105					110		
	Gly	Phe	Gly	Val	Thr	Ala	Glu	Met	Leu	Asn	Glu	Val	Arg	Pro	Pro	Ala
10			115					120					125			
	Ala	Met	Asn	Ala	Val	Asn	His	Phe	Leu	Trp	Asp	Ile	Asn	Phe	Arg	Gly
		130					135					140				
15	Thr	Leu	Ala	Glu	Ser	Ile	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Trp	Ala
	145					150					155					160
	Thr	Gly	Asp	Trp	Thr	Ile	Gln	Val	Tyr	Lys	Gly	Ile	Gln	Thr	Tyr	Thr
20					165					170					175	
	Glu	His	Pro	Glu	Val	Thr	Ile	Asn	Lys	Arg	Ser	Leu	Ala	Trp	Leu	Arg
25				180					185					190		
	Ala	His	Ala	His	Tyr	Asp	Asp	Leu	His	Pro	Tyr	Glu	Ala	Met	Glu	Leu
			195					200					205			
30	Ile	Lys	Arg	Leu	Cys	Asp	Lys	Asp	Pro	Ala	Leu	Gln	Lys	Lys	Ala	Phe
	210						215					220				
	Leu	Ala	Ala	Gln	Glu	Gly	Leu	Ala	Tyr	Tyr	Glu	Leu	Ala	Leu	Asp	Ala
35	225					230					235					240
	Cys	Tyr	Lys	Leu	Arg											
40					245											
	<210>	81														
	<211>	247														
	<212>	PRT														
45	<213>	Myxococcus xanthus														
	<400>	81														
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	Thr	Ala	His	Pro	Arg	Trp	Leu	Glu	Ser	Met	Leu	Glu	Ser	Val	Arg	Asp
				20					25					30		
55	Glu	Trp	Asn	Ala	Ala	Cys	Trp	Pro	Pro	Leu	Phe	Arg	Ala	Thr	Ala	Asp
			35					40					45			

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	Gly	Gln	Arg	Pro	Pro	Leu	Arg	His	Trp	Arg	Arg	Val	Leu	Ser	His	Phe	
	50						55					60					
5	Phe	Leu	Ile	Val	Glu	Ser	Phe	Pro	Lys	Tyr	Met	Gly	Leu	Ser	Leu	Ala	
	65					70					75					80	
	Lys	Thr	Thr	Tyr	Gly	Gln	Arg	Pro	Gly	Asp	Ala	Ser	Ala	Arg	Arg	Trp	
10					85					90					95		
	Leu	Leu	Gln	Asn	Leu	Gly	Val	Glu	Ala	Lys	His	Ala	Glu	Trp	Phe	Ile	
				100					105					110			
15	Asp	Trp	Met	Arg	Gly	Ile	Gly	Leu	Ala	Pro	Glu	Asp	Val	Phe	Thr	Gln	
			115					120					125				
	Arg	Pro	Leu	Pro	Glu	Val	Arg	Ala	Leu	His	Glu	Phe	Leu	Leu	Asp	Thr	
20							135					140					
	Cys	Ala	His	Gly	Thr	Leu	Ala	Glu	Gly	Val	Ala	Ala	Ser	Asn	Trp	Ala	
25	145					150					155					160	
	Val	Glu	Gly	Ile	Thr	Gly	Val	Trp	Thr	Arg	Glu	Val	Val	Glu	Pro	Phe	
					165					170					175		
30	Arg	Ala	Tyr	Ala	Glu	Asp	Gly	Ala	Arg	Ile	Asp	Ala	Tyr	Ser	Met	Met	
				180					185					190			
	Trp	Leu	Lys	Val	His	Ala	Arg	Tyr	Asp	Asp	Gln	His	Pro	Glu	Glu	Ala	
35			195					200					205				
	Leu	Glu	Ile	Ile	Lys	Leu	Ser	Thr	Asp	Ala	Gly	Thr	Gly	Glu	Pro	Phe	
40		210					215					220					
	Arg	Val	Gln	Ala	Ala	Ala	Arg	Lys	Ser	Leu	Gln	Met	Tyr	Ala	Ala	Ala	
	225					230					235					240	
45	Leu	His	Ala	Cys	Cys	Asn	Asp										
					245												
50	<210>	82															
	<211>	258															
	<212>	PRT															
	<213>	Myxococcus stipitatus															
	<400>	82															
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	1				5					10					15		

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

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 <211> 222  
 <212> PRT  
 <213> *Myxococcus fulvus*

<400> 83

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

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<210> 84  
 <211> 252  
 <212> PRT  
 <213> *Vogesella indigofera*

<400> 84

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

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

5 Asp Cys Cys Leu Gln Leu Glu Gln Ala Val Pro Gly  
245 250

10 <210> 85  
<211> 256  
<212> PRT  
<213> Rudaea cellulosilytica

<400> 85

15 Met Asn Ser Arg Phe Glu Arg Thr Gly Pro Ile Asp Glu Leu Ser Ser  
1 5 10 15

20 Tyr Pro Ala Trp Ala Gln Asp Met Val Asp Ser Cys Ala Asp Ala Lys  
20 25 30

Arg Glu Val Val Glu His Glu Leu Phe Ala Leu Met Arg Glu Ala Gln  
35 40 45

25 Leu Ser Thr Glu Ser Thr Ala Ser Phe Met Thr Gly Val Trp Pro Val  
50 55 60

30 Ile Glu Arg Phe Pro Ala Tyr Met Ser Ala Ser Leu Met Lys Thr Arg  
65 70 75 80

35 Tyr Gly Arg Ser Glu Gly Asp Asn Met Ala Arg Arg Trp Leu Val Arg  
85 90 95

Asn Ile Arg Val Glu Gln Asn His Ala Glu Tyr Trp Leu Asp Trp Ala  
100 105 110

40 Glu Gly Ala Gly Val Ser Arg Asp Lys Val Leu Asn Gly Trp Val Pro  
115 120 125

45 Thr Asp Ala Met Val Leu Ala Asn Trp Cys Glu Glu Val Ser Thr Gly  
130 135 140

50 Asp Gly Leu Ala Ala Gly Ile Ile Ala Thr Asn Tyr Ala Val Glu Gly  
145 150 155 160

Ala Thr Gly Glu Trp Ser Gln Arg Val Tyr Glu Gly Ala Ala Tyr Arg  
165 170 175

55 Glu Ser Met Pro Thr Pro Gln Ala Leu Arg Trp Leu Lys Leu His Ala  
180 185 190

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	Ala	Tyr	Asp	Asp	Glu	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	Val	Cys	Ala	
			195					200					205				
5	Leu	Val	Gly	Asn	Asn	Pro	Val	Pro	Gln	Gln	Val	Ala	His	Leu	Arg	Glu	
		210					215					220					
	Cys	Val	Arg	Arg	Ser	Tyr	Thr	Ser	Met	Arg	Ile	Val	Ile	Asp	Arg	Cys	
10	225					230					235					240	
	Leu	Ala	Ala	Ser	Arg	Tyr	Gly	Met	Asp	Ile	Leu	Arg	Glu	Val	Ala	Ala	
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15																	
	<210>		86														
	<211>		258														
	<212>		PRT														
	<213>		Rhodanobacter sp.														
20																	
	<400>		86														
	Met	His	Thr	Pro	Phe	Glu	Arg	Thr	Gly	Pro	Leu	Thr	Glu	Leu	Gly	Ser	
	1				5					10					15		
25																	
	Tyr	Pro	Gln	Trp	Ala	Gln	Asp	Met	Val	Ala	Asp	Cys	Glu	Ser	Thr	Lys	
				20					25					30			
	Gln	Ser	Val	Leu	Asp	His	Glu	Leu	Trp	Ala	Met	Met	Arg	Glu	Val	Arg	
30			35					40					45				
	Leu	Gly	Gln	Thr	Ser	Thr	Ala	Ser	Phe	Met	Val	Gly	Val	Trp	Pro	Phe	
35		50					55					60					
	Ile	Glu	Arg	Phe	Pro	Ser	Phe	Met	Ala	Leu	Asn	Leu	Leu	Lys	Thr	Arg	
	65					70					75					80	
40																	
	Tyr	Gly	Arg	Ser	Leu	Gly	Asp	Asp	Met	Ala	Arg	Arg	Trp	Leu	Val	Arg	
					85					90					95		
	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Ala	Trp	Ala	
45				100					105					110			
	Glu	Gly	Ser	Gly	Val	Pro	Arg	Glu	Glu	Val	Leu	Ser	Ala	Arg	Pro	Pro	
50			115					120					125				
	His	Gly	Thr	Gln	Thr	Leu	Ala	Asn	Trp	Cys	Glu	Glu	Ile	Ser	Thr	Ile	
		130					135					140					
55																	
	Gly	Thr	Leu	Ala	Ala	Gly	Met	Ile	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
	145					150					155					160	



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	Val	Thr	Gly	Glu	Trp	Ser	Arg	Pro	Ile	Tyr	Glu	Ser	Thr	Ala	Tyr	Ala	
					165					170					175		
5	Glu	Ser	Phe	Pro	Pro	Arg	Thr	Arg	Thr	Gly	Ser	Leu	Arg	Trp	Leu	Gln	
				180						185				190			
10	Leu	His	Ala	Ala	Tyr	Asp	Asp	Ile	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
15	Val	Cys	Thr	Leu	Leu	Gly	Arg	Thr	Pro	Lys	Ile	Asp	Glu	Val	Thr	His	
		210					215					220					
20	Leu	Gly	Glu	Cys	Val	Arg	Arg	Ser	Tyr	Thr	Ser	Met	Arg	Ile	Val	Gly	
	225					230					235					240	
25	Asp	Leu	Cys	Met	Gln	Ala	Arg	Ser	Pro	Ser	Thr	Leu	Met	Lys	Glu	Val	
					245					250					255		
30	Ala	Ala															
	<210>	87															
	<211>	258															
	<212>	PRT															
	<213>	Rhodanobacter sp.															
	<400>	87															
35	Met	His	Thr	Arg	Phe	Glu	Arg	Thr	Gly	Pro	Leu	Thr	Glu	Leu	Ser	Ser	
	1				5					10					15		
40	Tyr	Pro	Gln	Trp	Ala	Gln	Asp	Met	Val	Val	Asp	Cys	Glu	Ser	Thr	Lys	
				20					25					30			
45	Gln	Ser	Val	Leu	Asp	His	Glu	Leu	Trp	Ala	Met	Met	Arg	Glu	Val	Arg	
			35					40					45				
50	Leu	Asp	His	Ala	Ser	Thr	Ala	Asn	Phe	Met	Val	Gly	Val	Trp	Pro	Phe	
		50					55					60					
55	Ile	Glu	Arg	Phe	Pro	Ser	Phe	Met	Ala	Leu	Asn	Leu	Leu	Lys	Thr	Arg	
	65					70					75					80	
60	Tyr	Gly	Arg	Ser	Leu	Gly	Asp	Asp	Val	Ala	Arg	Arg	Trp	Leu	Val	Arg	
					85					90					95		
65	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	Tyr	Trp	Leu	Ala	Trp	Ala	
				100					105					110			

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	Glu	Gly	Ser	Gly	Val	Pro	Arg	Asp	Asp	Val	Leu	Asn	Ala	Arg	Pro	Pro	
			115					120					125				
5	His	Gly	Thr	Gln	Thr	Leu	Ala	Asn	Trp	Cys	Glu	Glu	Ile	Ser	Ala	Asn	
		130					135					140					
10	Gly	Thr	Leu	Ala	Ala	Gly	Met	Ile	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
	145					150					155					160	
15	Val	Thr	Gly	Glu	Trp	Ser	Arg	Pro	Ile	Tyr	Glu	Ser	Thr	Ala	Tyr	Ala	
					165					170					175		
20	Glu	Ser	Phe	Pro	Pro	Gly	Ile	Arg	Thr	Gly	Ser	Leu	Arg	Trp	Leu	Gln	
				180					185					190			
25	Leu	His	Ala	Ala	Tyr	Asp	Asp	Ile	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
30	Val	Cys	Thr	Leu	Leu	Gly	Met	Thr	Pro	Lys	Ala	Asp	Glu	Val	Ala	His	
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35	Leu	Gly	Glu	Cys	Val	Arg	Arg	Ser	Tyr	Thr	Ser	Met	Arg	Ile	Val	Gly	
	225					230					235					240	
40	Asp	Leu	Cys	Met	Gln	Thr	His	Arg	Pro	Ser	Thr	Trp	Met	Lys	Glu	Ala	
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				20					25					30			
65	Gln	Ser	Val	Leu	Asp	His	Glu	Leu	Trp	Ala	Met	Met	Arg	Glu	Val	Arg	
			35					40					45				
70	Leu	Asp	His	Ala	Ser	Thr	Ala	Asn	Phe	Met	Val	Gly	Val	Trp	Pro	Phe	
	50						55					60					

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5	Tyr	Gly	Arg	Ser	Leu	Gly	Asp	Asp	Val	Ala	Arg	Arg	Trp	Leu	Val	Arg	
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10				100					105					110			
	Glu	Gly	Ser	Gly	Val	Pro	Arg	Asp	Asp	Val	Leu	Asn	Ala	Arg	Pro	Pro	
			115					120					125				
15	His	Gly	Thr	Gln	Thr	Leu	Ala	Asn	Trp	Cys	Glu	Glu	Ile	Ser	Ala	Asn	
	130						135					140					
	Gly	Thr	Leu	Ala	Ala	Gly	Met	Ile	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
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	Val	Thr	Gly	Glu	Trp	Ser	Arg	Pro	Ile	Tyr	Glu	Ser	Thr	Ala	Tyr	Ala	
25					165					170					175		
	Glu	Ser	Phe	Pro	Pro	Arg	Ile	Arg	Thr	Gly	Ser	Leu	Arg	Trp	Leu	Gln	
				180					185					190			
30	Leu	His	Ala	Ala	Tyr	Asp	Asp	Ile	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
	Val	Cys	Thr	Leu	Leu	Gly	Met	Thr	Pro	Lys	Ala	Asp	Glu	Val	Ala	His	
35		210					215					220					
	Leu	Gly	Glu	Cys	Val	Arg	Arg	Ser	Tyr	Thr	Ser	Met	Arg	Ile	Val	Gly	
40	225					230					235					240	
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	1				5					10					15		

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

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

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	Ala	Ser	Phe	Ala	Pro	Gly	Ala	Arg	Arg	Arg	Ala	Thr	Arg	Trp	Leu	Thr	
				180					185					190			
5	Leu	His	Ala	His	Asp	Asp	Ala	Pro	Pro	Trp	Glu	Ala	Leu	Glu	Ile	Val	
			195					200					205				
10	Cys	Thr	Leu	Val	Gly	Thr	His	Ala	Asp	Pro	Ser	Thr	Val	Ala	Ala	Leu	
		210					215					220					
15	Arg	Asp	Ala	Ile	Cys	Lys	Ser	Tyr	Asp	Tyr	Met	Arg	Leu	Ile	Leu	Asp	
	225					230					235					240	
20	Arg	Cys	Leu	Gln	Ala	Glu	Val	Ser	Arg	Pro	Pro	Ala	Ser	Pro	Arg	Lys	
					245					250					255		
25	Ala	Phe	Val	Gln	Leu	Gly											
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	<400>	92															
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	1				5					10					15		
35	His	Ser	Tyr	Pro	Glu	Trp	Thr	Arg	Glu	Leu	Val	Arg	His	Cys	Asp	Ala	
				20					25					30			
40	Phe	Lys	Arg	Gln	Val	Val	Glu	His	Glu	Leu	Phe	Arg	Gln	Met	Arg	Asp	
			35					40					45				
45	Gly	Val	Leu	Pro	Ala	Ala	Ile	His	Gln	Ala	Phe	Leu	Arg	Gly	Gly	Trp	
		50					55					60					
50	Pro	Val	Ile	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Lys	Asn	Leu	Leu	Lys	
	65					70					75					80	
55	Val	Arg	Tyr	Gly	Gln	His	Arg	Gly	His	Asp	Met	Ala	Arg	Arg	Tyr	Leu	
					85					90					95		
60	Ile	Arg	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Asp	His	Trp	Val	Gln	
				100					105					110			
65	Trp	Ala	Ala	Ala	Ser	Gly	Val	Asp	Thr	Asp	Ser	Leu	Leu	Arg	Asn	Pro	
			115					120					125				

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	Gln	Ala	Leu	Glu	Thr	Leu	Ser	Leu	Ser	His	Trp	Cys	His	Gln	Val	Cys	
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	145					150					155					160	
10	Glu	Gly	Ala	Thr	Gly	Glu	Trp	Cys	Ala	Ala	Val	Cys	Ser	Lys	Glu	Asp	
					165					170					175		
15	Tyr	Ala	Arg	Gln	Phe	Pro	Lys	Glu	Lys	Arg	Ala	Lys	Ala	Met	Lys	Trp	
				180					185					190			
20	Leu	Ala	Leu	His	Ala	His	Tyr	Asp	Asp	Glu	His	Pro	Trp	Glu	Ala	Leu	
			195					200					205				
25	Glu	Ile	Ile	Val	Thr	Leu	Val	Gly	Glu	Asn	Pro	Asn	Ala	Gln	Gln	Val	
	210						215					220					
30	Ala	Glu	Leu	Arg	His	Ala	Ile	Cys	Gln	Ser	His	Arg	Tyr	Met	Arg	Leu	
	225					230					235					240	
35	Leu	Leu	Asp	His	Tyr	Met	Ser	Gln	Pro	Ala	Pro	Glu	Thr	Cys	Ala	Arg	
				245						250					255		
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60	Ala	Ser	Tyr	Pro	Glu	Trp	Thr	Gln	Glu	Met	Val	His	Tyr	Cys	Asp	Arg	
				20					25					30			
65	Phe	Lys	Ser	Glu	Val	Val	Glu	His	Asp	Leu	Phe	Thr	Gln	Met	Lys	Glu	
			35					40					45				
70	Ala	Arg	Leu	Glu	His	Ser	Ile	His	Lys	Ala	Phe	Leu	Ser	Gly	Gly	Trp	
	50						55					60					
75	Pro	Val	Ile	Asp	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Met	Asn	Leu	Leu	Lys	
	65					70					75					80	



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	Ile	Arg	Tyr	Gly	Gln	Gly	Glu	Gly	His	Asp	Met	Ala	Arg	Arg	Tyr	Leu	
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5	Ile	Arg	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Asp	His	Trp	Val	Asn	
				100					105					110			
10	Trp	Ala	Ala	Glu	Ser	Gly	Val	Asp	Ile	Gln	Ala	Met	Leu	His	Asn	Gln	
			115					120					125				
15	His	Ala	Leu	Glu	Thr	Leu	Ser	Leu	Ser	Gln	Trp	Cys	Trp	Gln	Val	Cys	
		130					135					140					
20	Asp	Arg	Glu	Ser	Leu	Ala	Val	Ala	Met	Ala	Ala	Thr	Asn	Tyr	Ala	Ile	
	145					150					155					160	
25	Glu	Gly	Ala	Thr	Gly	Glu	Trp	Ser	Ala	Arg	Val	Cys	Ser	Glu	Asp	Arg	
					165					170					175		
30	Tyr	Ala	Asn	Leu	Phe	Asp	Glu	Glu	Val	Arg	Gly	Lys	Ala	Met	Lys	Trp	
				180					185					190			
35	Leu	Lys	Leu	His	Ala	Lys	Tyr	Asp	Asp	Ala	His	Pro	Trp	Glu	Ala	Leu	
			195					200					205				
40	Glu	Ile	Ile	Val	Thr	Leu	Val	Gly	Leu	His	Pro	Ser	Gln	Glu	Thr	Ile	
		210					215					220					
45	Thr	Lys	Leu	Arg	Asn	Ala	Ile	Cys	Lys	Ser	His	Gln	Phe	Met	Arg	Leu	
	225					230					235					240	
50	Leu	Leu	Asp	His	Tyr	Met	Arg	Pro	Ala	Thr	Gln	Leu	Gly	Gly	Arg	Gln	
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75	Ser	Ser	Tyr	Pro	Ala	Trp	Thr	Gln	Asp	Leu	Val	Tyr	His	Cys	Asp	Arg	
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	Tyr	Lys	Ser	Glu	Val	Val	Glu	His	Glu	Leu	Phe	Gly	Arg	Met	Gln	Gln	
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5	Ala	Cys	Leu	Asp	His	Ala	Thr	His	Lys	Ala	Phe	Leu	Ser	Gly	Gly	Trp	
		50					55					60					
10	Pro	Val	Ile	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Met	Asn	Leu	Leu	Lys	
	65					70					75					80	
15	Ile	Arg	Tyr	Gly	Gln	Gly	Pro	Gly	Gln	Asp	Met	Ala	Arg	Arg	Tyr	Leu	
				85						90					95		
20	Ile	Arg	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Asp	His	Trp	Val	Asn	
			100						105					110			
25	Trp	Ala	Ala	Ala	Ser	Gly	Val	Asp	Val	Pro	Ala	Met	Leu	His	Gly	Thr	
			115					120					125				
30	His	Ala	Leu	Glu	Thr	Leu	Cys	Leu	Ser	Gln	Trp	Cys	Trp	Gln	Val	Cys	
	130						135					140					
35	Asp	Arg	Asp	Ser	Leu	Ala	Val	Ala	Ile	Ala	Ala	Thr	Asn	Tyr	Ala	Ile	
	145					150					155					160	
40	Glu	Gly	Ala	Thr	Gly	Glu	Trp	Ser	Ala	Arg	Val	Cys	Ala	Glu	Pro	His	
					165					170					175		
45	Tyr	Ala	Gln	Leu	Phe	Asp	Glu	Thr	Val	Arg	Ala	Lys	Ala	Met	Lys	Trp	
				180					185					190			
50	Leu	Lys	Leu	His	Ala	Lys	Tyr	Asp	Asp	Ala	His	Pro	Trp	Glu	Ala	Leu	
			195					200					205				
55	Glu	Ile	Ile	Val	Thr	Leu	Val	Gly	Leu	Asn	Pro	Ser	Ala	Glu	Thr	Ile	
	210						215					220					
60	Ser	Arg	Leu	Arg	His	Ala	Ile	Cys	Arg	Ser	His	Gln	Phe	Met	Arg	Met	
	225					230					235					240	
65	Leu	Leu	Asp	Tyr	Tyr	Met	Arg	Ser	Ala	Thr	Pro	Pro	Val	Ala	Ala	Gly	
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70	Leu	Met	Arg	Pro	Ala												
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5 <400> 95

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

55

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	225		230		235		240									
5	Leu	Leu	Asp	His	Tyr	Met	Ser	Gln	Pro	Ala	Pro	Glu	Ile	Cys	Ala	Arg
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20	His	Ser	Tyr	Pro	Glu	Trp	Thr	Arg	Glu	Leu	Val	Arg	His	Cys	Asp	Ala
				20					25					30		
25	Phe	Lys	Arg	Gln	Val	Val	Glu	His	Glu	Leu	Phe	Arg	Gln	Met	Arg	Asp
			35					40					45			
30	Gly	Val	Leu	Pro	Ala	Ala	Ile	His	Gln	Ala	Phe	Leu	Arg	Gly	Gly	Trp
	50						55					60				
35	Pro	Val	Ile	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Lys	Asn	Leu	Leu	Lys
	65					70					75				80	
40	Val	Arg	Tyr	Gly	Gln	His	Arg	Gly	His	Asp	Met	Ala	Arg	Arg	Tyr	Leu
					85					90					95	
	Ile	Arg	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Asp	His	Trp	Val	Gln
				100					105					110		
45	Trp	Ala	Ala	Ala	Ser	Gly	Val	Asp	Ala	Asp	Ser	Leu	Leu	Arg	Asn	Pro
		115						120					125			
50	Gln	Ala	Leu	Glu	Thr	Leu	Ser	Leu	Ser	His	Trp	Cys	His	Gln	Val	Cys
	130						135					140				
	Glu	Arg	Glu	Ser	Leu	Glu	Val	Ala	Met	Ala	Ala	Thr	Asn	Tyr	Ala	Ile
	145					150					155				160	
55	Glu	Gly	Ala	Thr	Gly	Glu	Trp	Cys	Ala	Ala	Val	Cys	Ser	Lys	Glu	Asp
					165					170					175	
	Tyr	Ala	Arg	Gln	Phe	Pro	Lys	Glu	Lys	Arg	Ala	Lys	Ala	Met	Lys	Trp

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	180		185		190
5	Leu Ala Leu His Ala His Tyr Asp Asp Glu His Pro Trp Glu Ala Leu	195	200	205	
10	Glu Ile Ile Val Thr Leu Val Gly Glu Asp Pro Ser Ala Arg Gln Val	210	215	220	
15	Ala Glu Leu Arg His Ala Ile Cys Gln Ser His Arg Tyr Met Arg Leu	225	230	235	240
20	Leu Leu Asp His Tyr Met Ser Gln Pro Thr Pro Glu Ala Cys Ala Arg	245	250	255	
25	Val Ser Ala Glu Pro Ala Leu Ala	260			
30	<210> 97				
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35	<400> 97				
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45	Tyr Pro Val Trp Ala Gln Glu Met Met His Ser Thr Val Lys Ala Lys	20	25	30	
50	His Lys Val Val Asp His Glu Leu Phe Ala Met Met Lys Glu Ala Ala	35	40	45	
55	Leu Pro Glu Pro Glu Thr Asn Lys Phe Leu Val Gly Gly Trp Pro Val	50	55	60	
	Ile Glu Gln Phe Pro Gln Phe Met Ala Val Asn Leu Cys Lys Val Gln	65	70	75	80
	Tyr Gly Arg Ser Arg Gly Glu Asp Met Ala Arg Lys Tyr Leu Met Arg	85	90	95	
	Asn Ile Arg Val Glu Gln His His Ala Asp Leu Trp Thr Gln Trp Ala	100	105	110	
	Ala Ala Cys Gly Val Asp Lys Lys Asp Leu Leu Asp Ser Ala Val Pro	115	120	125	
	Val Glu Thr Gln Ala Leu Asn His Trp Cys Trp His Ser Cys Glu Arg				

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	130		135		140											
5	Ser	Ser	Leu	Ala	Thr	Ser	Met	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Gly
	145					150					155					160
	Ala	Thr	Gly	Glu	Trp	Ser	Thr	Leu	Ile	Cys	Ser	Ser	Asp	Thr	Tyr	Glu
10					165					170					175	
	Asn	Ser	Phe	Pro	Pro	Glu	Leu	Arg	Lys	Gln	Ala	Thr	Arg	Trp	Leu	Arg
				180					185					190		
15	Leu	His	Ala	Gln	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile
			195					200					205			
	Ile	Cys	Ser	Leu	Ile	Gly	Asn	Arg	Ala	Glu	Pro	Lys	Tyr	Val	Ala	Tyr
20		210					215					220				
	Leu	Ala	Gln	Cys	Ile	Ser	Asn	Ser	Tyr	Glu	Tyr	Met	Ala	Leu	Ser	Leu
	225					230					235					240
25	Asp	Arg	Cys	Met	Ser	His	Glu	Pro	Val	Ala	Ala	His				
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30	<210>	98														
	<211>	244														
	<212>	PRT														
	<213>	Burkholderia sp.														
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40	Tyr	Pro	Ala	Trp	Leu	Gly	Asp	Val	Leu	Asn	Asp	Thr	Ser	Glu	Ala	Lys
				20					25					30		
	Gln	Ala	Ile	Val	Lys	His	Pro	Ile	Phe	Ala	Ala	Met	Arg	Glu	Ala	Lys
			35					40					45			
45	Leu	Glu	Ala	Arg	Gln	Ala	Glu	Ala	Phe	Leu	Val	Asn	Gly	Trp	Pro	Val
	50						55					60				
50	Val	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Met	Asn	Leu	Gln	Lys	Val	Arg
	65					70					75				80	
	Tyr	Gly	His	Ser	Arg	Gly	Glu	Asp	Leu	Ala	Arg	Arg	Tyr	Leu	Thr	Arg
					85					90					95	
55	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Asp	Tyr	Trp	Val	Asp	Trp	Ala

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	100		105		110
5	Ala Ala His Asp Val Ser Lys Arg Ala Leu Met Lys Ala Asn Gly Pro	115	120	125	
10	Thr Leu Ala Tyr Ala Leu Ser His Trp Cys Trp Lys Ser Ser Ser Thr	130	135	140	
15	Asp Pro Leu Ala Ala Ser Ile Ala Ala Thr Asn Phe Ala Ile Glu Gly	145	150	155	160
20	Val Thr Gly Glu Trp Ala Thr Leu Val Cys Ser Ser Glu Thr Tyr Ala	165	170	175	
25	Asn Ser Phe Pro Val Ser Ile Arg Arg Lys Ala Met Arg Trp Leu Ser	180	185	190	
30	Leu His Ala His Tyr Asp Asp Ala His Pro Trp Glu Ala Leu Glu Ile	195	200	205	
35	Val Ala Thr Leu Leu Gly Asn Ala Pro Gly Val Asp Glu Val His Glu	210	215	220	
40	Val Arg Arg Ser Ile Ala Met Ser Tyr Glu Tyr Phe Lys Met Ser Leu	225	230	235	240
45	Asp Cys Cys Leu				
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55	<211> 248				
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60	<400> 99				
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70	Tyr Pro Ala Trp Ala Gln Asp Met Val Leu Ala Thr Ala Pro Ala Lys	20	25	30	
75	Asn Lys Val Val Ala His Glu Leu Phe Ala Arg Met Arg Glu Ala Ser	35	40	45	
80	Leu Pro Ala Gln Ala Thr Tyr Asn Phe Leu Val Gly Gly Trp Pro Val	50	55	60	
85	Ile Glu Gln Phe Pro Gln Tyr Met Ala Val Asn Leu Cys Lys Ile Gln				

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	65		70		75		80									
5	Tyr	Gly	Arg	Ser	Ala	Gly	Glu	Asn	Met	Ala	Arg	Arg	Tyr	Leu	Met	Arg
					85					90					95	
10	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Asp	His	Trp	Val	Glu	Trp	Ala
				100					105					110		
15	Lys	Ala	Cys	Gly	Ile	Ser	Met	Arg	Asp	Leu	Phe	Asp	Ser	Gln	Ala	Pro
			115					120					125			
20	Val	Glu	Ser	Gln	Ala	Leu	Asn	His	Trp	Cys	Trp	His	Ser	Cys	Glu	Arg
		130					135					140				
25	Ala	Ser	Leu	Ala	Thr	Ser	Met	Ala	Val	Thr	Asn	Leu	Ala	Ile	Glu	Gly
	145					150					155					160
30	Ala	Thr	Gly	Glu	Trp	Ala	Asn	Leu	Ile	Cys	Ser	Ser	Asp	Ala	Tyr	Glu
					165					170					175	
35	Asn	Ser	Phe	Ala	Pro	Glu	Leu	Arg	Arg	Pro	Ala	Thr	Arg	Trp	Leu	Arg
				180					185					190		
40	Leu	His	Ala	Gln	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile
			195					200					205			
45	Ile	Cys	Ser	Leu	Ile	Gly	Arg	Arg	Ala	Glu	Pro	Lys	Tyr	Val	Asp	Ile
		210					215					220				
50	Leu	Val	Gln	Gly	Leu	Ser	Asn	Ser	Tyr	Gln	Tyr	Met	Ala	Leu	Ser	Leu
	225					230					235					240
55	Asp	Arg	Ser	Met	Ala	Pro	Ser	Gly								
					245											
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	<212>	PRT														
	<213>	Janthinobacterium agaricidamnosum														
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60	Met	Ser	Asn	Glu	Phe	Lys	Arg	Arg	Gly	Pro	Leu	Lys	Glu	Ala	Glu	Ser
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65	Tyr	Pro	Pro	Trp	Leu	Gln	Gln	Val	Leu	Arg	Asp	Thr	Ser	Gln	Ala	Arg
				20					25					30		
70	Gln	Leu	Val	Ala	Gly	His	Gly	Val	Phe	Ala	Gly	Met	Arg	Asp	Ala	Arg



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	35	40	45
5	Leu Gly Ala Arg Glu Phe Tyr Ala Phe Phe Val Asn Gly Trp Pro Val 50 55 60		
10	Val Glu Gln Phe Pro Gln Tyr Met Ala Met Asn Leu Leu Lys Ala Arg 65 70 75 80		
15	Phe Gly Arg Ser Glu Gly Glu Asp Met Ala Arg Arg Tyr Leu Thr Arg 85 90 95		
20	Asn Ile Arg Val Glu Gln Asn His Ala Asp Tyr Trp Val Asp Trp Ala 100 105 110		
25	Gly Met His Asp Val Ser Lys Ser Thr Leu Leu Arg Ala Glu Gly Pro 115 120 125		
30	Pro Ala Ala Phe Ala Leu Ser His Trp Cys Trp Ser Ser Ser Ser Ala 130 135 140		
35	Asp Leu Leu Ala Gln Ser Met Ala Ala Thr Asn Tyr Ala Ile Glu Gly 145 150 155 160		
40	Val Thr Gly Asp Trp Ala Thr Leu Val Cys Ser Ala Ser His Tyr Glu 165 170 175		
45	Asp Ser Phe Pro Ala Ala Ser Arg Lys Lys Ala Met Arg Trp Leu Gln 180 185 190		
50	Leu His Ala His Tyr Asp Asp Ala His Pro Trp Glu Ala Leu Asp Ile 195 200 205		
55	Val Ala Thr Leu Leu Gly Ser Glu Pro Ser Gln Glu Ser Ile Asp Gly 210 215 220		
	Val Arg Asn Ser Ile Leu Thr Ser Phe Ser His Phe Lys Ala Ser Leu 225 230 235 240		
	Asp Cys Cys Val		
	<210> 101 <211> 249 <212> PRT <213> Janthinobacterium sp.		
	<400> 101		
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	1		5						10					15			
5	Tyr	Pro	Arg	Trp	Ala	Gln	Asp	Met	Met	Leu	Ser	Thr	Leu	Glu	Ala	Lys	
				20					25					30			
	Glu	Lys	Val	Val	Lys	His	Asp	Leu	Phe	Ala	Met	Met	Arg	Asp	Ala	Val	
			35					40					45				
10	Leu	Thr	Pro	Lys	Ala	Met	Arg	Asn	Phe	Leu	Ile	Gly	Gly	Trp	Pro	Val	
		50					55					60					
15	Val	Val	Gln	Phe	Pro	Gln	Phe	Met	Ala	Val	Asn	Leu	Cys	Lys	Ile	Gln	
	65					70					75					80	
	Tyr	Gly	Arg	Ser	Leu	Gly	Glu	Asn	Met	Ala	Arg	Lys	Tyr	Leu	Met	Lys	
20					85					90					95		
	Asn	Ile	Arg	Val	Glu	Gln	Asn	His	Ala	Glu	His	Trp	Val	Glu	Trp	Ala	
				100					105					110			
25	Lys	Ala	Cys	Ala	Val	Ser	Glu	Arg	Asp	Leu	Leu	Asp	Ser	Tyr	Leu	Pro	
			115					120					125				
30	Val	Glu	Ser	Gln	Ala	Leu	Ser	His	Trp	Cys	Trp	His	Ser	Ser	Glu	His	
		130					135					140					
	Thr	Ser	Leu	Ala	Thr	Ser	Met	Ala	Ala	Thr	Asn	Leu	Ala	Ile	Glu	Gly	
35						150					155					160	
	Ala	Thr	Gly	Glu	Trp	Ala	Ser	Leu	Val	Cys	Ser	Ala	Pro	Asp	Tyr	Glu	
					165					170					175		
40	Asn	Ser	Phe	Ala	Pro	Glu	Glu	Arg	Lys	Lys	Ala	Met	Arg	Trp	Leu	Lys	
				180					185					190			
45	Leu	His	Ala	His	Tyr	Asp	Asp	Thr	His	Pro	Trp	Glu	Ala	Leu	Asp	Ile	
			195					200					205				
	Ile	Ala	Ser	Leu	Ile	Gly	Trp	Glu	Ile	Glu	Pro	Lys	Tyr	Ala	Glu	Leu	
50		210				215						220					
	Leu	Gly	Gln	Cys	Val	Met	Asn	Ser	Tyr	Lys	Cys	Met	Thr	Leu	Ser	Leu	
	225					230					235					240	
55	Asn	Cys	Tyr	Leu	Ser	Glu	Ile	Cys	Gly								
					245												

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 <212> PRT  
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<400> 102

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

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	Leu	Arg	Leu	Ala	Ile	Cys	Thr	Ser	Tyr	Arg	Tyr	Met	Ala	Met	Thr	Leu
	225					230					235					240
5	Asp	Arg	Cys	Met	Gln	Met	Ala	Pro	Ala	Lys	Gln	Asp				
					245					250						
10	<210>	103														
	<211>	251														
	<212>	PRT														
	<213>	Aquaspirillum sp.														
	<400>	103														
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	1				5					10					15	
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				20					25					30		
25	Ala	Arg	Val	Thr	Gly	His	Val	Leu	Phe	Gln	Arg	Met	Arg	Asp	Ala	Thr
			35					40					45			
30	Leu	Pro	Tyr	Pro	Val	Met	Arg	His	Phe	Leu	Ile	Gly	Val	Trp	Pro	Val
	50						55					60				
35	Ile	Glu	Gln	Phe	Pro	Gln	Tyr	Met	Ala	Phe	Asn	Leu	Leu	Lys	Val	Arg
	65					70					75					80
40	Tyr	Gly	Arg	His	Pro	Gly	Glu	Asp	Leu	Ala	Arg	Thr	Trp	Leu	Ile	Arg
					85					90					95	
45	Asn	Leu	Arg	Val	Glu	Gln	His	His	Ala	Asp	Tyr	Trp	Val	Asp	Trp	Ala
				100					105					110		
50	Glu	Ala	Ser	Asp	Val	Ser	Arg	Asp	Ala	Leu	Ile	Ala	Gly	Thr	Asp	Asp
			115					120					125			
55	Pro	Ala	Thr	Ser	Ala	Leu	Ala	His	Trp	Cys	Trp	Arg	Thr	Cys	Glu	Arg
		130					135					140				
	Glu	Ala	Leu	Ala	Ile	Ser	Val	Ala	Ala	Thr	His	Tyr	Ala	Ile	Glu	Gly
	145					150					155					160
	Ala	Thr	Gly	Glu	Trp	Ser	Asn	Leu	Val	Cys	Ser	Thr	Pro	Thr	Tyr	Ala
					165					170					175	
	Asn	Leu	Phe	Ala	Glu	Ser	Glu	Arg	Lys	Lys	Ala	Thr	Arg	Trp	Leu	Arg
				180					185					190		

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Gln His Ala Gln Tyr Asp Asp Thr His Pro Trp Glu Ala Leu Asp Ile  
195 200 205

5 Met Cys Thr Leu Leu Gly Thr Gln Pro Asp Pro Ala Gln Val Thr Pro  
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10 Ile Arg Thr Ala Ile Cys Thr Ser Tyr Arg Tyr Met Ala Met Thr Leu  
225 230 235 240

Asp Arg Cys Met Ala Leu Glu Gln Pro Ala Ser  
245 250

15 <210> 104  
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<213> Pseudogulbenkiania subflava

20 <400> 104

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25 Tyr Pro Pro Trp Ala Arg Glu Leu Val Arg Leu Cys Gln Glu Ser Lys  
20 25 30

30 Ser Gln Val Val Glu His Glu Leu Phe Arg Arg Leu Arg Asp Gly Glu  
35 40 45

35 Leu Asp Thr Ala Ile Leu His His Phe Leu Ile Gly Val Trp Pro Val  
50 55 60

Ile Glu Gln Phe Pro Gln Tyr Met Ala Leu Asn Leu Leu Lys Ile Arg  
65 70 75 80

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

45 Asn Ile Arg Val Glu Gln Ser His Ala Glu His Trp Val Glu Trp Ala  
100 105 110

Leu Ala Ser Gly Val Thr Arg Glu Asp Leu Met Phe Ser Pro Val Pro  
115 120 125

50 Val Pro Met Leu Ala Leu Cys His Trp Cys Trp His Thr Cys Asp Arg  
130 135 140

55 Asp Thr Leu Val Leu Gly Met Ala Ala Thr Asn Phe Ala Ile Glu Gly  
145 150 155 160

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Ala Thr Gly Glu Trp Ala Val Lys Val Cys Ser Arg Asp Leu Tyr Glu  
165 170 175

5 Gln Ser Phe Ala Pro Ala Glu Arg Val Arg Ala Met Arg Trp Leu Lys  
180 185 190

10 Leu His Ala Gln Tyr Asp Asp Ala His Pro Trp Glu Ala Leu Glu Ile  
195 200 205

Ile Ser Thr Leu Leu Gly Asn His Pro Thr Glu Arg Asp Phe Ala His  
210 215 220

15 Leu His Ala Val Ile Cys Asn Ser Tyr Asp Tyr Met Arg Met Thr Leu  
225 230 235 240

20 Asp Tyr Cys Leu Asp Ala Ser Val Leu Pro Pro Pro Arg Glu Ala Arg  
245 250 255

25 Phe Asp Gly Ala Pro Val Leu Pro Gln Leu Gln Gly Arg Phe Ala Tyr  
260 265 270

Leu Gly

30 <210> 105  
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35 <400> 105

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40 Tyr Pro Ile Trp Ala Arg Glu Leu Met Arg Leu Cys Glu Glu Ser Lys  
20 25 30

45 Arg Gln Val Val Glu His Glu Leu Phe Arg Lys Met Arg Asp Gly Glu  
35 40 45

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

50 Ile Glu Gln Phe Pro Gln Tyr Met Ala Leu Asn Leu Leu Lys Ile Arg  
65 70 75 80

55 Tyr Gly Arg Thr Arg Gly Gln Asp Leu Ala Arg Arg Tyr Leu Val Arg  
85 90 95

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	Asn	Ile	Arg	Val	Glu	Gln	Ser	His	Ala	Glu	His	Trp	Val	Glu	Trp	Ala	
				100					105					110			
5	Leu	Ala	Ser	Gly	Val	Lys	Arg	Glu	Asp	Leu	Met	Phe	Ser	Pro	Val	Pro	
			115					120					125				
10	Val	Pro	Met	Leu	Ala	Leu	Cys	His	Trp	Cys	Trp	His	Thr	Cys	Asp	Arg	
		130					135					140					
15	Asp	Thr	Leu	Val	Leu	Gly	Ile	Ala	Ala	Thr	Asn	Tyr	Ala	Ile	Glu	Gly	
	145					150					155				160		
20	Ala	Thr	Gly	Glu	Trp	Ala	Leu	Lys	Val	Cys	Ser	Arg	Asp	Leu	Tyr	Glu	
					165					170					175		
25	Gln	Ser	Phe	Thr	Pro	Ala	His	Arg	Ala	Lys	Ala	Met	Arg	Trp	Leu	Lys	
				180					185					190			
30	Leu	His	Ala	Gln	Tyr	Asp	Asp	Ala	His	Pro	Trp	Glu	Ala	Leu	Glu	Ile	
			195					200					205				
35	Ile	Cys	Thr	Leu	Leu	Gly	Asn	Asn	Pro	Thr	Ala	Arg	Asp	Phe	Ala	His	
		210					215					220					
40	Leu	His	Ala	Ala	Ile	Cys	Asn	Ser	Tyr	Gln	Tyr	Met	Arg	Met	Thr	Leu	
						230					235					240	
45	Asp	Tyr	Cys	Leu	Asp	Ala	Ser	Val	Met	Pro	Pro	Pro	Arg	Glu	Ala	Arg	
					245					250					255		
50	Phe	Glu	Gly	Glu	Ala	Leu	Leu	Pro	Arg	Leu	Gln	Gly	Arg	Phe	Ala	Tyr	
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55	Leu	Gly															
60	<210>	106															
65	<211>	269															
70	<212>	PRT															
75	<213>	Methylbium sp.															
80	<400>	106															
85	Met	Ser	Asp	Asp	Phe	Ile	Arg	Glu	Gly	Glu	Leu	Thr	Asp	Ile	Arg	Ser	
	1				5					10					15		
90	Tyr	Pro	Ala	Trp	Ala	Gln	Asp	Leu	Val	Glu	Ser	Cys	Ala	Glu	Thr	Arg	
				20					25					30			

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



## Claims

## 1. A hand-dishwashing composition comprising:

a) a surfactant system comprising at least one anionic surfactant; and  
 b) a non-heme fatty acid decarboxylase; wherein said decarboxylase comprises an amino acid selected from the group consisting of: a) leucine or isoleucine at position 41, b) alanine at position 57, c) glycine, alanine, isoleucine, leucine, valine, serine, or threonine at position 239, and d) combinations thereof; wherein said positions are numbered with reference to SEQ ID NO: 1; and wherein said decarboxylase catalyzes the conversion of at least one fatty acid selected from the group consisting of: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and mixtures thereof.

2. The composition according to claim 1, wherein said decarboxylase comprises a polypeptide sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 100% identity to one or more sequences selected from the group consisting of: SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, and their functional fragments thereof.

3. The composition according to claim 2, wherein said decarboxylase comprises a polypeptide sequence having at least 80%, 90%, 95%, 98%, 100% identity to one or more sequences selected from the group consisting of: SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, and their functional fragments; preferably comprising a polypeptide sequence having at least 80%, 90%, 95%, 98%, 100% identity to SEQ ID NO: 4.

4. The composition according to any preceding claim, wherein the composition further comprises one or more co-enzymes selected from the group consisting of: fatty-acid peroxidases (EC 1.11.1.3), unspecific peroxygenases (EC 1.11.2.1), plant seed peroxygenases (EC 1.11.2.3), fatty acid peroxygenases (EC 1.11.2.4), linoleate diol synthases (EC 1.13.11.44), 5,8-linoleate diol synthases (EC 1.13.11.60 and EC 5.4.4.5), 7,8-linoleate diol synthases (EC 1.13.11.60 and EC 5.4.4.6), 9,14-linoleate diol synthases (EC 1.13.11.B1), 8,11-linoleate diol synthases, oleate diol synthases, other linoleate diol synthases, unspecific monooxygenase (EC 1.14.14.1), alkane 1-monooxygenase (EC 1.14.15.3), oleate 12-hydroxylases (EC 1.14.18.4), fatty acid amide hydrolase (EC 3.5.1.99), oleate hydratases (EC 4.2.1.53), linoleate isomerases (EC 5.2.1.5), linoleate (10E,12Z)-isomerases (EC 5.3.3.B2), fatty acid decarboxylases (OleT-like), alpha-dioxygenases, amylases, lipases, proteases, cellulases, and mixtures thereof; preferably fatty-acid peroxidases (EC 1.11.1.3), unspecific peroxygenases (EC 1.11.2.1), plant seed peroxygenases (EC 1.11.2.3), and fatty acid peroxygenases (EC 1.11.2.4), heme fatty acid decarboxylases (OleT-like), alpha-dioxygenases, and mixtures thereof.

5. The composition according to any preceding claim, wherein said one or more non-heme fatty acid decarboxylases are present in an amount of from 0.0001 wt% to 1 wt%, preferably from 0.001 wt% to 0.2 wt%, by weight of the hand dish-washing composition, based on active protein.

6. The composition according to any preceding claims, wherein the composition comprises from 5% to 50%, preferably 8% to 45%, more preferably from 15% to 40%, by weight of the total composition of a surfactant system.

7. The composition according to any preceding claims, wherein the anionic surfactant comprises alkyl sulphated anionic surfactant selected from the group consisting of: alkyl sulphate, alkyl alkoxy sulphate, and mixtures thereof.

8. The composition according to claim 7, wherein the alkyl sulphated anionic surfactant has an average alkyl chain length of from 8 to 18, preferably from 10 to 14, more preferably from 12 to 14, most preferably from 12 to 13 carbon atoms.

9. The composition according to any of claims 7 or 8, wherein the alkyl sulphated anionic surfactant has an average degree of alkoxylation, of less than 5, preferably less than 3, more preferably from 0.5 to 2.0, most preferably from 0.5 to 0.9.

10. The composition according to any of claims 7 to 9, wherein the alkyl sulphated anionic surfactant has a weight average degree of branching of more than 10%, preferably more than 20%, more preferably more than 30%, even

more preferably between 30% and 60%, most preferably between 30% and 50%.

5 11. The composition according to any preceding claims, wherein the surfactant system further comprises a co-surfactant, wherein the co-surfactant is selected from the group consisting of: an amphoteric surfactant, a zwitterionic surfactant, and mixtures thereof.

10 12. The composition according to claim 11, wherein the co-surfactant is an amphoteric surfactant, preferably an amphoteric surfactant selected from amine oxide surfactant, more preferably wherein the amine oxide surfactant is selected from the group consisting of: alkyl dimethyl amine oxide, alkyl amido propyl dimethyl amine oxide, and mixtures thereof.

13. The composition according to any of claims 11 or 12, wherein the weight ratio of the anionic surfactant to the co-surfactant is from 1:1 to 8:1, preferably from 2:1 to 5:1, more preferably from 2.5:1 to 4:1.

15 14. A method of manually washing dishware comprising the steps of delivering a detergent composition according to any preceding claims into a volume of water to form a wash solution and immersing the dishware in the solution.

20 15. The method according to claim 14, wherein the non-heme fatty acid decarboxylase is present at a concentration of from 0.005 ppm to 15 ppm, preferably from 0.02 ppm to 0.5 ppm, in an aqueous wash liquor during the washing process.



## EUROPEAN SEARCH REPORT

Application Number  
EP 19 19 9988

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