Abstract: The present invention relates to a composition comprising: (i) a lipase; and (ii) a bleach catalyst that is capable of accepting an oxygen atom from a peroxycacid and transferring the oxygen atom to an oxidizeable substrate.
A COMPOSITION COMPRISING A LIPASE AND A BLEACH CATALYST

FIELD OF THE INVENTION

The present invention relates to a composition comprising a lipase and a bleach catalyst. More specifically, the present invention relates to composition comprising a lipase and a bleach catalyst that is capable of accepting an oxygen atom from a peroxycacid and transferring the oxygen atom to an oxidizeable substrate. The compositions of the present invention are typically suitable for use as laundry detergent compositions and exhibit a good cleaning performance and a reduced malodor profile, especially on problematic residual dairy soils.

BACKGROUND OF THE INVENTION

Dingy soils such as body soils and other hydrophobic soils, including dairy soils, are extremely difficult to remove from fabric during a laundering process. The appearance of lipase enzymes suitable for detergent applications in the 1980’s (e.g. Lipolase and Lipolase Ultra, ex Novo Nordisk—now Novozymes) gave the formulator a new approach to improve grease removal. Lipase enzymes catalyse the hydrolysis of triglycerides which form a major component of many commonly encountered fatty soils such as sebum, animal fats (e.g. lard, ghee, butter) and vegetable oils (e.g. olive oil, sunflower oil, peanut oil). However, these enzymes show limited performance in the first wash cycle (being effective mainly during the drying stage of the laundering process) and give rise to a post-wash malodor. Without wishing to be bound by theory, the malodor arises from fatty acids released by the hydrolysis of fats and is particularly noticeable for dairy soils like milk, cream, butter and yogurt; dairy fats contain triglycerides functionalized with short chain (e.g. C₄) fatty acyl units which release malodorous volatile fatty acids after lipolysis. For a general review of the use of lipases in solid laundry detergents see the following reference: Enzymes in Detergency, ed. J.H. van Ee et al, Vol 69 Marcel Dekker Surfactant Series, Marcel Dekker, New York, 1997, pp93-132 (ISBN 0-8247-9995-X).

More recently so-called 'first wash' lipases have been commercialised such as Lipoprime™ and Lipex™ (ex. Novozymes) which show performance benefits in the initial wash cycle. The Lipex™ enzyme is described in more detail in WO 00/60063 and US 6,939,702 B1 (Novozymes). Laundry detergent formulations comprising the Lipex™ enzyme are described in more detail in IP.com publication IP 6443D (Novozymes). However in order to better exploit
lipase technology, both the odour profile on residual dairy stains and the cleaning performance on complex soils still needs to be improved.


There is a continuing need for laundry detergent compositions that exhibit a good overall cleaning profile, a good cold water temperature bleaching performance, good greasy soil cleaning performance and a reduced malodor profile on residual fatty soils, especially dairy soils.

The Inventors have found that by using lipase in combination with a bleach catalyst that is capable of accepting an oxygen atom from a peroxyacid and transferring the oxygen atom to an oxidizeable substrate improves the cleaning performance of the detergent composition whilst maintaining a reduced malodor profile on residual fatty soils, especially dairy soils.

In another embodiment of the present invention, the Inventors have found that the rubber sump hose compatibility profile is improved when a diacyl and/or a tetraacyl peroxide species is in combination with a lipase.

In an especially preferred embodiment of the present invention, the Inventors have found that using a lipase in combination with (i) a bleach catalyst that is capable of accepting an oxygen atom from a peroxyacid and transferring the oxygen atom to an oxidizeable substrate and (ii) a diacyl and/or tetraacyl peroxide species, significantly improves the cleaning performance of the composition, reduces the malodor profile of the composition and improves the rubber sump hose compatibility profile of the composition.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the alignment of lipases.
SEQUENCE LISTINGS

SEQ ID NO: 1 shows the DNA sequence encoding lipase from *Thermomyces lanoginosus.*

SEQ ID NO: 2 shows the amino acid sequence of a lipase from *Thermomyces lanoginosus.*

SEQ ID NO: 3 shows the amino acid sequence of a lipase from *Absidia reflexa.*

SEQ ID NO: 4 shows the amino acid sequence of a lipase from *Absidia corymbifera.*

SEQ ID NO: 5 shows the amino acid sequence of a lipase from *Rhizomucor miehei.*

SEQ ID NO: 6 shows the amino acid sequence of a lipase from *Rhizopus oryzae.*

SEQ ID NO: 7 shows the amino acid sequence of a lipase from *Aspergillus niger.*

SEQ ID NO: 8 shows the amino acid sequence of a lipase from *Aspergillus tubingensis.*

SEQ ID NO: 9 shows the amino acid sequence of a lipase from *Fusarium oxysporum.*

SEQ ID NO: 10 shows the amino acid sequence of a lipase from *Fusarium heterosporum.*

SEQ ID NO: 11 shows the amino acid sequence of a lipase from *Aspergillus oryzae.*

SEQ ID NO: 12 shows the amino acid sequence of a lipase from *Penicillium camemberti.*

SEQ ID NO: 13 shows the amino acid sequence of a lipase from *Aspergillus foetidus.*

SEQ ID NO: 14 shows the amino acid sequence of a lipase from *Aspergillus niger.*

SEQ ID NO: 15 shows the amino acid sequence of a lipase from *Aspergillus oryzae.*

SEQ ID NO: 16 shows the amino acid sequence of a lipase from *Landerina penisapora.*

SUMMARY OF THE INVENTION

In a first embodiment, the present invention provides a composition comprising: (i) a lipase; and (ii) a bleach catalyst that is capable of accepting an oxygen atom from a peroxycacid and transferring the oxygen atom to an oxidizeable substrate.

In a second embodiment, the present invention provides a composition comprising: (i) a lipase; and (ii) a diacyl and/or tetraacyl peroxide species.

DETAILED DESCRIPTION OF THE INVENTION

Composition

The composition comprises: (i) a lipase; and (ii) a bleach catalyst that is capable of accepting an oxygen atom from a peroxycacid and transferring the oxygen atom to an oxidizeable substrate. The lipase and the bleach catalyst are described in more detail below.
The composition may be suitable for use as a laundry detergent composition, laundry additive composition, dish-washing composition, or hard surface cleaning composition. The composition is typically a detergent composition. The composition may be a fabric treatment composition. Preferably the composition is a laundry detergent composition.

The composition can be any form such as liquid or solid, although preferably the composition is in solid form. Typically, the composition is in particulate form such as an agglomerate, a spray-dried powder, an extrudate, a flake, a needle, a noodle, a bead, or any combination thereof. The composition may be in compacted particulate form, such as in the form of a tablet or bar. The composition may be in some other unit dose form, such as in the form of a pouch, wherein the composition is typically at least partially, preferably essentially completely, enclosed by a water-soluble film such as polyvinyl alcohol. Preferably, the composition is in free-flowing particulate form; by free-flowing particulate form, it is typically meant that the composition is in the form of separate discrete particles. The composition may be made by any suitable method including agglomeration, spray-drying, extrusion, mixing, dry-mixing, liquid spray-on, roller compaction, spherisation, tabletting or any combination thereof.

The composition typically has a bulk density of from 450g/l to 1,000g/l, preferred low bulk density detergent compositions have a bulk density of from 550g/l to 650g/l and preferred high bulk density detergent compositions have a bulk density of from 750g/l to 900g/l. The composition may also have a bulk density of from 650g/l to 750g/l. During the laundering process, the composition is typically contacted with water to give a wash liquor having a pH of from above 7 to less than 13, preferably from above 7 to less than 10.5. This is the optimal pH to provide good cleaning whilst also ensuring a good fabric care profile.

Preferably, the composition comprises: (i) from 0% to less than 10%, preferably to 7%, or to 4%, or from 1%, or from 1.5%, by weight of the composition, of tetraacetylene diamine and/or oxybenzene sulphonate bleach activators. Most preferably, the composition is essentially free of tetraacetylene diamine and/or oxybenzene sulphonate bleach activators. By "is essential free of" it is typically meant "comprises no deliberately incorporated". Keeping the levels of these types of bleach activators to a minimum maintains the good dye safety profile of the composition.

Preferably, upon contact with water the composition forms a wash liquor having a pH of from 7 to 10.5. Compositions having this reserve alkalinity profile and pH profile exhibit a good stability profile for lipase.
Preferably, the composition comprises from 0% or from 1%, or from 2%, or from 3%, or from 4%, or from 5%, and to 30%, or to 20%, or to 10%, by weight of the composition, of a source of carbonate anion. The above described levels of a source of carbonate anion ensure that the composition has a good overall cleaning performance and a good bleaching performance.

Preferably, the composition comprises a dye transfer inhibitor. Suitable dye transfer inhibitors are selected from the group consisting of: polyvinylpyrrolidone, preferably having a weight average molecular weight of from 40,000 Da to 80,000 Da, preferably from 50,000 Da to 70,000 Da; polyvinylimidazole, preferably having a weight average molecular weight of from 10,000 Da to 40,000 Da, preferably from 15,000 Da to 25,000 Da; polyvinyl pyridine N-oxide polymer, preferably having a weight average molecular weight of from 30,000 Da to 70,000 Da, preferably from 40,000 Da to 60,000 Da; a co-polymer of polyvinylpyrrolidone and vinyl imidazole, preferably having a weight average molecular weight of from 30,000 Da to 70,000 Da, preferably from 40,000 Da to 60,000 Da; and any combination thereof. Compositions comprising a dye transfer inhibitor show a further improved dye safety profile.

The composition may comprise from 0% to less than 5%, preferably to 4%, or to 3%, or to 2%, or even to 1%, by weight of the composition, of zeolite-builder. Whilst the composition may comprise zeolite-builder at a level of 5 wt% or greater, preferably the composition comprises less than 5 wt% zeolite-builder. It may be preferred for the composition to be essentially free of zeolite-builder. By: "essentially free of zeolite-builder", it is typically meant that the composition comprises no deliberately incorporated zeolite-builder. This is especially preferred when the composition is a solid laundry detergent composition and it is desirable for the composition to be very highly soluble, to minimize the amount of water-insoluble residues (for example, which may deposit on fabric surfaces), and also when it is highly desirable to have transparent wash liquor. Suitable zeolite-builders include zeolite A, zeolite X, zeolite P and zeolite MAP.

The composition may comprise from 0% to less than 10%, or less than 5%, preferably to 4%, or to 3%, or to 2%, or even to 1%, by weight of the composition, of phosphate-builder. Whilst the composition may comprise phosphate-builder at a level of 10 wt% or greater, preferably the composition comprises less than 10 wt% phosphate-builder. It may even be preferred for the composition to be essentially free of phosphate-builder. By: "essentially free of phosphate-builder", it is typically meant that the composition comprises no deliberately added
phosphate-builder. This is especially preferred if it is desirable for the composition to have a very good environmental profile. Suitable phosphate-builders include sodium tripolyphosphate.

The composition may comprise from 0% to less than 5%, or preferably to 4%, or to 3%, or even to 2%, or to 1%, by weight of the composition, of silicate salt. Whilst the composition may comprise silicate salt at a level of 5wt% or greater, preferably the composition comprises less than 5wt% silicate salt. It may even be preferred for the composition to be essentially free of silicate salt. By: "essentially free from silicate salt", it is typically meant that the composition comprises no deliberately added silicate salt. This is especially preferred when the composition is a solid laundry detergent composition and it is desirable to ensure that the composition has very good dispensing and dissolution profiles and to ensure that the composition provides a clear wash liquor upon dissolution in water. The silicate salts include water-insoluble silicate salts. The silicate salts also include amorphous silicate salts and crystalline layered silicate salts (e.g. SKS-6). The silicate salts include sodium silicate.

The composition typically comprises adjunct ingredients. These adjunct ingredients include: detersive surfactants such as anionic detersive surfactants, non-ionic detersive surfactants, cationic detersive surfactants, zwitterionic detersive surfactants, amphoteric detersive surfactants; preferred anionic detersive surfactants are alkoxylated anionic detersive surfactants such as linear or branched, substituted or unsubstituted C₁₂₋₁₈ alkyl alkoxyated sulphates having an average degree of alkoxylation of from 1 to 30, preferably from 1 to 10, more preferably a linear or branched, substituted or unsubstituted C₁₂₋₁₈ alkyl ethoxylated sulphates having an average degree of ethoxylation of from 1 to 10, most preferably a linear unsubstituted C₁₂₋₁₈ alkyl ethoxylated sulphates having an average degree of ethoxylation of from 3 to 7, other preferred anionic detersive surfactants are alkyl sulphates, alkyl sulphonates, alkyl phosphonates, alkyl carboxylates or any mixture thereof, preferred alkyl sulphates include linear or branched, substituted or unsubstituted C₁₀₋₁₃ alkyl sulphates, another preferred anionic detersive surfactant is a C₁₀₋₁₃ linear alkyl benzene sulphonate; preferred non-ionic detersive surfactants are C₆₋₈ alkyl alkoxylated alcohols having an average degree of alkoxylation of from 1 to 20, preferably from 3 to 10, most preferred are C₁₂₋₁₄ alkyl ethoxylated alcohols having an average degree of alkoxylation of from 3 to 10; preferred cationic detersive surfactants are mono-C₆₋₁₃ alkyl mono-hydroxyethyl di-methyl quaternary ammonium chlorides, more preferred are mono-C₆-18 alkyl mono-hydroxyethyl di-methyl quaternary ammonium chloride, mono-C₁₀₋₁₂ alkyl mono-hydroxyethyl di-methyl quaternary ammonium chloride and mono-C₁₀₋₁₃ alkyl mono-
hydroxyethyl di-methyl quaternary ammonium chloride; source of peroxygen'such as percarbonate salts and/or perborate salts, preferred is sodium percarbonate, the source of peroxygen is preferably at least partially coated, preferably completely coated, by a coating ingredient such as a carbonate salt, a sulphate salt, a silicate salt, borosilicate, or mixtures, including mixed salts thereof; bleach activators such as tetraacetyl ethylene diamine, oxybenzene sulphonate bleach activators such as nonanoyl oxybenzene sulphonate, caprolactam bleach activators, imide bleach activators such as N-nonanoyl-N-methyl acetamide; enzymes such as amylases, arabinases, xylanases, galactanases, glucanases, carbohydrases, cellulases, laccases, oxidases, peroxidases, proteases, glucanases, pectate lyases and mannanases, especially preferred are proteases; Suds suppressing systems such as silicone based Suds suppressors; fluorescent whitening agents; photobleach; filler salts such as sulphate salts, preferably sodium sulphate; fabric-softening agents such as clay, silicone and/or quaternary ammonium compounds, especially preferred is montmorillonite clay optionally in combination with a silicone; flocculants such as polyethylene oxide; dye transfer inhibitors such as polyvinylpyrrolidone, poly A-vinylpyridine N-oxide and/or co-polymer of vinylpyrrolidone and vinylimidazole; fabric integrity components such as hydrophobically modified cellulose and oligomers produced by the condensation of imidazole and epichlorhydrin; soil dispersants and soil anti-redeposition aids such as alkoxylated polyamines and ethoxylated ethyleneimine polymers; anti-redeposition components such as carboxymethyl cellulose and polyesters; perfumes; sulphamic acid or salts thereof; citric acid or salts thereof; carbonate salts, especially preferred is sodium carbonate; and dyes such as orange dye, blue dye, green dye, purple dye, pink dye, or any mixture thereof.

A second embodiment of the present invention relates to a composition comprising: (i) a lipase; and (ii) a diacetyl peroxide.

Lipase

The lipase of the composition of the present invention is a lipase variant with no C-terminal extension but with mutations introduced in certain regions of a parent lipase whereby the tendency to odor generation is reduced.
Parent lipase

The parent lipase may be a fungal lipase with an amino acid sequence having at least 50% homology as defined in the section "Homology and augment" to the sequence of the *T. lanuginosus* lipase shown in SEQ ID NO: 2.

The parent lipase may be a yeast polypeptide such as Candida, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces, or Yarrowia polypeptide; or more preferably a filamentous fungal polypeptide such as Acremonium, Aspergillus, Aureobasidium, Cryptococcus, Filobasidium, Fusarium, Hemicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Piromyces, Schizophyllum, Talaromyces, Thermoaascus, Thielavia, Tolypocladium, or Trichoderma polypeptide.

In a preferred aspect, the parent lipase is a *Saccharomyces* carlsbergensis, *Saccharomyces* cerevisiae, *Saccharomyces* diastaticus, *Saccharomyces* douglasii, *Saccharomyces* kluyveri, *Saccharomyces* norbensis, or *Saccharomyces* oviformis polypeptide having lipase activity.


In another preferred aspect, the parent lipase is a Thermomyces lipase.

In a more preferred aspect, the parent lipase is a *Thermomyces* lanuginosus lipase. In an even more preferred embodiment the parent lipase is the lipase of SEQ ID NO: 2.

Identification of regions and substitutions.
The positions referred to in Region I through Region TV below are the positions of the amino acid residues in SEQ ID NO:2. To find the corresponding (or homologous) positions in a different lipase, the procedure described in "Homology and alignment" is used.

Substitutions in Region I

Region I consists of amino acid residues surrounding the N-terminal residue El. In this region it is preferred to substitute an amino acid of the parent lipase with a more positive amino acid. Amino acid residues corresponding to the following positions are comprised by Region I: 1 to 11 and 223-239. The following positions are of particular interest: 1, 2, 4, 8, 11, 223, 227, 229, 231, 233, 234 and 236. In particular the following substitutions have been identified: XIN/*, X4V, X227G, X23 IR and X233R.

In a preferred embodiment the parent lipase has at least 80%, such as 85% or 90%, such as at least 95% or 96% or 97% or 98% or 99%, identity to SEQ ID NO:2. In a most preferred embodiment the parent lipase is identical to SEQ ID NO: 2.

Substitutions in Region II

Region II consists of amino acid residues in contact with substrate on one side of the acyl chain and one side of the alcohol part. In this region it is preferred to substitute an amino acid of the parent lipase with a more positive amino acid or with a less hydrophobic amino acid.

Amino acid residues corresponding to the following positions are comprised by Region II: 202 to 211 and 249 to 269. The following positions are of particular interest: 202, 210, 211, 253, 254, 255, 256, 259. In particular the following substitutions have been identified: X202G, X210K/VWA, X255Y/V/A, X256K/R and X259G/M/Q/V.

In a preferred embodiment the parent lipase has at least 80%, such as 85% or 90%, such as at least 95% or 96% or 97% or 98% or 99%, identity to SEQ ID NO:2. In a most preferred embodiment the parent lipase is identical to SEQ ID NO: 2.

Substitutions in Region III

Region III consists of amino acid residues that form a flexible structure and thus allowing the substrate to get into the active site. In this region it is preferred to substitute an amino acid of the parent lipase with a more positive amino acid or a less hydrophobic amino acid. Amino acid residues corresponding to the following positions are comprised by Region
III: 82 to 102. The following positions are of particular interest: 83, 86, 87, 90, 91, 95, 96, 99. In particular the following substitutions have been identified: X83T, X86V and X90A/R.

In a preferred embodiment the parent lipase has at least 80%, such as 85% or 90%, such as at least 95% or 96% or 97% or 98% or 99%, identity to SEQ ID NO:2 . In a most preferred embodiment the parent lipase is identical to SEQ ID NO: 2.

Substitutions in Region IV

Region IV consists of amino acid residues that bind electrostatically to a surface. In this region it is preferred to substitute an amino acid of the parent lipase with a more positive amino acid. Amino acid residues corresponding to the following positions are comprised by Region IV: 27 and 54 to 62. The following positions are of particular interest: 27, 56, 57, 58, 60. In particular the following substitutions have been identified: X27R, X58N/AG/T/P and X60V/S/G/N/R/K7A/L.

In a preferred embodiment the parent lipase has at least 80%, such as 85% or 90%, such as at least 95% or 96% or 97% or 98% or 99%, identity to SEQ ID NO:2 . In a most preferred embodiment the parent lipase is identical to SEQ ID NO: 2.

Amino acids at other positions

The parent lipase may optionally comprise substitutions of other amino acids, particularly less than 10 or less than 5 such substitutions. Examples are substitutions corresponding to one or more of the positions 24, 37, 38, 46, 74, 81, 83, 115, 127, 131, 137, 143, 147, 150, 199, 200, 203, 206, 211, 263, 264, 265, 267 and 269 of the parent lipase. In a particular embodiment there is a substitution in at least one of the positions corresponding to position 81, 143, 147, 150 and 249. In a preferred embodiment the at least one substitution is selected from the group consisting of X81Q/E, X143S/C/N/D/A, X147M/Y, X150G/K and X249R/I/L.

The variant may comprise substitutions outside the defined Regions I to IV, the number of substitutions outside of the defined Regions I to IV is preferably less than six, or less than five, or less than four, or less than three, or less than two, such as five, or four, or three, or two or one. Alternatively, the variant does not comprise any substitution outside of the defined Regions I to IV.
Further substitutions may, e.g., be made according to principles known in the art, e.g., substitutions described in WO 92/05249, WO 94/25577, WO 95/22615, WO 97/04079 and WO 97/07202.

Parent lipase variants

In one aspect, said variant, when compared to said parent, comprising a total of at least three substitutions, said substitutions being selected from one or more of the following groups of substitutions:

a) at least two, or at least three, or at least four, or at least five, or at least six, such as two, three, four, five or six, substitutions in Region I,

b) at least one, at least two, or at least three, or at least four, or at least five, or at least six, such as one, two, three, four, five or six, substitution in Region II,

c) at least one, at least two, or at least three, or at least four, or at least five, or at least six, such as one, two, three, four, five or six, substitution in Region III,

d) and/or at least one, at least two, or at least three, or at least four, or at least five, or at least six, such as one, two, three, four, five or six, substitution in Region IV.

The variant may comprise substitutions, compared to the variant's parent, corresponding to those substitutions listed below in Table 1.

<table>
<thead>
<tr>
<th>Region I</th>
<th>Region II</th>
<th>Region III</th>
<th>Region IV</th>
<th>Outside regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>X4V + X227G +</td>
<td>X210K +</td>
<td>X83T+</td>
<td>X58A + X60S</td>
<td>X150G</td>
</tr>
<tr>
<td>X231R + X233R</td>
<td>X256K</td>
<td>X86V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X227G + X231R +</td>
<td>X256K</td>
<td>X86V</td>
<td>X58N + X60S</td>
<td>X150G</td>
</tr>
<tr>
<td>X233R</td>
<td>X255Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X231R + X233R</td>
<td>X202G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X227G + X231R +</td>
<td>X256K</td>
<td>X86V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X233R</td>
<td>X255V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4V + X231R +</td>
<td></td>
<td></td>
<td>X58N + X60S</td>
<td></td>
</tr>
<tr>
<td>X233R</td>
<td>X90R</td>
<td>X58N + X60S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X231R + X233R</td>
<td>X255V</td>
<td>X90A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In a further particular embodiment the parent lipase is identical to SEQ ID NO:2, and the variants of Table 1 will thus be:

<table>
<thead>
<tr>
<th>Region I</th>
<th>Region II</th>
<th>Region III</th>
<th>Region IV</th>
<th>Outside regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q4V + L227G + T231R + N233R</td>
<td>E210K + P256K</td>
<td>S83T + I86V</td>
<td>S58A + V60S</td>
<td>A150G</td>
</tr>
<tr>
<td>L227G + T231R + N233R</td>
<td>P256K</td>
<td>I86V</td>
<td>S58A + V60S</td>
<td>A150G</td>
</tr>
<tr>
<td>T231R + N233R</td>
<td>I255Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T231R + N233R</td>
<td>I202G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L227G + T231R + N233R</td>
<td>P256K</td>
<td>I86V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4V + T231R + N233R</td>
<td></td>
<td></td>
<td>S58A + V60S</td>
<td></td>
</tr>
<tr>
<td>T231R + N233R</td>
<td>I90R</td>
<td>S58A + V60S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T231R + N233R</td>
<td>I255V</td>
<td>I90A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L227G + T231R + N233R</td>
<td>P256K</td>
<td>I86V</td>
<td>S58A + V60S</td>
<td>A150G</td>
</tr>
<tr>
<td>T231R + N233R</td>
<td>F211L</td>
<td>S58A + V60S</td>
<td></td>
<td>L147M</td>
</tr>
<tr>
<td>X231R + X233R</td>
<td></td>
<td></td>
<td></td>
<td>X150K</td>
</tr>
</tbody>
</table>

Table 2: Some particular variants of SEQ ID NO:2
Nomenclature for amino acid modifications

In describing lipase variants according to the invention, the following nomenclature is used for ease of reference: Original amino acid(s):position(s): substituted amino acid(s)

According to this nomenclature, for instance the substitution of glutamic acid for glycine in position 195 is shown as G195E. A deletion of glycine in the same position is shown as G195*, and insertion of an additional amino acid residue such as lysine is shown as G195GK. Where a specific lipase contains a "deletion" in comparison with other lipases and an insertion is made in such a position this is indicated as *36D for insertion of an aspartic acid in position 36.

Multiple mutations are separated by pluses, i.e.: R170Y+G195E, representing mutations in positions 170 and 195 substituting tyrosine and glutamic acid for arginine and glycine, respectively.

X231 indicates the amino acid in a parent polypeptide corresponding to position 231, when applying the described alignment procedure. X231R indicates that the amino acid is replaced with R. For SEQ ID NO: 2 X is T, and X231R thus indicates a substitution of T in position 231 with R. Where the amino acid in a position (e.g. 231) may be substituted by another amino acid selected from a group of amino acids, e.g. the group consisting of R and P and Y, this will be indicated by X231R/P/Y.

In all cases, the accepted IUPAC single letter or triple letter amino acid abbreviation is employed.

Amino acid grouping

In this specification, amino acids are classified as negatively charged, positively charged or electrically neutral according to their electric charge at pH 10. Thus, negative amino acids are E, D, C (cysteine) and Y, particularly E and D. Positive amino acids are R, K and H, particularly R and K. Neutral amino acids are G, A, V, L, I, P, F, W, S, T, M, N, Q and C when forming part of a disulfide bridge. A substitution with another amino acid in the same group (negative, positive or neutral) is termed a conservative substitution.

The neutral amino acids may be divided into hydrophobic or non-polar (G, A, V, L, I, P, F, W and C as part of a disulfide bridge) and hydrophilic or polar (S, T, M, N, Q).
Amino acid identity

The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "identity".

For purposes of the present invention, the alignment of two amino acid sequences is determined by using the Needle program from the EMBOSS package (http://emboss.org) version 2.8.0. The Needle program implements the global alignment algorithm described in Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453. The substitution matrix used is BLOSUM62, gap opening penalty is 10, and gap extension penalty is 0.5.

The degree of identity between an amino acid sequence of the present invention ("invention sequence"; e.g. amino acids 1 to 269 of SEQ ID NO:2) and a different amino acid sequence ("foreign sequence") is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence" or the length of the "foreign sequence", whichever is the shortest. The result is expressed in percent identity.

An exact match occurs when the "invention sequence" and the "foreign sequence" have identical amino acid residues in the same positions of the overlap. The length of a sequence is the number of amino acid residues in the sequence (e.g. the length of SEQ ID NO:2 is 269).

The parent lipase has an amino acid identity of at least 50 % with the T. lanuginosus lipase (SEQ ID NO: 2), particularly at least 55 %, at least 60 %, at least 75 %, at least 85 %, at least 90 %, more than 95 % or more than 98 %. In a particular embodiment the parent lipase is identical to the T. lanuginosus lipase (SEQ ID NO:2).

The above procedure may be used for calculation of identity as well as homology and for alignment. In the context of the present invention homology and alignment has been calculated as described below.

Homology and alignment

For purposes of the present invention, the degree of homology may be suitably determined by means of computer programs known in the art, such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, CD., (1970), Journal of Molecular Biology, 48, 443-45), using GAP with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.
In the present invention, corresponding (or homologous) positions in the lipase sequences of *Absidia reflexa*, *Absidia corymbefera*, *Rhizmucor miehei*, *Rhizopus delemar*, *Aspergillus niger*, *Aspergillus tubigensis*, *Fusarium oxysporum*, *Fusarium heterosporum*, *Aspergillus oryzae*, *Penicillium camemberti*, *Aspergillus foetidus*, *Aspergillus niger*, *Thermomyces lanuginosus* (synonym: *Humicola lanuginose*) and *Lanterina penisapora* are defined by the alignment shown in Figure 1.

To find the homologous positions in lipase sequences not shown in the alignment, the sequence of interest is aligned to the sequences shown in Figure 1. The new sequence is aligned to the present alignment in Figure 1 by using the GAP alignment to the most homologous sequence found by the GAP program. GAP is provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, CD., (1970), Journal of Molecular Biology, 48, 443-45). The following settings are used for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

The parent lipase has a homology of at least 50% with the *T. lanuginosus* lipase (SEQ ID NO: 2), particularly at least 55%, at least 60%, at least 75%, at least 85%, at least 90%, more than 95% or more than 98%. In a particular embodiment the parent lipase is identical to the *T. lanuginosus* lipase (SEQ ID NO:2).

Hybridization

The present invention also relates to isolated polypeptides having lipase activity which are encoded by polynucleotides which hybridize under very low stringency conditions, preferably low stringency conditions, more preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) nucleotides 178 to 660 of SEQ ID NO: 1, (ii) the cDNA sequence contained in nucleotides 178 to 660 of SEQ ID NO: 1, (iii) a subsequence of (i) or (ii), or (iv) a complementary strand of (i), (ii), or (iii) (J. Sambrook, E.F. Fritsch, and T. Maniatus, 1989, Molecular Cloning, A Laboratory Manual, 2d edition, Cold Spring Harbor, New York). A subsequence of SEQ ID NO: 1 contains’ at least 100 contiguous nucleotides or preferably at least 200 contiguous nucleotides. Moreover, the subsequence may encode a polypeptide fragment which has lipase activity.
For long probes of at least 100 nucleotides in length, very low to very high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 ug/ml sheared and denatured salmon sperm DNA, and either 25% formamide for very low and low stringencies, 35% formamide for medium and medium-high stringencies, or 50% formamide for high and very high stringencies, following standard Southern blotting procedures for 12 to 24 hours optimally.

For long probes of at least 100 nucleotides in length, the carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS preferably at least at 45°C (very low stringency), more preferably at least at 50°C (low stringency), more preferably at least at 55°C (medium stringency), more preferably at least at 60°C (medium-high stringency), even more preferably at least at 65°C (high stringency), and most preferably at least at 70°C (very high stringency).

**DNA sequence, Expression vector, Host cell, Production of lipase**

The invention provides a DNA sequence encoding the lipase of the invention, an expression vector harboring the DNA sequence, and a transformed host cell containing the DNA sequence or the expression vector. These may be obtained by methods known in the art.

The invention also provides a method of producing the lipase by culturing the transformed host cell under conditions conducive for the production of the lipase and recovering the lipase from the resulting broth. The method may be practiced according to principles known in the art.

**Lipase activity**

- Lipase activity on tributyrin at neutral pH (LU)

A substrate for lipase is prepared by emulsifying tributyrin (glycerin tributyrate) using gum Arabic as emulsifier. The hydrolysis of tributyrin at 30°C at pH 7 or 9 is followed in a pH-stat titration experiment. One unit of lipase activity (1 LU) equals the amount of enzyme capable of releasing 1 micro mol butyric acid/min at pH 7.

- Benefit Risk

The Benefit Risk factor describing the performance compared to the reduced risk for odour smell is defined as: BR = RP_{v/g} / R. Lipase variants described herein may have BRs greater than 1, greater than 1.1, or even greater than 1 to about 1000.

- Average Relative Performance
The procedure for calculating average relative performance (RPavg) is found in Example 5 of the present specification. Lipase variants described herein may have (RPavg) of at least 0.8, at least 1.1, at least 1.5, or even at least 2 to about 1000.

Bleach catalyst

The bleach catalyst is capable of accepting an oxygen atom from a peroxycid and/or salt thereof, and transferring the oxygen atom to an oxidizable substrate. Suitable bleach catalysts include, but are not limited to: iminium cations and polyions; iminium zwitterions; modified amines; modified amine oxides; N-sulphonyl imines; N-phosphonyl imines; N-acyl imines; thiadiazole dioxides; perfluoroimines; cyclic sugar ketones and mixtures thereof.

Suitable iminium cations and polyions include, but are not limited to, N-methyl-3,4-dihydroisoquinolinium tetrafluoroborate, prepared as described in Tetrahedron (1992), 49(2), 423-38 (see, for example, compound 4, p. 433); N-methyl-3,4-dihydroisoquinolinium p-toluene sulphonate, prepared as described in U.S. Pat. 5,360,569 (see, for example, Column 11, Example 1); and N-octyl-3,4-dihydroisoquinolinium p-toluene sulphonate, prepared as described in U.S. Pat. 5,360,568 (see, for example, Column 10, Example 3).

Suitable iminium zwitterions include, but are not limited to, N-(3-sulfopropyl)-3,4-dihydroisoquinolinium inner salt, prepared as described in U.S. Pat. 5,576,282 (see, for example, Column 31, Example II); N-[2-(sulphoxy)dodecyl]-3,4-dihydroisoquinolinium inner salt, prepared as described in U.S. Pat. 5,817,614 (see, for example, Column 32, Example V); 2-[3-[(2-ethylhexyl)oxy]-2-(sulphoxy)propyl]-3,4-dihydroisoquinolinium, inner salt, prepared as described in WO05/047264 (see, for example, page 18, Example 8), and 2-[3-[(2-butyloctyl)oxy]-2-(sulphoxy)propyl]-3,4-dihydroisoquinolinium, inner salt.

Suitable modified amine oxygen transfer catalysts include, but are not limited to, 1,2,3,4-tetrahydro-2-methyl-1-isoquinolinol, which can be made according to the procedures described in Tetrahedron Letters (1987), 28(48), 6061-6064. Suitable modified amine oxide oxygen transfer catalysts include, but are not limited to, sodium 1-hydroxy-N-oxy-N-[2-(sulphoxy)decyl]-1,2,3,4-tetrahydroisoquinoline.

Suitable N-sulphonyl imine oxygen transfer catalysts include, but are not limited to, 3-methyl-1,2-benzisothiazole 1,1-dioxide, prepared according to the procedure described in the Journal of Organic Chemistry (1990), 55(4), 1254-61.
Suitable N-phosphonyl imine oxygen transfer catalysts include, but are not limited to, [R-(E)]-N-[(2-chloro-5-nitrophenyl)methylene]-P-phenyl-P-(2,4,6-trimethylphenyl)-phosphinic amide, which can be made according to the procedures described in the Journal of the Chemical Society, Chemical Communications (1994), (22), 2569-70.

Suitable N-acyl imine oxygen transfer catalysts include, but are not limited to, [N(E)]-N-(phenylmethylene)acetamide. which can be made according to the procedures described in Polish Journal of Chemistry (2003), 77(5), 577-590.

Suitable thiadiazole dioxide oxygen transfer catalysts include but are not limited to, 3-methyl-4-phenyl-1,2,5-thiadiazole 1,1-dioxide, which can be made according to the procedures described in U.S. Pat. 5,753,599 (Column 9, Example 2).

Suitable perfluoroimine oxygen transfer catalysts include, but are not limited to, (Z)-2,2,3,3,4,4,4-heptafluoro-N-(nonafluorobutyl)butanimidoyl fluoride, which can be made according to the procedures described in Tetrahedron Letters (1994), 35(34), 6329-30.

Suitable cyclic sugar ketone oxygen transfer catalysts include, but are not limited to, 1,2:4,5-di-0-isopropylidene-D-erythro-2,3-hexodiuro-2,6-pyranose as prepared in U.S. Pat. 6,649,085 (Column 12, Example 1).

Preferably, the bleach catalyst comprises an iminium and/or carbonyl functional group and is typically capable of forming an oxaziridinium and/or dioxirane functional group upon acceptance of an oxygen atom, especially upon acceptance of an oxygen atom from a peroxyacid and/or salt thereof. Preferably, the bleach catalyst comprises an oxaziridinium functional group and/or is capable of forming an oxaziridinium functional group upon acceptance of an oxygen atom, especially upon acceptance of an oxygen atom from a peroxyacid and/or salt thereof.

Preferably, the bleach catalyst comprises a cyclic iminium functional group, preferably wherein the cyclic moiety has a ring size of from five to eight atoms (including the nitrogen atom), preferably six atoms. Preferably, the bleach catalyst comprises an arylinium functional group, preferably a bi-cyclic arylinium functional group, preferably a 3,4-dihydroisoquinolinium functional group. Typically, the imine functional group is a quaternary imine functional group and is typically capable of forming a quaternary oxaziridinium functional group upon acceptance of an oxygen atom, especially upon acceptance of an oxygen atom from a peroxyacid and/or salt thereof.

Preferably, the bleach catalyst has a chemical structure corresponding to the following chemical formula
wherein: n and m are independently from O to 4, preferably n and m are both 0; each R^1 is independently selected from a substituted or unsubstituted radical selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, fused aryl, heterocyclic ring, fused heterocyclic ring, nitro, halo, cyano, sulphonato, alkoxy, keto, carboxylic_{3} and carboalkoxy radicals; and any two vicinal R^1 substituents may combine to form a fused aryl, fused carbocyclic or fused heterocyclic ring; each R^2 is independently selected from a substituted or unsubstituted radical independently selected from the group consisting of hydrogen, hydroxy, alkyl, cycloalkyl, alkaryl, aryl, aralkyl, alkenes, heterocyclic ring, alkoxy, arylcarbonyl groups, carboxyalkyl groups and amide groups; any R^2 may be joined together with any other of R^2 to form part of a common ring; any geminal R^2 may combine to form a carbonyl; and any two R^2 may combine to form a substituted or unsubstituted fused unsaturated moiety; R^3 is a C_1 to C_20 substituted or unsubstituted alkyl; R^4 is hydrogen or the moiety Q_{f}-A. wherein: Q is a branched or unbranched alkylene, t = 0 or 1 and A is an anionic group selected from the group consisting of OSOs^{n}, SO_{3}^{-}, CO_{2}^{n}, OCO_{2}^{n}, OPO_{3}^{2-}, OPO_{3}H^{+} and OPO_{2}^{-}; R^5 is hydrogen or the moiety -CR^{1}_{f}-Y_{b}-G_{c}-Y_{d}-[(CR^{9}_{f}R^{10}_{f})y-O]_{k}-R^8, wherein: each Y is independently selected from the group consisting of O, S, N-H, or N-R^8; and each R^8 is independently selected from the group consisting of alkyl, aryl and heteroaryl, said moieties being substituted or unsubstituted, and whether substituted or unsubstituted said moieties having less than 21 carbons; each G is independently selected from the group consisting of CO, SO_{2}, SO, PO and PO_{2}; R^9 and R^{10} are independently selected from the group consisting of H and C_{1}-C_{4} alkyl; R^{11} and R^{12} are independently selected from the group consisting of H and alkyl, or when taken together may join to form a carbonyl; b = O or 1; c can = O or 1, but c must = O if b = O; y is an integer from 1 to 6; k is an integer from O to 20; R^6 is H, or an alkyl, aryl or heteroaryl moiety; said moieties being substituted or unsubstituted; and X, if present, is a suitable charge balancing counterion, preferably X is present when R^4 is hydrogen,
suitable X, include but are not limited to: chloride, bromide, sulphate, methosulphate, sulphonate, p-toluenesulphonate, borontetraflouride and phosphate.

In one embodiment of the present invention, the bleach catalyst has a structure corresponding to general formula below:

wherein \( R^{13} \) is a branched alkyl group containing from three to 24 carbon atoms (including the branching carbon atoms) or a linear alkyl group containing from one to 24 carbon atoms; preferably \( R^{13} \) is a branched alkyl group containing from eight to 18 carbon atoms or linear alkyl group containing from eight to eighteen carbon atoms; preferably \( R^{13} \) is selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, iso-nonyl, iso-decyl, iso-tridecyl and iso-pentadecyl; preferably \( R^{13} \) is selected from the group consisting of 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, iso-tridecyl and iso-pentadecyl.

Oxybenzene sulphonate and/or oxybenzoic bleach activators

The composition preferably comprises (i) oxybenzene sulphonate bleach activators and/or oxybenzoic bleach activators and (ii) a source of peroxygen. Typically, the oxybenzoic acid bleach activator is in its salt form. Preferred oxybenzene sulphonate bleach activators include bleach activators having the general formula:

\[
R-(\text{C}=\text{O})-L
\]

wherein \( R \) is an alkyl group, optionally branched, having, when the bleach activator is hydrophobic, from 6 to 14 carbon atoms, or from 8 to 12 carbon atoms and \( L \) is leaving group. Examples of suitable leaving groups are benzoic acid and derivatives thereof, especially salts thereof. Another especially preferred leaving group is oxybenzene sulphonate. Suitable bleach activators include dodecanoyl oxybenzene sulphonate, decanoyl oxybenzene sulphonate, a salt of decanoyl oxybenzoic acid, 3,5,5-trimethyl hexanoyloxybenzene sulphonate, nonanoylamidocaproyloxybenzene sulphonate, and nonanoyloxybenzene sulphonate (NOBS).

Suitable bleach activators are also disclosed in WO 98/17767. The incorporation of these bleach
activators into the composition is especially preferred when the composition comprises low levels of zeolite builder and phosphate builder. The Inventors have found that combining these bleach activators with a source of peroxxygen and a bleach catalyst as described in more detail above and a lipase, especially in an under-built detergent composition (such as a detergent composition comprising low levels of zeolite-builder and phosphate-builder), improves the overall cleaning performance, improves the rubber sump hose compatibility profile and reduces the malodor profile of the composition.

Diacyl peroxide

In another embodiment the composition comprises: (i) a lipase; and (ii) a diacyl and/or tetraacyl peroxide species. The Inventors have found that these composition exhibit excellent rubber hose compatibility. Diacyl peroxides and also tetraacyl peroxides are known to attack rubber, such as the rubber sump hoses of automatic washing machines, and over multiple washing cycles this can lead to failure of the rubber sump hose. The Inventors have found that combining the diacyl peroxides and/or tetraacyl peroxides with lipase overcomes this problem of rubber sump hose incompatibility.

The diacyl peroxide bleaching species is preferably selected from diacyl peroxides of the general formula:

\[ \text{R}^\text{a}(\text{O})\text{O}-(\text{O})\text{C-R}^2 \]

in which \( \text{R}^1 \) represents a \( \text{C}_6\text{-C}_{1g} \) alkyl, preferably \( \text{C}_6\text{-C}_{12} \) alkyl group containing a linear chain of at least 5 carbon atoms and optionally containing one or more substituents (e.g. \( \text{-N}^+\text{(CH}_3\text{)}_3 \), \( \text{-COOH} \) or \( \text{-CN} \)) and/or one or more interrupting moieties (e.g. \( \text{-CONH-} \) or \( \text{-CH=CH-} \)) interpolated between adjacent carbon atoms of the alkyl radical, and \( \text{R}^2 \) represents an aliphatic group compatible with a peroxide moiety, such that \( \text{R}^1 \) and \( \text{R}^2 \) together contain a total of 8 to 30 carbon atoms. In one preferred aspect \( \text{R}^1 \) and \( \text{R}^2 \) are linear unsubstituted \( \text{C}_6\text{-C}_{12} \) alkyl chains. Most preferably \( \text{R}^1 \) and \( \text{R}^2 \) are identical. Diacyl peroxides, in which both \( \text{R}^1 \) and \( \text{R}^2 \) are \( \text{C}_6\text{-C}_{12} \) alkyl groups, are particularly preferred. Preferably, at least one of, most preferably only one of, the \( \text{R} \) groups (\( \text{R}_1 \) or \( \text{R}_2 \)), does not contain branching or pendant rings in the alpha
position, or preferably neither in the alpha nor beta positions or most preferably in none of the alpha or beta or gamma positions. In one further preferred embodiment the DAP may be asymmetric, such that preferably the hydrolysis of R1 acyl group is rapid to generate peracid, but the hydrolysis of R2 acyl group is slow.

The tetraacyl peroxide bleaching species is preferably selected from tetraacyl peroxides of the general formula:

\[ R3-C(O)-OO-C(O)-(CH_2)_n-C(O)-OO-C(O)-R3 \]

in which R3 represents a C1-Cg alkyl, preferably C3 - C7, group and n represents an integer from 2 to 12, preferably 4 to 10 inclusive.

Preferably, the diacyl and/or tetraacyl peroxide bleaching species is present in an amount sufficient to provide at least 0.5 ppm, more preferably at least 10 ppm, and even more preferably at least 50 ppm by weight of the wash liquor. In a preferred embodiment, the bleaching species is present in an amount sufficient to provide from about 0.5 to about 300 ppm, more preferably from about 30 to about 150 ppm by weight of the wash liquor.

Pre-formed peroxyacid

The pre-formed peroxyacid or salt thereof is typically either a peroxycarboxylic acid or salt thereof, or a peroxy sulphonic acid or salt thereof.

The pre-formed peroxyacid or salt thereof is preferably a peroxy carboxylic acid or salt thereof, typically having a chemical structure corresponding to the following chemical formula:

\[ \text{O} \quad R \quad \text{O} \quad \text{Q} \quad \text{Y} \]

wherein: R is selected from alkyl, aralkyl, cycloalkyl, ary1 or heterocyclic groups; the R group can be linear or branched, substituted or unsubstituted; and Y is any suitable counter-ion that achieves electric charge neutrality, preferably Y is selected from hydrogen, sodium or potassium. Preferably, R is a linear or branched, substituted or unsubstituted C6-9 alkyl.

Preferably, the peroxyacid or salt thereof is selected from peroxyhexanoic acid, peroxyheptanoic
acid, peroxyoctanoic acid, peroxynonanoic acid, peroxydecanoic acid, any salt thereof, or any combination thereof. Preferably, the peroxycid or salt thereof has a melting point in the range of from 30°C to 60°C.

The pre-formed peroxycid or salt thereof can also be a peroxysulfonic acid or salt thereof, typically having a chemical structure corresponding to the following chemical formula:

\[
\begin{array}{c}
\text{O} \\
\text{R}_{15} \rightarrow \text{S} \leftarrow \text{O} \\
\end{array}
\]

wherein: \( R_{15} \) is selected from alkyl, aralkyl, cycloalkyl, aryl or heterocyclic groups; the \( R_{15} \) group can be linear or branched, substituted or unsubstituted; and \( Z \) is any suitable counter-ion that achieves electric charge neutrality, preferably \( Z \) is selected from hydrogen, sodium or potassium. Preferably \( R_{15} \) is a linear or branched, substituted or unsubstituted C\(_{6—9}\) alkyl.

EXAMPLES

**LIPASE VARIANTS EXAMPLES**

Chemicals used as buffers and substrates are commercial products of at least reagent grade.

- Media and Solutions: LAS (Surfac PS™) and Zeolite A (Wessalith P™). Other ingredients used are standard laboratory reagents.

- Materials. EMPA221 from EMPA St Gallen, Lerchfeldstrasse 5, CH-9014 St. Gallen, Switzerland

**Example 1: Production of enzyme**

A plasmid containing the gene encoding the lipase is constructed and transformed into a suitable host cell using standard methods of the art.

Fermentation is carried out as a fed-batch fermentation using a constant medium temperature of 34°C and a start volume of 1.2 liter. The initial pH of the medium is set to 6.5. Once the pH has increased to 7.0 this value is maintained through addition of 10% H\(_3\)PO\(_4\).
level of dissolved oxygen in the medium is controlled by varying the agitation rate and using a fixed aeration rate of 1.0 liter air per liter medium per minute. The feed addition rate is maintained at a constant level during the entire fed-batch phase.

The batch medium contained maltose syrup as carbon source, urea and yeast extract as nitrogen source and a mixture of trace metals and salts. The feed added continuously during the fed-batch phase contains maltose syrup as carbon source whereas yeast extract and urea is added in order to assure a sufficient supply of nitrogen.

Purification of the lipase may be done by use of standard methods known in the art, e.g. by filtering the fermentation supernatant and subsequent hydrophobic chromatography and anion exchange, e.g. as described in EP 0 851 913, Example 3.


The enzyme variants of the present application are tested using the Automatic Mechanical Stress Assay (AMSA). With the AMSA test the wash performance of a large quantity of small volume enzyme-detergent solutions can be examined. The AMSA plate has a number of slots for test solutions and a lid firmly squeezing the textile swatch to be washed against all the slot openings. During the washing time, the plate, test solutions, textile and lid are vigorously shaken to bring the test solution in contact with the textile and apply mechanical stress. For further description see WO 02/42740 especially the paragraph "Special method embodiments" at page 23-24. The containers, which contain the detergent test solution, consist of cylindrical holes (6 mm diameter, 10 mm depth) in a metal plate. The stained fabric (test material) lies on the top of the metal plate and is used as a lid and seal on the containers. Another metal plate lies on the top of the stained fabric to avoid any spillage from each container. The two metal plates together with the stained fabric are vibrated up and down at a frequency of 30 Hz with an amplitude of 2 mm.

The assay is conducted under the experimental conditions specified below:

<table>
<thead>
<tr>
<th>Test solution</th>
<th>0.5 g/l LAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.52 g/l Na2CO3</td>
</tr>
<tr>
<td></td>
<td>1.07 g/l Zeolite A</td>
</tr>
<tr>
<td></td>
<td>0.52 g/l Tn sodium Citrate</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th>Test solution volume</th>
<th>160 micro l</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>As is (≈9.9)</td>
</tr>
<tr>
<td>Wash time</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Water hardness</td>
<td>15°dH</td>
</tr>
<tr>
<td>Ratio of Ca²⁺/Mg²⁺/NaHCO₃</td>
<td>4:1:7.5</td>
</tr>
<tr>
<td>Enzyme concentration in test solution</td>
<td>0.125, 0.25, 0.50, 1.0 mg enzyme protein/liter (mg ep/l)</td>
</tr>
</tbody>
</table>
| Drying               | Performance: After washing the textile pieces is immediately flushed in tap water and air-dried at 85°C in 5 min  
                           Odor: After washing the textile pieces is immediately flushed in tap water and dried at room temperature (20°C) for 2 hours |
| Test material        | Cream turmeric swatch as described below (EMPA221 used as cotton textile) |

Cream-turmeric swatches are prepared by mixing 5 g of turmeric (Santa Maria, Denmark) with 100 g cream (38% fat, Aria, Denmark) at 50°C, the mixture is left at this temperature for about 20 minutes and filtered (50°C) to remove any undissolved particles. The mixture is cooled to 20°C) woven cotton swatches, EMPA221, are immersed in the cream-turmeric mixture and afterwards allowed to dry at room temperature over night and frozen until use. The preparation of cream-turmeric swatches is disclosed in the patent application PA 2005 00775, filed 27 May 2005.

The performance of the enzyme variant is measured as the brightness of the colour of the textile samples washed with that specific enzyme variant. Brightness can also be expressed as the intensity of the light reflected from the textile sample when luminated with white light. When
the textile is stained the intensity of the reflected light is lower, than that of a clean textile. Therefore the intensity of the reflected light can be used to measure wash performance of an enzyme variant.

Color measurements are made with a professional flatbed scanner (PFU DL2400pro), which is used to capture an image of the washed textile samples. The scans are made with a resolution of 200 dpi and with an output color depth of 24 bits. In order to get accurate results, the scanner is frequently calibrated with a Kodak reflective IT8 target.

To extract a value for the light intensity from the scanned images, a special designed software application is used (Novozymes Color Vector Analyzer). The program retrieves the 24 bit pixel values from the image and converts them into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

\[ \text{Int} = \sqrt{r^2 + g^2 + b^2} \]

The wash performance (P) of the variants is calculated in accordance with the formula:

\[ P = \text{Int(v)} - \text{Int(r)} \]

Int(v) is the light intensity value of textile surface washed with tested enzyme and Int(r) is the light intensity value of textile surface washed without the tested enzyme.

A relative performance score is given as the result of the AMSA wash in accordance with the definition: Relative Performance scores (RP) are summing up the performances (P) of the tested enzyme variants against the reference enzyme: \[ \text{RP} = \frac{P(\text{test enzyme})}{P(\text{Reference enzyme})} \]

RPavg indicates the average relative performance compared to the reference enzyme at all four enzyme concentrations (0.125, 0.25, 0.5, 1.0 mg ep/1)

\[ \text{RPavg} = \text{avg}(\text{RP}(0.125), \text{RP}(0.25), \text{RP}(0.5), \text{RP}(\text{LO})) \]

A variant is considered to exhibit improved wash performance, if it performs better than the reference. In the context of the present invention the reference enzyme is the lipase of SEQ ID NO:2 with the substitutions T23IR + N233R.

The butyric acid release from the lipase washed swatches are measured by Solid Phase Micro Extraction Gas Chromatography (SPME-GC) using the following method. Four textile pieces (5 mm in diameter), washed in the specified solution in Table 3 containing 1 mg/l lipase, are transferred to a Gas Chromatograph (GC) vial. The samples are analysed on a Varian 3800 GC equipped with a Stabilwax- DA w/Integra-Guard column (30m, 0.32 mm ID and 0.25 micro-m df) and a Carboxen PDMS SPME fibre (75 micro-m). Each sample is preincubated for 10 min at 40°C followed by 20 min sampling with the SPME fibre in the head-space over the textile pieces. The sample is subsequently injected onto the column (injector temperature=250 °C). Column flow = 2 ml Helium/min. Column oven temperature gradient: 0 min = 40°C, 2 min = 40°C, 22 min = 240°C, 32 min = 240°C. The butyric acid is detected by FID detection and the amount of butyric acid is calculated based on a butyric acid standard curve.

The Risk Performance Odour, R, of a lipase variant is the ratio between the amount of released butyric acid from the lipase variant washed swatch and the amount of released butyric acid from a swatch washed with the lipase of SEQ ID NO: 2 with the substitutions T231R + N233R (reference enzyme), after both values have been corrected for the amount of released butyric acid from a non-lipase washed swatch. The risk (R) of the variants is calculated in accordance with the below formula:

\[
\text{Odour} = \text{measured in micro g butyric acid developed at 1 mg enzyme protein / 1}
\]
\[
\text{corrected for blank}
\]
\[
\text{Xtest enzyme} = \text{OdOUT}_{\text{test enzyme}} - \text{Blank}
\]
\[
\text{reference enzyme} = \text{OdOUT}_{\text{reference enzyme}} - \text{Blank}
\]
\[
R = \frac{\text{Xtest enzyme}}{\text{reference enzyme}}
\]

A variant is considered to exhibit reduced odor compared to the reference, if the R factor is lower than 1.

**Example 4: Activity (LU) relative to absorbance at 280nm**

The activity of a lipase relative to the absorbance at 280 nm is determined by the following assay LU/A280:

The activity of the lipase is determined as described above in the section Lipase activity.

The absorbance of the lipase at 280 nm is measured (A280) and the ratio LU/A280 is calculated. The relative LU/A280 is calculated as the LU/A280 of the variant divided by the LU/A280 of a
reference enzyme. In the context of the present invention the reference enzyme is the lipase of SEQ ID NO:2 with the substitutions T231R + N233R.

**Example 5: BR - Benefit Risk**

The Benefit Risk factor describing the performance compared to the reduced risk for odour smell is thus defined as: \( BR = \frac{RP_{avg}}{R} \).

A variant is considered to exhibit improved wash performance and reduced odor, if the BR factor is higher than 1.

Applying the above methods the following results are obtained:

<table>
<thead>
<tr>
<th>Variant</th>
<th>Mutations in SEQ ID NO: 2</th>
<th>Average RP (RP_avg)</th>
<th>BR</th>
<th>LU/A280</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I202G + T231R + N233R</td>
<td>0.84</td>
<td>1.41</td>
<td>not determined</td>
</tr>
<tr>
<td>2</td>
<td>I86V + L227G + T231R + N233R + P256K</td>
<td>1.08</td>
<td>1.52</td>
<td>1700</td>
</tr>
<tr>
<td>3</td>
<td>Q4V + S58N + V60S + T231R + N233R</td>
<td>0.87</td>
<td>1.73</td>
<td>1950</td>
</tr>
<tr>
<td>4</td>
<td>S58N + V60S + I90R + T231R + N233R</td>
<td>1.06</td>
<td>1.27</td>
<td>2250</td>
</tr>
<tr>
<td>5</td>
<td>I255Y + T231R + N233R</td>
<td>1.19</td>
<td>1.17</td>
<td>3600</td>
</tr>
<tr>
<td>6</td>
<td>I90A + T231R + N233R + I255V</td>
<td>1.13</td>
<td>1.14</td>
<td>2700</td>
</tr>
<tr>
<td>Reference</td>
<td>T231R + N233R</td>
<td>1.00</td>
<td>1.00</td>
<td>3650</td>
</tr>
</tbody>
</table>
Table 4

The reference lipase and variants 7 and 8 in Table 4 are described in WO 2000/060063.

Example 6
5 BR - Benefit Risk

The Benefit Risk was measured for the variants listed in Table 5. The Benefit Risk factor was measured in the same way as described in Example 5 and it was found to be above 1 for all the listed variants.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Mutations in SEQ ID NO: 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>T231R + N233R</td>
</tr>
<tr>
<td>9</td>
<td>L97V+ T231R+N233R</td>
</tr>
<tr>
<td>10</td>
<td>A150G+T231R+N233R</td>
</tr>
<tr>
<td>11</td>
<td>I90R+T231R+N233R</td>
</tr>
<tr>
<td>12</td>
<td>I202V+T231R+N233R</td>
</tr>
<tr>
<td>13</td>
<td>L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>14</td>
<td>I90A+ T231R+ N233R</td>
</tr>
<tr>
<td>15</td>
<td>T231R+N233R+ I255P</td>
</tr>
<tr>
<td>16</td>
<td>I90V+I255V+T231R+N233R</td>
</tr>
<tr>
<td>17</td>
<td>F211L+ L227G+ T231R+ N233R+ I255L+ P256K</td>
</tr>
<tr>
<td>18</td>
<td>S58N+ V60S+ T231R+ N233R+ Q249L</td>
</tr>
<tr>
<td>19</td>
<td>S58N+ V60S+ T231R+ N233R+ Q249I</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>20</td>
<td>A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>21</td>
<td>K46L+ S58N+ V60S+ T231R+ N233R+ Q249L+ D254I</td>
</tr>
<tr>
<td>22</td>
<td>Q4L+ E43T+ K46I+ S58N+ V60S+ T231R+ N233R+ Q249L+ D254I</td>
</tr>
<tr>
<td>23</td>
<td>Q4L+ S58N+ V60S+ T231R+ N233R+ Q249L+ D254I</td>
</tr>
<tr>
<td>24</td>
<td>K46I+ S58N+ V60S+ T231R+ N233R+ Q249L+ D254L</td>
</tr>
<tr>
<td>25</td>
<td>K46L+ S58N+ V60S+ K223I+ T231R+ N233R+ D254I</td>
</tr>
<tr>
<td>26</td>
<td>E43T+ K46I+ S58N+ V60S+ T231R+ N233R+ Q249L+ D254I</td>
</tr>
<tr>
<td>27</td>
<td>S58N+ V60S+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>29</td>
<td>S58A+ V60A+ 186V+ T231R+ N233R</td>
</tr>
<tr>
<td>30</td>
<td>K24R+ K46R+ S58N+ V60S+ K74R+ 186V+ K98R+ K127R+ D137K+ K223R+ T231R+ N233R</td>
</tr>
<tr>
<td>31</td>
<td>S58A+ V60A+ 186V+ A150G+ T231R+ N233R</td>
</tr>
<tr>
<td>32</td>
<td>S58N+ V60V+ D62G+ T231R+ N233R</td>
</tr>
<tr>
<td>33</td>
<td>Q4V+ S58N+ V60S+ 186V+ T231R+ N233R+ Q249L</td>
</tr>
<tr>
<td>34</td>
<td>Q4V+ S58N+ V60S+ 186V+ A150G+ T231R+ N233R+ I255V</td>
</tr>
<tr>
<td>35</td>
<td>Q4V+ S58N+ V60S+ 190A+ A150G+ T231R+ N233R+ I255V</td>
</tr>
<tr>
<td>36</td>
<td>Y53A+ S58N+ V60S+ T231R+ N233R+ P256L</td>
</tr>
<tr>
<td>37</td>
<td>I202L+ T231R+ N233R+ I255A</td>
</tr>
<tr>
<td>38</td>
<td>S58A+ V60S+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>39</td>
<td>D27R+ S58N+ V60S+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>40</td>
<td>V60K+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>41</td>
<td>Q4V+ S58A+ V60S+ S83T+ 186V+ A150G+ E210K+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>42</td>
<td>Q4V+ V60K+ S83T+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>43</td>
<td>D27R+ V60K+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>44</td>
<td>Q4N+ L6S+ S58N+ V60S+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>45</td>
<td>E1N+ V60K+ 186V+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>46</td>
<td>V60K+ 186V+ A150G+ K223N+ G225S+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>47</td>
<td>E210V+ T231R+ N233R+ Q249R</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>48</td>
<td>S58N+ V60S+ E210V+ T231R+ N233R+ Q249R</td>
</tr>
<tr>
<td>49</td>
<td>Q4V+ V60K+ I90R+ T231R+ N233R+ I255V</td>
</tr>
<tr>
<td>50</td>
<td>Q4V+ V60K+ A150G+ T231R+ N233R</td>
</tr>
<tr>
<td>51</td>
<td>V60K+ S83T+ T231R+ N233R</td>
</tr>
<tr>
<td>52</td>
<td>V60K+ A150G+ T231R+ N233R+ I255V</td>
</tr>
<tr>
<td>53</td>
<td>T231R+ N233G+ D234G</td>
</tr>
<tr>
<td>54</td>
<td>S58N+ V60S+ I86V+ A150G+ E210K+ L227G+ T231R+ N233R+ Q249R+ P256K</td>
</tr>
<tr>
<td>55</td>
<td>S58N+ V60S+ I86V+ A150G+ E210K+ L227G+ T231R+ N233R+ I255A+ P256K</td>
</tr>
<tr>
<td>56</td>
<td>S58N+ V60S+ I86V+ A150G+ G156R+ E210K+ L227G+ T231R+ N233R+ I255A+ P256K</td>
</tr>
<tr>
<td>57</td>
<td>S58T+ V60K+ I86V+ N94K+ A150G+ E210V+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>58</td>
<td>S58T+ V60K+ I86V+ D102A+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>59</td>
<td>S58T+ V60K+ I86V+ D102A+ A150G+ E210V+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>60</td>
<td>S58T+ V60K+ S83T+ I86V+ N94K+ A150G+ E210V+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>61</td>
<td>S58A+ V60S+ I86V+ T143S+ A150G+ L227G+ T231R+ N233R+ P256K</td>
</tr>
<tr>
<td>62</td>
<td>G91S+ D96V+ D254R</td>
</tr>
<tr>
<td>63</td>
<td>V60L+ G91M+ T231W+ Q249L</td>
</tr>
<tr>
<td>64</td>
<td>T37A+ D96A+ T231R+ N233R+ Q249G</td>
</tr>
<tr>
<td>65</td>
<td>E56G+ E87D+ T231R+ N233R+ D254A</td>
</tr>
<tr>
<td>66</td>
<td>E210K+ T231R+ N233R</td>
</tr>
<tr>
<td>67</td>
<td>D27H+ E87Q+ D96N+ T231R+ N233R+ D254V</td>
</tr>
<tr>
<td>68</td>
<td>F181L+ E210V+ T231R+ N233R</td>
</tr>
<tr>
<td>69</td>
<td>D27N+ D96G+ T231R+ N233R</td>
</tr>
<tr>
<td>70</td>
<td>D96N+ T231R+ N233R</td>
</tr>
<tr>
<td>71</td>
<td>T231R+ N233H+ D234G</td>
</tr>
<tr>
<td>72</td>
<td>S58K+ V60L+ E210V+ Q249R</td>
</tr>
<tr>
<td>73</td>
<td>S58H+ V60L+ E210V+ Q249R</td>
</tr>
<tr>
<td>74</td>
<td>Q4V+ F55V+ I86V+ T231R+ N233R+ I255V</td>
</tr>
</tbody>
</table>
Table 5

<table>
<thead>
<tr>
<th>75</th>
<th>Q4V+ S58T+ V60K+ T199L+ N200A+ E210K+ T231R+ N233R+ I255A+ P256K</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>Q4V+ D27N+ V60K+ T231R+ N233R</td>
</tr>
<tr>
<td>77</td>
<td>I90F+ I202P+ T231R+ N233R+ I255L</td>
</tr>
<tr>
<td>78</td>
<td>S58N+ V60S+ D158N+ T231R+ N233R</td>
</tr>
<tr>
<td>79</td>
<td>S58N+ V60S+ S115K+ T231R+ N233R</td>
</tr>
<tr>
<td>80</td>
<td>S58N+ V60S+ L147M+ A150G+ F211L+ T231R+ N233R</td>
</tr>
<tr>
<td>81</td>
<td>V60K+ A150G+ T231R+ N233R</td>
</tr>
<tr>
<td>82</td>
<td>I90V+L227G+T231R+N233R+P256K</td>
</tr>
<tr>
<td>83</td>
<td>T231R+N233R+I255S</td>
</tr>
<tr>
<td>84</td>
<td>I86G+T231R+N233R</td>
</tr>
<tr>
<td>85</td>
<td>V60K+ I202V+ E210K+ T231R+ N233R+ I255A+ P256K</td>
</tr>
<tr>
<td>86</td>
<td>I90G+ I202L+ T231R+ N233R+ I255S</td>
</tr>
<tr>
<td>87</td>
<td>S58G+ V60G+ T231R+ N233R</td>
</tr>
</tbody>
</table>

The reference lipase is described in WO 2000/060063.

5 BLEACH CATALYST EXAMPLES

Example 6: Preparation of Sulphuric acid mono-r2-f3.4-dihydro-iso(quinolin-2-yl)-l-f2-ethyloxyloxymethyl-D-ethyl] ester, internal salt

Preparation of 2-ethylhexyl glycidyl ether: To a flame dried, 500 mL round bottomed flask equipped with an addition funnel charged with epichlorohydrin (15.62 g, 0.17 moles), is added 2-ethylhexanol (16.5 g, 0.127 moles) and stannic chloride (0.20 g, 0.001 moles). The reaction is kept under an argon atmosphere and warmed to 90°C using an oil bath. Epichlorohydrin is dripped into the stirring solution over 60 minutes followed by stirring at 90°C for 18 hours. The reaction is fitted with a vacuum distillation head and 1-chloro-3-(2-ethyl-hexyloxy)-propan-2-ol is distilled under 0.2mm Hg. The 1-chloro-3-(2-ethyl-hexyloxy)-propan-2-ol (4.46 g, 0.020 moles) is dissolved in tetrahydrofuran (50 mL) and stirred at room temperature under an argon atmosphere. To the stirring solution is added potassium tert-butoxide (2.52 g, 0.022 moles) and the suspension is stirred at room temperature for 18 hours. The reaction is then evaporated to dryness, residue dissolved in hexanes and washed with water (100 mL). The hexanes phase is
separated, dried with Na2SC>4, filtered and evaporated to dryness to yield the crude 2-ethylhexyl glycidyl ether, which can be further purified by vacuum distillation.

Preparation of Sulphuric acid mono-[2-(3,4-dihydro-isoquin-2-yl)-l-(2-ethylhexyloxyethyl)-ethyl] ester, internal salt: To a flame dried 250 mL three neck round bottomed flask, equipped with a condenser, dry argon inlet, magnetic stir bar, thermometer, and heating bath is added 3,4-dihydroisoquinoline (0.40 mol.; prepared as described in Example 1 of U.S. 5,576,282), 2-ethylhexyl glycidyl ether (0.38 mol, prepared as described above), SO3-DMF complex (0.38 mol), and acetonitrile (500 mL). The reaction is warmed to 80°C and stirred at temperature for 72 hours. The reaction is cooled to room temperature, evaporated to dryness and the residue recrystallized from ethyl acetate and/or ethanol to yield the desired product. The solvent acetonitrile may be replaced with other solvents, including but not limited to, 1,2-dichloroethane.

Example 7: Preparation of Sulphuric acid mono-[2-(3,4-dihydro-isoquin-2-yl)-l-(2-butyl-octyloxyethyl)-ethyl] ester, internal salt

The desired product is prepared according to Example 1 but substituting 2-butyloctanol for 2-hexyloctanol.

COMPOSITION EXAMPLE

The lipase incorporated in the compositions below is the lipase variant 1 to 5 described in example 5 Table 4, and combinations thereof.

Example 8: Laundry detergent compositions

The following laundry detergent compositions A, B, C and D are suitable for use in the present invention. Typically, these compositions are dosed into water at a concentration of from 80g/l to 120g/l during the laundering process.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach catalyst made according to example 6 or 7</td>
<td>0.1 wt%</td>
<td>0.05 wt%</td>
<td>0.03 wt%</td>
<td>0.05 wt%</td>
</tr>
<tr>
<td>Lipase (9mg/g active)</td>
<td>0.15 wt%</td>
<td>0.2 wt%</td>
<td>0.3 wt%</td>
<td>0.2 wt%</td>
</tr>
<tr>
<td>Material</td>
<td>9.0wt%</td>
<td>8.0wt%</td>
<td>7.5wt%</td>
<td>7.0wt%</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Sodium linear C&lt;sub&gt;12-13&lt;/sub&gt; alkyl benzenesulphonate (LAS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tallow alkyl sulphate (TAS)</td>
<td>1.0wt%</td>
<td>1.0wt%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;14-15&lt;/sub&gt; alkyl ethoxylated alcohol having an average degree of ethoxylation of 7 (AE7)</td>
<td>2.5wt%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;14-15&lt;/sub&gt; alkyl ethoxylated alcohol sulphate having an average degree of ethoxylation of 3 (AE&lt;sub&gt;3&lt;/sub&gt;S)</td>
<td></td>
<td>4wt%</td>
<td>3.0wt%</td>
<td>2.5wt%</td>
</tr>
<tr>
<td>Mono-C&lt;sub&gt;12-14&lt;/sub&gt; alkyl monohydroxyethyl di-methyl quaternary ammonium chloride</td>
<td>1.5wt%</td>
<td>1.0wt%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zeolite 4A</td>
<td>15wt%</td>
<td>12.5wt%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric Acid</td>
<td>3.0wt%</td>
<td>2.0wt%</td>
<td>3.0wt%</td>
<td>3.0wt%</td>
</tr>
<tr>
<td>Sodium Percarbonate</td>
<td>20wt%</td>
<td>15wt%</td>
<td>17.5wt%</td>
<td>14wt%</td>
</tr>
<tr>
<td>TAED (tetraacetylene diaminomine)</td>
<td>2.5wt%</td>
<td>3wt%</td>
<td>2.3wt%</td>
<td>1.6wt%</td>
</tr>
<tr>
<td>NOBS (nonanoyloxybenzene sulphonate)</td>
<td>0.0%</td>
<td>1.0wt%</td>
<td>0.0wt%</td>
<td>1.5wt%</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>20wt%</td>
<td>25wt%</td>
<td>20wt%</td>
<td>25wt%</td>
</tr>
<tr>
<td>Polymeric carboxylate</td>
<td>2.0wt%</td>
<td>1.5wt%</td>
<td>3.0wt%</td>
<td>2.5wt%</td>
</tr>
<tr>
<td>A compound having the following general structure:</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
<td>0.75wt%</td>
<td>1.0wt%</td>
</tr>
<tr>
<td>bis((C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;O)(C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;O)&lt;sub&gt;n&lt;/sub&gt;)(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;-C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;x-N&lt;sup&gt;+&lt;/sup&gt;-(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;-&lt;/sup&gt;-bis((C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;O)(C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;O)&lt;sub&gt;n&lt;/sub&gt;)&lt;sub&gt;x&lt;/sub&gt;, wherein n = from 20 to 30, and x = from 3 to 8, or sulphated or sulphonated variants thereof</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td></td>
<td></td>
<td>1.5wt%</td>
<td>1.0wt%</td>
</tr>
<tr>
<td>Other enzymes</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
<td>0.75wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Ethylene diamine disuccinic acid</td>
<td>0.5wt%</td>
<td>0.1wt%</td>
<td>0.2wt%</td>
<td>0.25wt%</td>
</tr>
</tbody>
</table>
The following laundry detergent compositions E, F, G, and H are suitable for use in the present invention. Typically, these compositions are dosed into water at a concentration of from 80g/l to 120g/l during the laundering process.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulphate</td>
<td>0.75wt%</td>
<td>0.5wt%</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Hydroxyethane di(methylene phosphonic acid)</td>
<td>0.5wt%</td>
<td>0.25wt%</td>
<td>0.2wt%</td>
<td>0.4wt%</td>
</tr>
<tr>
<td>Fluorescent whitening agent</td>
<td>0.2wt%</td>
<td>0.1wt%</td>
<td>0.15wt%</td>
<td>0.25wt%</td>
</tr>
<tr>
<td>Silicone suds suppressing agent</td>
<td>0.1wt%</td>
<td>0.05wt%</td>
<td>0.1wt%</td>
<td>0.1wt%</td>
</tr>
<tr>
<td>Soap</td>
<td>0.5wt%</td>
<td>0.25wt%</td>
<td>0.0wt%</td>
<td>0.3wt%</td>
</tr>
<tr>
<td>Photobleach</td>
<td>0.01wt%</td>
<td>0.0001wt%</td>
<td>0.0005wt%</td>
<td>0.0015wt%</td>
</tr>
<tr>
<td>Perfume</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
<td>0.75wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>13wt%</td>
<td>15wt%</td>
<td>30wt%</td>
<td>30wt%</td>
</tr>
<tr>
<td>Water and miscellaneous</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
</tr>
</tbody>
</table>

The following laundry detergent compositions E, F, G, and H are suitable for use in the present invention. Typically, these compositions are dosed into water at a concentration of from 80g/l to 120g/l during the laundering process.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach catalyst made according to example 6 or 7</td>
<td></td>
<td></td>
<td>0.01wt%</td>
<td>0.05wt%</td>
</tr>
<tr>
<td>Diacyl peroxide</td>
<td>2 wt%</td>
<td>1wt%</td>
<td>0.5wt%</td>
<td>1wt%</td>
</tr>
<tr>
<td>Lipase (9mg/g active enzyme)</td>
<td>0.5wt%</td>
<td>0.3wt%</td>
<td>0.2wt%</td>
<td>0.1wt%</td>
</tr>
<tr>
<td>Sodium linear C_{12-13} alkyl benzenesulphonate (LAS)</td>
<td>8.0wt%</td>
<td>5.0wt%</td>
<td>7.5wt%</td>
<td>7.0wt%</td>
</tr>
<tr>
<td>C_{14-15} alkyl ethoxylated alcohol sulphate having an average degree of ethoxylation of 3 (AE_{3}S)</td>
<td>5.0wt%</td>
<td>2.5wt%</td>
<td>3.5wt%</td>
<td>6.0wt%</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>3.0wt%</td>
<td>2.0wt%</td>
<td>5.0wt%</td>
<td>2.5wt%</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>20wt%</td>
<td>25wt%</td>
<td>22.5wt%</td>
<td>25wt%</td>
</tr>
<tr>
<td>Polymeric carboxylate</td>
<td>2.0wt%</td>
<td>3.5wt%</td>
<td>3.5wt%</td>
<td>2.5wt%</td>
</tr>
<tr>
<td>A compound having the following general structure:</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
<td>0.75wt%</td>
<td>1.0wt%</td>
</tr>
</tbody>
</table>
The following laundry detergent compositions I, J, K and L are suitable for use in the present invention. Typically, these compositions are dosed into water at a concentration of from 20g/l to 60g/l during the laundering process.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Percarbonate</td>
<td>0wt%</td>
<td>15wt%</td>
<td>17.5wt%</td>
<td>14wt%</td>
</tr>
<tr>
<td>TAED (tetraacetylene diamine)</td>
<td>0wt%</td>
<td>3wt%</td>
<td>2.3wt%</td>
<td>1.6wt%</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>0.5wt%</td>
<td>1.0wt%</td>
<td>1.5wt%</td>
<td>1.0wt%</td>
</tr>
<tr>
<td>Other Enzymes</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
<td>0.2wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Ethylene diamine disuccinic acid</td>
<td>0.05wt%</td>
<td>0.1wt%</td>
<td>0.2wt%</td>
<td>0.15wt%</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.35wt%</td>
<td>0.1wt%</td>
<td>1.0wt%</td>
<td>0.25wt%</td>
</tr>
<tr>
<td>Hydroxyethane di(methylene phosphonic acid)</td>
<td>0.1wt%</td>
<td>0.25wt%</td>
<td>0.2wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Fluorescent whitening agent</td>
<td>0.2wt%</td>
<td>0.1wt%</td>
<td>0.15wt%</td>
<td>0.25wt%</td>
</tr>
<tr>
<td>Silicone suds suppressing agent</td>
<td>0.1wt%</td>
<td>0.05wt%</td>
<td>0.1wt%</td>
<td>0.2wt%</td>
</tr>
<tr>
<td>Soap</td>
<td>0.5wt%</td>
<td>0.25wt%</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Photobleach</td>
<td>0.01wt%</td>
<td>0.0001wt%</td>
<td>0.0005wt%</td>
<td>0.0015wt%</td>
</tr>
<tr>
<td>Perfume</td>
<td>1.0wt%</td>
<td>0.5wt%</td>
<td>0.75wt%</td>
<td>0.5wt%</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>45wt%</td>
<td>30wt%</td>
<td>20wt%</td>
<td>22wt%</td>
</tr>
<tr>
<td>Water and miscellaneous</td>
<td>to 100wt%</td>
<td>To 100wt%</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
</tr>
<tr>
<td>Ingredient</td>
<td>I</td>
<td>J</td>
<td>K</td>
<td>L</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Bleach catalyst made according to example 6 or 7</td>
<td>0.15 wt%</td>
<td>0.10 wt%</td>
<td>0.1 wt%</td>
<td>0.15 wt%</td>
</tr>
<tr>
<td>Diacyl peroxide</td>
<td>1 wt%</td>
<td></td>
<td>0.5 wt%</td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td>0.5 wt%</td>
<td>0.3 wt%</td>
<td>0.1 wt%</td>
<td>0.2 wt%</td>
</tr>
<tr>
<td>Sodium linear C_{12-13} alkyl benzenesulphonate (LAS)</td>
<td>15 wt%</td>
<td>17.5 wt%</td>
<td>20 wt%</td>
<td>10.0 wt%</td>
</tr>
<tr>
<td>C_{14-15} alkyl ethoxylated alcohol sulphate having an average degree of ethoxylation of 3 (AE_{3/5})</td>
<td>7.0 wt%</td>
<td>7.5 wt%</td>
<td>5.0 wt%</td>
<td>5.0 wt%</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>7.0 wt%</td>
<td>5.0 wt%</td>
<td>7.5 wt%</td>
<td>3.0 wt%</td>
</tr>
<tr>
<td>Sodium Percarbonate</td>
<td>20 wt%</td>
<td>15 wt%</td>
<td>0 wt%</td>
<td>14 wt%</td>
</tr>
<tr>
<td>TAED (tetraacetylenediethylenediamine)</td>
<td>2.5 wt%</td>
<td>3 wt%</td>
<td>0 wt%</td>
<td>1.6 wt%</td>
</tr>
<tr>
<td>NOBS (nonanoyloxybenzene sulphonate)</td>
<td>0.0 wt%</td>
<td>2.0 wt%</td>
<td>0.0 wt%</td>
<td>0 wt%</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>22.5 wt%</td>
<td>25 wt%</td>
<td>20 wt%</td>
<td>10 wt%</td>
</tr>
<tr>
<td>Polymeric carboxylate</td>
<td>7.0 wt%</td>
<td>7.5 wt%</td>
<td>5.0 wt%</td>
<td>3.0 wt%</td>
</tr>
<tr>
<td>A compound having the following general structure:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bis((C_{2}H_{5}O)(C_{2}H_{4}O)n)(CH_{3})-N^{+}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{6}H_{2x}-N^{+}-(CH_{3})-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bis((C_{2}H_{5}O)(C_{2}H_{4}O)n)_{x} wherein n = from 20 to 30, and x = from 3 to 8, or sulphated or sulphonated variants thereof</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>2.5 wt%</td>
<td>3.0 wt%</td>
<td>1.5 wt%</td>
<td>1.0 wt%</td>
</tr>
<tr>
<td>Other Enzymes</td>
<td>2.5 wt%</td>
<td>1.5 wt%</td>
<td>3.0 wt%</td>
<td>0.75 wt%</td>
</tr>
<tr>
<td>Ethylene diamine disuccinic acid</td>
<td>0.25 wt%</td>
<td>0.1 wt%</td>
<td>0.5 wt%</td>
<td>0.15 wt%</td>
</tr>
<tr>
<td>Hydroxyethane di(methylene phosphonic acid)</td>
<td>0.5 wt%</td>
<td>0.75 wt%</td>
<td>0.25 wt%</td>
<td>0.2 wt%</td>
</tr>
</tbody>
</table>
Bleaching detergent compositions having the form of granular laundry detergents are exemplified by the following formulations. Any of the below compositions is used to launder fabrics at a concentration of 600 - 10000 ppm in water, with typical median conditions of 2500 ppm, 25°C, and a 25:1 water:cloth ratio. The typical pH is about 10 but can be adjusted by altering the proportion of acid to Na-salt form of alkylbenzenesulfonate.

<table>
<thead>
<tr>
<th>Fluorescent whitening agent</th>
<th>0.5wt%</th>
<th>0.75wt%</th>
<th>0.25wt%</th>
<th>0.15wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicone suds suppressing agent</td>
<td>0.05wt%</td>
<td>0.10wt%</td>
<td>0.02wt%</td>
<td>0.02wt%</td>
</tr>
<tr>
<td>Photobleach</td>
<td>0.025wt%</td>
<td>0.050wt%</td>
<td>0.02wt%</td>
<td>0.0015wt %</td>
</tr>
<tr>
<td>Water, filler (including sodium sulphate) and miscellaneous</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
<td>to 100wt%</td>
</tr>
</tbody>
</table>

Bleaching detergent compositions having the form of granular laundry detergents are exemplified by the following formulations. Any of the below compositions is used to launder fabrics at a concentration of 600 - 10000 ppm in water, with typical median conditions of 2500 ppm, 25°C, and a 25:1 water:cloth ratio. The typical pH is about 10 but can be adjusted by altering the proportion of acid to Na-salt form of alkylbenzenesulfonate.
While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention. All documents cited are, in relevant part, incorporated herein by reference, the citation of any document is not to be construed as an admission that it is prior art with respect to the present invention.

<table>
<thead>
<tr>
<th>Diethylenetriaminepentacetic acid</th>
<th>0.6</th>
<th>0.3</th>
<th>0.6</th>
<th>0.25</th>
<th>0.6</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Percarbonate</td>
<td>0.0</td>
<td>5.2</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Photobleach</td>
<td>0.003</td>
<td>0.0015</td>
<td>0.0015</td>
<td>0.0020</td>
<td>0.0045</td>
<td>0.0010</td>
</tr>
<tr>
<td>Sodium Perborate Monohydrate</td>
<td>4.4</td>
<td>0.0</td>
<td>3.85</td>
<td>2.09</td>
<td>0.78</td>
<td>3.63</td>
</tr>
<tr>
<td>NOBS</td>
<td>1.9</td>
<td>0.0</td>
<td>1.66</td>
<td>0.0</td>
<td>0.33</td>
<td>0.75</td>
</tr>
<tr>
<td>TAED</td>
<td>0.58</td>
<td>1.2</td>
<td>0.51</td>
<td>0.0</td>
<td>0.015</td>
<td>0.28</td>
</tr>
<tr>
<td>Organic Catalyst **</td>
<td>0.0185</td>
<td>0.0185</td>
<td>0.0162</td>
<td>0</td>
<td>0.0111</td>
<td>0.0074</td>
</tr>
<tr>
<td>Diacyl peroxide ***</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sulfate/Moisture</td>
<td>Balance to 100%</td>
<td>Balance to 100%</td>
<td>Balance to 100%</td>
<td>Balance to 100%</td>
<td>Balance to 100%</td>
<td>Balance to 100%</td>
</tr>
</tbody>
</table>

* Lipase variant 1 to 5 described in example 5 Table 4, and combinations thereof.

** Organic catalyst prepared according to Examples 6 or 7 or mixtures thereof.

*** Diacyl peroxide is preferably dinonanoylperoxide.
What is claimed is:

1. A composition comprising:
   a) a variant of a parent lipase, said variant, when compared to said parent, comprising a total of at least three substitutions, said substitutions being selected from one or more of the following groups of substitutions:
      (i) at least two substitutions in Region I,
      (ii) at least one substitution in Region II,
      (iii) at least one substitution in Region III, and/or
      (iv) at least one substitution in Region IV; and
   b) a bleach catalyst that is capable of accepting an oxygen atom from a peroxycacid and transferring the oxygen atom to an oxidizeable substrate.

2. A detergent composition according to Claim 1, wherein said substitutions in Region I comprise substitutions in the positions corresponding to the positions 231 and 233.

3. A detergent composition according to Claim 2, wherein said substitutions at positions 231 and 233 are substituted with an R.

4. A detergent composition according to Claim 2, wherein said variant comprises a substitution in the position corresponding to position 4 of SEQ ID NO:2.

5. A detergent composition according to Claim 4, wherein said variant corresponding to position 4 of SEQ ID NO:2 is V.

6. A detergent composition according to Claim 6, wherein said variant comprises a substitution in the position corresponding to position 227 of SEQ ID NO:2.

7. A detergent composition according to Claim 6, wherein said variant corresponding to position 227 of SEQ ID NO:2 is G.
8. A detergent composition according to Claim 1, wherein said at least one substitution in Region II comprises a substitution selected from the group consisting of substitutions in positions corresponding to the positions 202, 211, 255 and 256.

9. A detergent composition according to Claim 8, wherein said at least one substitution in Region II comprises a substitution selected from the group consisting of X202G, X21 IL, X255Y/V and X256K.

10. A detergent composition according to Claim 1, wherein said at least one substitution in Region II comprises a substitution in the position corresponding to the position 210.

11. A detergent composition according to Claim 10, wherein said substitution corresponding to position 210 comprises X210K.

12. A detergent composition according to Claim 1, wherein said at least one substitution in Region III comprises a substitution selected from the group consisting of substitutions in positions corresponding to the positions 86 and 90.

13. A detergent composition according to Claim 12, wherein said at least one substitution in Region III comprises a substitution selected from the group consisting of X86V and X90A/R.

14. A detergent composition according to Claim 1, wherein said at least one substitution in Region III comprises a substitution in the position corresponding to the position 83.

15. A detergent composition according to Claim 14, wherein said substitution corresponding to position 83 comprises X83T.

16. A detergent composition according to Claim 1, wherein said at least one substitution in Region IV comprises a substitution selected from the group consisting of substitutions in positions corresponding to the positions 27, 58 and 60.
17. A detergent composition according to Claim 16, wherein said at least one substitution in Region IV comprises a substitution selected from the group consisting of X27R, X58N/A/G/P/T and X60S/V/G/N/R/K/A/L.

18. A detergent composition according to Claim 1, comprising at least two substitutions in Region IV corresponding to the positions 27, 58 and 60.

19. A detergent composition according to Claim 18, composing at least two substitutions in Region IV selected from the group consisting of X27R, X58N/A/G/P/T and X60S/V/G/N/R/K/A/L.

20. A detergent composition according to Claim 1, wherein said variant comprises at least one substitution outside the defined Regions I to IV.

21. A detergent composition according to Claim 20, wherein said at least one substitution outside the defined Regions I to IV is selected from the group consisting of substitutions in positions corresponding to position 81, 147, 150 and 249.

22. A detergent composition according to Claim 20, wherein said at least one substitution outside the defined Regions I to IV is selected from the group consisting of X81Q/E, X147M/Y, X150G and X249R/I/L.

23. A detergent composition according to Claim 2, wherein said parent lipase is at least 90% identical to SEQ ID NO:2.

24. A detergent composition according to Claim 1, wherein the parent lipase is identical to SEQ ID NO: 2 and said variant comprises one of the following groups of substitutions:

   a) T231R + N233R + I255Y
   b) I202G + T231R + N233R
   c) I86V + L227G + T231R +N233R+ P256K
   d) Q4V + S58N + V60S + T231R +N233R
   e) S58N + V60S + I90R + T231R + N233R
   f) I90A + T231R + N233R + I255Y
   g) S58N + V60S + I86V + A150G + L227G + T231R +N233R+ P256K
   h) S58N + V60S + L147M + F21 1L + T231R + N233R
25. A detergent composition according to Claim 1, wherein the parent lipase is identical to SEQ ID NO: 2 and said variant comprises one of the following groups of substitutions:
   a) Q4V + S58A + V60S + S83T + I86V + A150G + E210K + L227G + T231R + N233R + P256K
   b) S58N + V60S + I86V + A150G + L227G + T231R + N233R + P256K.

26. A detergent composition according to Claim 1, wherein the lipase variant is characterized in that the Benefit Risk, when measured as given in the specification, is larger than 1.

27. A detergent composition comprising:
   a) polypeptide having lipase activity and which further has a Average Relative Performance of at least 0.8 and a Benefit Risk of at least 1.1 at the test conditions given in the specification; and
   b) a bleach catalyst that is capable of accepting an oxygen atom from a per oxyacid and transferring the oxygen atom to an oxidizeable substrate.

28. A composition according to Claim 27, wherein the bleach catalyst comprises a moiety selected from the group consisting of iminium cations and poly ions; iminium zwitterions; modified amines; modified amine oxides; N-sulphonyl imines; N-phosphoryl imines; N-acyl imines; thiadiazole dioxides; perfluoroimines; cyclic sugar ketones and mixtures thereof.

29. A composition according to Claim 27, wherein the bleach catalyst comprises an iminium and/or a carbonyl functional group.

30. A composition according to Claim 1, wherein the bleach catalyst comprises an oxaziridinium and/or a dioxirane functional group, and/or is capable of forming an oxaziridinium and/or a dioxirane functional group upon acceptance of an oxygen atom.

31. A composition according to Claim 1, wherein the bleach catalyst has a chemical structure corresponding to the chemical formula:
wherein: n and m are independently from 0 to 4; each R1 is independently selected from a substituted or unsubstituted radical selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, fused aryl, heterocyclic ring, fused heterocyclic ring, nitro, halo, cyano, sulphonato, alkoxy, keto, carboxylic, and carboalkoxy radicals, and any two vicinal R1 substituents may combine to form a fused aryl, fused carboxycyclic or fused heterocyclic ring; each R2 is independently selected from a substituted or unsubstituted radical independently selected from the group consisting of hydrogen, hydroxy, alkyl, cycloalkyl, alkaryl, aryl, aralkyl, alkenes, heterocyclic ring, alkoxy, arylcarbonyl groups, carboxyalkyl groups and amide groups; any R2 may be joined together with any other of R2 to form part of a common ring; any geminal R2 may combine to form a carbonyl; and wherein any two R2 may combine to form a substituted or unsubstituted fused unsaturated moiety; R3 is a C1 to C20 substituted or unsubstituted alkalyl; R4 is hydrogen or the moiety Qt-A, wherein: Q is a branched or unbranched alkyne, t = 0 or 1, and A is an anionic group selected from the group consisting of OSO$_3^-$, SO$_3^-$, CO$_2^-$, OCO$_2^-$, OPO$_3$$^{2-}$, OPO$_3$$^H^+$ and OPO$_2^-$; R5 is hydrogen or the moiety \( \text{CR}^4\text{R}^{12}_1\text{Y}_{G_{b}}\text{Y}_{c}^{-}\{(\text{CR}^9\text{R}^{10})_{2}\text{O})_{k}\}^{-}\text{R}^8 \), wherein: each Y is independently selected from the group consisting of O, S, N-H, or N-R$_8$; and each R$_8$ is independently selected from the group consisting of alkyl, aryl and heteroaryl, said moieties being substituted or unsubstituted, and whether substituted or unsubstituted said moieties having less than 21 carbons; each G is independently selected from the group consisting of CO, SO$_2$, SO, PO and PO$_2$; R$^9$ and R$^{10}$ are independently selected from the group consisting of hydrogen and C$_1$-C$_4$ alkyl; R$^1$ and R$^{12}$ are independently selected from the group consisting of hydrogen and alkyl, or when taken together may join to form a carbonyl; b = Oor 1; c can = Oor 1, but c must =

\[
\text{Oif } b = a; y \text{ is an integer of from 1 to 6; } k \text{ is an integer of from Oto 20; } R^6 \text{ is H, or }
\]
an alkyl, aryl or heteroaryl moiety; said moieties being substituted or unsubstituted; and X, if present, is a suitable charge balancing counterion.

32. A composition according to Claim 1, wherein the bleach catalyst has a chemical structure corresponding to the chemical formula:

\[
\begin{align*}
\text{wherein } R^{13} & \text{ is a branched alkyl group containing from 3 to 24 carbons, or a linear alkyl group containing from 1 to 24 carbons.}
\end{align*}
\]

33. A composition according to Claim 1, wherein the bleach catalyst has a chemical structure corresponding to the chemical formula:

\[
\begin{align*}
\text{wherein } R^{13} & \text{ is selected from the group consisting of 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, iso-tridecyl and iso-pentadecyl.}
\end{align*}
\]

34. A composition according to Claim 1, wherein the composition comprises less than 5%, by weight of the composition, of a source of peroxxygen.

35. A composition according to Claim 1, wherein the composition comprises from 5% to 10%, by weight of the composition, of a source of carbonate anion.
36. A composition according to Claim 1, wherein the composition comprises a dye transfer inhibitor.

37. A composition according to Claim 1, wherein the composition comprises:
   a) less than 5%, by weight of the composition, of zeolite builder;
   b) optionally, less than 5%, by weight of the composition, of phosphate builder; and
   c) optionally, less than 5%, by weight of the composition, of silicate salt.

38. A composition according to Claim 1, wherein the composition comprises a diacyl and/or a tetraacyl peroxide species.

39. A composition according to Claim 1, wherein the composition comprises an oxybenzene sulphonate bleach activator and a source of peroxygen.

40. A composition according to Claim 1, wherein the composition comprises a pre-formed peroxyacid.

41. A composition comprising:
   a) lipase; said lipase comprising a variant of a parent lipase, said variant, when compared to said parent, comprising a total of at least three substitutions, said substitutions being selected from one or more of the following groups of substitutions:
      (i) at least two substitutions in Region I,
      (ii) at least one substitution in Region II,
      (iii) at least one substitution in Region III, and/or
      (iv) at least one substitution in Region IV; and
   b) a diacyl and/or tetraacyl peroxide species.

42. A composition according to Claim 41, wherein the composition comprises a bleach catalyst that is capable of accepting an oxygen atom from a peroxyacid and transferring the oxygen atom to an oxidizable substrate.
43. A composition according to Claim 41, wherein: said lipase comprises a variant of a parent lipase, said variant, when compared to said parent, comprising a total of at least three substitutions, said substitutions being selected from one or more of the following groups of substitutions:
   a) at least two substitutions in Region I,
   b) at least one substitution in Region II,
   c) at least one substitution in Region III, and/or
   d) at least one substitution in Region IV.

44. A composition according to Claim 41, wherein the parent lipase is identical to SEQ ID NO: 2 and said variant comprises one of the following groups of substitutions:
   a) T231R + N233R + I255Y
   b) I202G + T231R + N233R
   c) I86V + L227G + T231R + N233R + P256K
   d) Q4V + S58N + V60S + T231R + N233R
   e) S58N + V60S + I90R + T231R + N233R
   f) I90A + T231R + N233R + I255V
   g) S58N + V60S + I86V + A150G + L227G + T231R + N233R + P256K
   h) S58N + V60S + L147M + F211L + T231R + N233R
   i) Q4V + S58A + V60S + S83T + I86V + A150G + E210K + L227G + T231R + N233R + P256K
   j) S58N + V60S + I86V + A150G + L227G + T231R + N233R + P256K.

45. A composition according to Claim 41, wherein the lipase variant is a polypeptide having lipase activity and which further has a Average Relative Performance of at least 0.8 and a Benefit Risk of at least 1.1 at the test conditions given in the specification.

46. A composition according to Claim 1, wherein said lipase variant is a variant of SEQ ID NO: 2 comprising at least one of the mutations Q4V, S58N/A/G/P/T, I90R or Q249I/L.
Figure 1

11. *Aspergillus foetidus* 13
12. *Aspergillus niger* 14
13. *Aspergillus oryzea* 15
14. *Thermomyces lanuginosus* 2
15. *Landerina penispora* 16

Figure 1. Alignment of lipase sequences.
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A COMPOSITION COMPRISING A LIPASE AND A BLEACH CATALYST

10283M

PatentIn version 3.3

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Pro Arg VaI Gly Asp Pro Ala Phe Ala Asn Tyr VaI VaI Ser Thr Gly

lie Pro Tyr Arg Arg Thr val Asn Glu Arg Asp lie VaI Pro His Leu

Pro Pro Ala Ala Phe Gly Phe Leu His Ala Gly Glu Gly Tyr Trp lie

Thr Asp Asn Ser Pro Glu Thr Val Gin Val cys Thr ser Asp Leu Glu

Thr ser Asp Cys Ser Asn Ser lie val Pro Phe Thr ser val Leu Asp

His Leu ser Tyr Phe Gly lie Asn Thr Gly Leu cys Thr

<210> 6
<211> 271
<212> PRT
<213> Rhizopus oryzae

<400> 6

ser Ala Ser Asp Gly Gly Lys val val Ala Ala Thr Thr Ala Gin lie

Gin Glu Phe Thr Lys Tyr Ala Gly, ile Ala Ala Thr Ala Tyr Cys Arg
10283M sequence listing.txt

20 25 30

Ser val VaT Pro Gly Asn l_ys Trp Asp Cys Val Gin Cys Gin Lys Trp

35

val Pro Asp Gly Lys lie lie Thr Thr Phe Thr Ser Leu Leu Ser Asp

50

Thr Asn Gly Tyr val Leu Arg Ser Asp Lys Gin Lys Thr lie Tyr Leu

65 70 75 80

val phe Arg Gly Thr Asn Ser Phe Arg ser Ala lie Thr Asp lie Val

85 90

Phe Asn Phe Ser Asp Tyr Lys Pro Val Lys Gly Ala l_ys Val His Ala

100 105 110

Gly Phe Leu Ser Ser Tyr Glu Gin Val Val Asn Asp Tyr Phe Pro Val

115 120 125

Val Gin Glu Gin Leu Thr Ala His Pro Thr Tyr Lys val lie val Thr

130 135 140

Gly His ser Leu Gly Ala Gin Ala Leu Leu Ala Gly Met Asp Leu

145 150 155 160

Tyr Gin Arg Glu Pro Arg Leu Ser Pro Lys Asn Leu Ser lie Phe Thr

165 170 175

val Gly Gly Pro Arg Val Gly Asn Pro Thr Phe Ala Tyr Tyr val Glu

180 185 190

ser Thr Gly lie Pro Phe Gin Arg Thr Val His Lys Arg Asp lie val

195 200 205

Pro His val Pro Pro Gin ser Phe Gly Phe Leu His Pro Gly val Glu

210 215 220

Ser Trp lie Lys ser Gly Thr Ser Asn Val Gin lie Cys Thr Ser Glu

225 230 235 240

lie Glu Thr Lys Asp cys Ser Asn Ser lie Val Pro Phe Thr Ser lie

245 250 255

Leu Asp His Leu Ser Tyr Phe Asp lie Asn Glu Gly ser Cys Leu

260 265 270

<210> 7
<211> 267
<212> PRT
<213> Aspergillus niger
Thr Ala Gly His Ala Leu Ala Ala ser Thr Gin Gly lie ser Glu Asp
Leu Tyr Ser Arg Leu Val Glu Met Ala Thr lie ser Gin Ala Ala Tyr
Ala Asp Leu Cys Asn lie Pro Ser Thr lie He Lys Gly Glu Lys lie
Tyr Asn ser Gin Thr Asp lie 40 Asn Gly Trp lie Leu Arg Asp Asp Ser
ser Lys Glu lie lie Thr Val Phe Arg Gly Thr Gly ser Asp Thr Asn
Leu Gin Leu Asp Thr 85 Asn Tyr Thr Leu Thr 90 Pro Phe Asp Thr Leu Pro
Gln cys Asn Gly 100 Cys Glu Val His 105 Gly Tyr Tyr lie Gly Trp val
Ser val Gin 115 Asp Gin Val Glu Ser Leu val Lys Gin Gin Val ser Gin
Tyr Pro 130 Asp Tyr Ala Leu Thr val Thr Gly His Ser Leu Gly Ala ser
Leu Ala Ala Leu Thr Ala Ala Gin Leu Ser Ala Thr Tyr Asp Asn lie
Arg Leu Tyr Thr Phe Gly Glu Pro Arg ser Gly Asn Gin Ala Phe Ala
Ser Tyr Met Asn 180 Asp Ala Phe Gin Ala ser ser Pro Asp Thr Thr Gin
Tyr Phe Arg val Thr His Ala Asn Asp Gly lie Pro Asn Leu Pro Pro
Val Glu Gin Gly Tyr Ala His Gly Gly val Glu Tyr Trp Ser val Asp
pro Tyr Ser Ala Gin 230 Thr phe val cys Thr Gly Asp Glu val Gin
Val 240 Cys Cys Glu Ala 245 Gly Gly Gin Gly Val Asn Asn Ala His Thr
Tyr Phe Gly Met 260 Thr ser Gly Ala cys Thr Trp
| Thr Ala Gly His Ala Leu Ala Ala Ser Thr Gin Gly He Ser Glu Asp |
| Leu Tyr ser Arg Leu VaI Glu Met Ala Thr lie Ser Gin Ala Ala Tyr |
| Ala Asp Leu cys Asn lie pro Ser Thr lie lie Lys Gin Glu Lys lie |
| Tyr Asn Ser Gin Thr Asp lie Asn Gly Trp lie Leu Arg Asp Asp Ser |
| Ser Lys Glu lie lie Thr VaI Phe Arg Gly Thr Gin Ser Asp Thr Asn |
| Leu Gin Leu Asp Thr Asn Tyr Thr Leu Thr Pro Phe Asp Thr Leu Pro |
| Gin Cys Asn Ser cys Glu VaI His Gin Gin Tyr Tyr lie Gin Trp lie |
| Ser VaI Gin Asp Gin VaI Glu Ser Leu val Gin Gin Gin val ser Gin |
| Phe Pro Asp Tyr Ala Leu Thr val Thr Gin His ser Leu Gin Ala ser |
| Leu Ala Ala Leu Thr Ala Gin Leu ser Ala Thr Tyr Asp Asn lie |
| Arg Leu Tyr Thr Phe Gin Glu Pro Arg ser Asn Gin Ala Phe Ala Ser |
| Tyr Met Asn Asp Ala Phe Gin Ala Ser Ser Pro Asp Thr Thr Gin Tyr |
| Phe Arg Val Thr His Ala Asn Asp Gly lie Pro Asn Gin val Gin Cys |
| Tyr Ser Ala Gin Asn Thr Phe Val cys Thr Gin Gin Cys |

10283M sequence listing.txt

8
266
PRT
Aspergillus tubingensis

Leu Tyr ser Arg Leu Val Glu Met Ala Thr lie Ser Gin Ala Ala Tyr
Ala Asp Leu cys Asn lie pro Ser Thr lie lie Lys Gin Glu Lys lie
Tyr Asn Ser Gin Thr Asp lie Asn Gly Trp lie Leu Arg Asp Asp Ser
Ser Lys Glu lie lie Thr Val Phe Arg Gly Thr Gin Ser Asp Thr Asn
Leu Gin Leu Asp Thr Asn Tyr Thr Leu Thr Pro Phe Asp Thr Leu Pro
Gin Cys Asn Ser cys Glu Val His Gin Gin Tyr Tyr lie Gin Trp lie
Ser Val Gin Asp Gin Val Glu Ser Leu Val Gin Gin Gin Val Ser Gin
Phe Pro Asp Tyr Ala Leu Thr Val Thr Gin His ser Leu Gin Ala ser
Leu Ala Ala Leu Thr Ala Gin Leu ser Ala Thr Tyr Asp Asn lie
Arg Leu Tyr Thr Phe Gin Glu Pro Arg ser Asn Gin Ala Phe Ala Ser
Tyr Met Asn Asp Ala Phe Gin Ala Ser Ser Pro Asp Thr Thr Gin Tyr
Phe Arg Val Thr His Ala Asn Asp Gly lie Pro Asn Leu Pro Pro Ala
Asp Glu Gin Tyr Ala His Gin Val Val Glu Tyr Trp ser Val Asp Pro
Tyr Ser Ala Gin Asn Thr Phe Val cys Thr Gin Gin Cys
10283M sequence listing.txt

Cys Glu Ala Gin Gly Gly Gin Gly val Asn Asn Ala His Thr Thr Tyr

Phe Gly Met Thr Ser Gly His Cys Thr Trp

<210>  9
<211> 276
<212> PRT
<213> Fusarium oxysporum
<400>  9

Ala val Gly Val Thr Thr Thr Asp Phe Ser Asn Phe Lys Phe Tyr lie

1  5  10  15

Gin His Gly Ala Ala Ala Tyr Cys Asn Ser Glu Ala Ala Ala Ala Gly ser

20  25  30

Lys lie Thr Cys Ser Asn Asn Gly Cys Pro Thr Val Gin gly Asn Gly

35  40  45

Ala Thr lie val Thr ser Phe Val Gly Ser Lys Thr Gly lie Gly Gly

50  55  60

Tyr val Ala Thr Asp Ser Ala Arg Lys Glu Hec val val Ser Phe Arg

65  70  75

Gly ser lie Asn lie Arg Asn Trp Leu Thr Thr Asn Leu Asp Phe Gly Gin

80  85  90  95

Glu Asp Cys Ser Leu Val Ser Gly Cys Gly Val His Ser Gly Phe Gin

100  105  110

Arg Ala a Trp 115 Asn Glu lie Ser Ser Gin Ala Thr Ala Ala val Ala Ser

120  125

Ala Arg 130 Lys Ala Asn Pro Ser 135 Phe Asn Val lie ser 140 Thr Gly His ser

140

Leu Gly Gly Ala Val Ala val Leu Ala Ala Ala Asn Leu Arg val Gly

145  150  155  160

Gly Thr Pro Val Asp lie Tyr Thr Tyr Gly Ser Pro Arg Val Gly Asn

165  170  175

Ala Gin Leu Ser Ala Phe Val Ser Asn Gin Ala Gly Gly Glu Tyr Arg

180  185  190

val Thr His Ala Asp Asp Pro val 195 Pro Arg Leu Pro Pro Leu lie Phe
Gly Tyr Arg His Thr Thr Pro Glu Phe Trp Leu Ser Gly Gly Gly 210
Gly Gly Gly
Asp Lys Val Asp Tyr Thr lie Ser Asp Val Lys Val cys Glu Gly Ala 225
Ala Asn Leu Gly Cys Asn Gly Gly Thr Leu Gly Leu Asp lie Ala Ala 245
His Leu His Tyr Phe Gin Ala Thr Asp Ala Cys Asn Ala Gly Gly Phe 260
ser Trp Arg Arg 275

<210> 10
<211> 273
<212> PRT
<213> Fusarium heterosporum
<400> 10
Thr val Thr Thr Gin Asp Leu ser Asn Phe Arg Phe Tyr Leu Gin His 1
Ala Asp Ala Ala Tyr Cys Asn Phe Asn Thr Ala val Gly Lys Pro Val 20
His cys Ser Ala Gly Asn Cys Pro Asp lie Glu Lys Asp Ala Ala lie 35
val Val Gly Ser Val Val Gly Thr Lys Thr Gly lie Gly Ala Tyr val 50
Ala Thr Asp Asn Ala Arg Lys Glu He Val Val ser val Arg Gly Ser 65
lie Asn val Arg Asn Trp lie Thr Asn Phe Asn Phe Gly Gin Lys Thr 80
cys Asp Leu val Ala Gly Cys Gly val His Thr Gly phe Leu Asp Ala 100
Trp Glu Glu Val Ala Ala Asn Val Lys Ala Ala val ser Ala Ala Lys 115
Thr Ala Asn pro Thr Phe Lys Phe val Val Thr Gly His Ser Leu Gly 130
Gly Ala val Ala Thr lie Ala Ala Ala Tyr Leu Arg Lys Asp Gly Phe 145
Pro Phe Asp Leu Tyr Thr Tyr Gly Ser Pro Arg Val Gly Asn Asp Phe
10283M sequence string

Phe Ala Asn Phe Val Thr Gin Gin Thr Gly Ala Glu Tyr Arg Val Thr

His Gly Asp Asp Pro Val Pro Arg Leu Pro pro lie val Phe Gly Tyr

Arg His Thr Ser Pro Glu Tyr Trp Leu Asn Gly Gly Pro Leu Asp Lys

Asp Tyr Thr Val Thr Glu He Lys val cys Gly Gly lie Ala Asn val

Met cys Asn Gly Gly Thr lie Gly Leu Asp lie Leu Ala His lie Thr

Tyr Phe Gin Ser Met Ala Thr Cys Ala Pro Pie Ala lie pro Trp Lys

Arg

<210> 11
<211> 278
<212> PRT
<213> Aspergillus oryzae

<400> 11

Asp lie pro Thr Thr Gin Leu Glu Asp Phe Lys Phe Trp Val Gin Tyr

Ala Ala Ala Thr Tyr Cys Pro Asn Asn Tyr val Ala Lys Asp Gly Glu

Lys Leu Asn 35 Cys Ser Val Gly Asn cys Pro Asp val Glu Ala Ala Gly

Ser Thr val Lys Leu ser Phe Ser Asp Thr lie Thr Asp Thr Ala

Gly Phe Val Ala val Asp Asn Thr Asn Lys Ala lie val val Ala Phe

Arg Gly ser Tyr Ser lie Arg Asn Trp Val Thr Asp Ala Thr Phe Pro

Gin Thr Asp pro Gly Leu Cys Asp Gly Cys Lys Ala Glu Leu Gly Phe

Trp Thr Ala Trp Lys Val Val Arg Asp Arg lie lie Lys Thr Leu Asp
10283M sequence listing.txt

Glu Leu Lys Pro Glu His Ser Asp Tyr Lys lie Val Val val Gly His
130 135 140
Ser Leu Gly Ala Ala lie Ala Ser Leu Ala Ala Ala Ala Asp Leu Arg Thr
145 150 155 160
Lys Asn Tyr Asp Ala lie Leu Tyr Ala Tyr Ala Ala Pro Arg Val Ala
165 170
Asn Lys Pro Leu Ala Glu Phe lie Thr Asn Gin Gly Asn Asn Tyr Arg
180 185 190
Phe Thr His Asn Asp Asp Pro val Pro Lys Leu Pro Leu Leu Thr Met
195 200 205
Gly Tyr val His lie ser Pro Glu Tyr Tyr lie Thr Ala Pro Asp' Asn
210 215 220
Thr Thr Val Thr Asp Asn Gin val Thr val Leu Asp Gly Tyr val Asn
225 230 235 240
Phe Lys Gly Asn Thr Gly Thr ser Gly Gly Leu Pro Asp Leu Leu Ala
245 250 255
Phe His Ser His val Trp Tyr Phe lie His Ala Asp Ala Cys Lys Gly
260 265 270
Pro Gly Leu Pro Leu Arg
275

<210> 12
<211> 278
<212> PRT
<213> Penici Ilum camemberti

<400> 12

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

<210> 13
<211> 270
<212> .PRT
<213> Aspergillus foetidus
<400> 13
Ser val Ser Thr Ser Thr Leu Asp Glu Leu Gin Leu Phe Ala Gin Trp ser Ala Ala Ala Tyr cys ser Asn Asn lie Asp ser Lys Asp ser Asn
Leu Thr cys Thr Ala Asn Ala cys Pro Ser Val Glu Glu Ala ser Thr
35

Thr Met Leu Leu Glu Phe Asp Leu Thr Asn Asp Phe Gly Gly Thr Ala
50

Gly Phe Leu Ala Ala Asp Asn Thr Asn Lys Arg Leu Val Val Ala Phe
65

Arg Gly Ser Ser Thr lie Glu Asn Trp lie Ala Asn Leu Asp Phe lie
85

Leu Glu Asp Asn Asp Asp Leu cys Thr Gly cys Lys Val His Thr Gly
100

Phe Trp Lys Ala Trp Glu ser Ala Ala Asp Glu Leu Thr ser Lys lie
115

Lys Ser Ala Met Ser Thr Tyr ser Gly Tyr Thr Leu Tyr Phe Thr Gly
130

His Ser Leu Gly Gly Ala Leu Ala Thr Leu Gly Ala Thr Val Leu Arg
145

Asn Asp Gly Tyr Ser Val Glu Leu Tyr Thr Tyr Gly cys Pro Arg lie
165

Gly Asn Tyr Ala Leu Ala Glu His lie Thr ser Gin Gly ser Gly Ala
180

Asn Phe Arg Val Thr His Leu Asn Asp lie Val Pro Arg Val Pro Pro
195

Met Asp Phe GTy Phe Ser Gin Pro Ser Pro Glu Tyr Trp lie Thr Ser
210

Gly Asn Gly Ala Ser val Thr Ala Ser Asp lie Glu val lie Glu Gly
225

lie Asn Ser Thr Ala Gly Asn Ala Gly Glu Ala Thr Val ser val Leu
245

Ala His Leu Trp Tyr Phe Phe Ala lie ser Glu cys Leu Leu
260
10283M sequence H sting .txt

Ser Ala Ala Tyr cys Ser Asn Asn lie Asp ser Asp Asp Ser Asn
val Thr cys Thr Ala Asp Ala Cys Pro ser val Glu Glu Ala ser Thr
Lys Met Leu Leu Gly Phe Asp Leu Thr Asn Asn Phe Gly Gly Thr Ala
Gly phe Leu Ala Ala Asp Thr Asn Lys Arg Leu val val Ala Phe
Arg Gly Ser Ser Thr lie Lys Asn Trp lie Ala Asp Leu Asp Phe lie
Leu Gin Asp Asn Asp Asp Leu Cys Thr Gly cys Lys val His Thr Gly
Phe Trp Lys Ala Trp Gly Ala Ala Ala Asp Asn Leu Thr Ser Lys lie
Lys Ser Ala Met Ser Thr Tyr Ser Gly Tyr Thr Leu Tyr Phe Thr Gly
His Ser Leu Gly Gly Ala Leu Ala Thr Leu Gly Ala Thr val Leu Arg
Asn Asp Gly Tyr ser val Glu Leu Tyr Thr Tyr Gly Cys Pro Arg Val
Gly Asn Tyr Ala Leu Ala Gly His lie Thr ser Gin Gly Ser Gly Ala
Asn Phe Pro val Thr His Leu Asn Asp lie val Pro Arg val Pro Pro
Met Asp Phe Gly Phe ser Gin Pro ser Pro Glu Tyr Trp lie Thr ser
Gly Thr Gly Ala ser val Thr Ala ser Asp lie Glu Leu lie Gly
225
230
235
240
225
230
235
240
lie Asn Ser Thr Ala Gly Asn Ala Gly Gly Ala Thr val Asp val Leu
Ala His Leu Trp Tyr phe Phe Ala lie Ser Glu Cys Leu Leu
<210>
<211> 269
<212> PRT
<213> Aspergillus oryzae

<400> 15

Asp val ser ser ser Leu Leu Asn Asp Leu Leu Phe Ala Gin Tyr
1

ser Ala Ala Ala Tyr cys Asp Glu Asn Leu Asn ser Thr Gly Thr Lys
5

Leu Thr cys ser val Gly Asp Cys 40 Pro Leu Val Glu Ala Ala Ser Thr
20

Gln Ser 50 Leu Asp Glu Phe Asn Glu Ser Ser Ser Tyr Gly Asn Pro Ala
25

Gly Tyr Leu Ala Ala Asp 70 Glu Thr Asn Lys Leu Leu Val Leu Ser Phe
35

Arg Gly Ser Ala 85 Leu Ala Asn Trp Val 90 Ala Asn Leu Asn Phe Gly
40

Leu Glu Asp Ala 100 ser Asp Leu Cys Ser Gly Cys Glu Val His ser Gly
45

Phe Trp Lys 115 Ala Trp Ser Glu lie Ala Asp Thr lie Thr 125 ser Lys val
50

Glu Ser Ala Leu Ser Asp 130 His ser Asp Ser Asp Tyr ser Leu Val Leu Thr Gly
55

His Ser Tyr Gly Ala Ala Leu Ala Ala Leu Ala Ala Thr Ala Leu Arg
60

Asn ser Gly His ser Val Glu Leu Tyr Asn Tyr Gly Gin Pro Arg Leu
65

Gly Asn Glu Ala 180 Leu Ala Thr Tyr lie Thr Asp Gin Asn Lys Gly Gly
70

Asn Tyr Arg 195 val Thr His Thr Asp lie Val Pro Lys Leu Pro pro
75

Thr Leu 210 Leu Gly Tyr His His Phe Ser Pro Glu Tyr Tyr lie ser ser
80

Ala Asp Glu Ala 225 Thr val Thr Thr Thr Asp Val 235 Thr Glu Val Thr Gly
85

lie Asp Ala Thr Gly 245 Gly Asn Asp Gly Thr Asp Gly Thr Ser lie Asp
Ala His Arg Trp Tyr Phe lie Tyr H e ser Glu cys ser

<210> 1 6
<211> 251
<212> PRT
<213> Landerina penisapora

<400> 16
Pro Gin Asp Ala Tyr Thr Ala ser His Ala Asp Leu Val Lys Tyr Ala

Thr Tyr Ala Gly Leu Ala Tyr Gin Thr Asp Ala Trp Pro Ala ser

Arg Thr Val Pro Lys Asp Thr Thr lie ser Ser Phe Asp His Thr

Leu Lys Gly ser ser Gly Tyr lie Ala Phe Asn Glu Pro Cys Lys Glu

lie lie val Ala Tyr Arg Gly Thr Asp Ser Leu lie Asp Trp Leu Thr

Asn Leu Asn Phe Asp Lys Thr Ala Trp Pro Ala Asn lie Ser Asn Ser

Leu Val His Glu Gly Phe Leu Asn Ala Tyr Leu val ser Met Gin Gin

val Gin Glu Ala val Asp Ser Leu Ala Lys cys Pro Asp Ala Thr

lie Ser phe Thr Gly His ser Leu Gly Gly Ala Leu Ala Cys lie Ser

Met val Asp Thr Ala Gin Arg His Arg Gly lie Lys Met Gin Met Phe

Thr Tyr Gly Gin Pro Arg Thr Gly Asn Gin Ala Phe Ala Glu Tyr val

Glu Asn Leu Gly His Pro Val Phe Arg Val val Tyr Arg His Asp lie

Val Pro Arg Met Pro Pro Met Asp Leu Gly Phe Gin His His Gly Gin

Glu Val Trp Tyr Glu Gly Asp Glu Asn lie Lys Phe Cys Lys Gly Glu
10283M sequence listing.txt

Gly Glu Asn Leu Thr Cys Glu Leu Gly Val Pro Phe ser Glu Leu Asn
225 230 235 240

Ala Lys Asp His Ser Glu Tyr Pro Gly Met His
245 250