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(54) Title: OVEREXPRESSED AND PURIFIED *ASPERGILLUS FICUUM* OXIDASE AND NUCLEIC ACID ENCODING THE SAME

(57) Abstract: The present invention relates to a new isolated nucleic acid sequence comprising a gene that encodes a new fungal oxidase enzyme, and nucleic acid fragments thereof. It also relates to said new fungal oxidase isolated and purified from *Aspergillus ficuum*, its amino acid sequence as shown in SEQ ID NO 40 or 41, and functional equivalents or derivatives thereof. The present invention also relates to constructs, vectors and hosts cells comprising a nucleic acid molecule of the invention, as well as methods for producing an oxidase of the invention. The present invention also relates to the use of an oxidase of the invention in industrial processes.



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OVEREXPRESSED AND PURIFIED *ASPERGILLUS FICUM* OXIDASE AND
NUCLEIC ACID ENCODING THE SAME

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Field of the invention

[001] The present invention relates to a new isolated nucleic acid sequence comprising a gene that encodes a new
15 fungal oxidase enzyme, and nucleic acid fragments thereof.

[002] It also relates to said new fungal oxidase isolated and purified from *Aspergillus ficum*, its amino acid sequence as shown in SEQ ID NO 40 or 41, and functional equivalents or derivatives thereof.

20 [003] The present invention also relates to constructs, vectors and hosts cells comprising a nucleic acid molecule of the invention, as well as methods for producing an oxidase of the invention.

[004] The present invention also relates to the use of
25 an oxidase of the invention in industrial processes.

Background of the invention

[005] Oxidases are enzymes that catalyze many kinds of biological oxidations.

30 [006] Among these oxidases, for example, laccases (also referred to as polyphenol oxidases ; EC 1.10.3.1. ; benzenediol:oxygen oxidoreductases) are multi-copper containing enzymes that catalyze the oxidation of a variety of

phenolic compounds with concomitant reduction of O_2 to H_2O . These polyphenol oxidases are widely spread and produced by a wide variety of (1) fungi including (a) ascomycetes such as *Aspergillus*, *Neurospora* or *Podospora*, (b) the deuteromycete
5 *Botrytis*, (c) basidiomycetes such as *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes*, *Phlebia* or *Pycnoporus*, (d) perfect forms of *Rhizoctonia*, but also by (2) plants such as *Rhus vernicifera*, *Liriodendron tulipifera*, *Nicotiana tabacum* or *Acer pseudoplatanus*, and by (3) bacteria such as
10 *Azospirillum lipoferum*. They are also widespread in bacteria (Alexandre and Zhulin, 2000, Tibtech 18: 41).

[007] Taking into account the biodiversity of their producers, oxidases and laccases exhibit a wide range of substrate specificities with different abilities to oxidize
15 phenolic substrates. Thus, laccases are involved in pigmentation, fruiting body formation, pathogenicity and lignin degradation and biosynthesis.

[008] Because of this substrate specificity, oxidases and laccases show potential in industrial applications (pulp and paper processing, dye transfer inhibition in detergents or
20 phenol polymerization), in environmental applications (environmental pollutants detoxification or waste water treatment), in food application (baking, brewing, prevention of wine discoloration, color enhancement of tea based
25 foodstuff, deoxygenation of food items, or juice manufacture) and in pharmaceutical applications (transformations of steroid and antibiotics) (see for examples: Sariaslani, 1989, Critic. Rev. Biotechnol. 9:171; Potus et al., 1999, Industries des
30 céréales, 115:3; Lopez et al., 2002, J. Biotechnol., 99:249; Duran et al., 2002, Enz. Microbial Technol., 31:907; Minussi et al., 2002, Trends Food Sci. Technol., 13:205; Biotechnology in the pulp and paper industry, 2002, Viikari et Lantto eds, Elsevier).

[009] Depending on their origins, fungal laccases have different temperature and pH optima, different redox potential and substrate specificities (Xu F. & al., 1996, Biochim Biophys Acta. 1292, p.303). A large number of fungal laccases
5 have been isolated and most of their corresponding genes have been cloned. Similarities and strong identities values found between their amino acid sequences show that closely related sequences belongs to organisms which are members of the same phylogenetic group. These values are sometimes higher between
10 enzymes from different species of the same genus than between different laccases produced by the same species (Eggert et al., 1998, Appl Environ Microbiol. 64: 1766).

[010] Depending on their wide range of substrate specificity, oxidases and laccases have great commercial
15 potential and the ability to express these enzymes at very high level is critical for commercial purposes. Attempts to express laccase genes in heterologous fungal systems frequently gave very low yields. Thus, the expression of *Phlebia radiata* laccase in *Trichoderma reesei* gave only 20 mg
20 per liter of active enzyme (Saloheimo et al., Bio/Technology 1991, 9: 987), while expression of *Coprinus cinereus* Lcc1 laccase in *Aspergillus oryzae* gave 8 to 135 mg per liter (Yaver et al., 1999, lcc1. Appl Environ Microbiol., 65(11):4943-8.) and that of *Myceliophthora thermophila* in
25 *Aspergillus oryzae* gave 11 to 19 mg per liter (Berka et al., 1997, Appl Environ Microbiol. 63: 3151). The laccase of *Pycnoporus cinnabarinus* has been expressed at a level of about 80 mg per liter in *Aspergillus* (Sigoillot C. et al., 2004, Appl. Microbiol. Biotechnol. 64: 346).

30 [011] At the present time there is still a need for new oxidases that can be used in the different applications described herein.

[012] Furthermore the expression of their corresponding genes at high level in industrial hosts such as *Aspergillus* is of great interest.

5 Summary of the invention

[013] The present invention relates to the isolation and characterization of a new gene encoding an *Aspergillus ficuum* oxidase.

[014] A new gene encoding an oxidizing enzyme
10 according to the invention has been isolated from *A. ficuum* and is 1,923 base pairs long with two introns and an open reading frame corresponding to 596 amino acids.

[015] An aspect of the invention relates to a nucleic acid molecule comprising or consisting of SEQ ID NO 1, 2, 3,
15 4, 5, 6, 7, 8, 9, 10, or 11 encoding an *A. ficuum* oxidase.

[016] Another aspect of the invention relates to a nucleic acid molecule encoding a polypeptide of the present invention.

[017] Another aspect of the invention relates to a
20 polynucleotide selected from the group consisting of:

- a nucleic acid molecule comprising a nucleotide sequence having at least 60%, advantageously at least 70%, more advantageously at least 80%, preferably at least 85%, more preferably at least 90% and even more preferably at
25 least 95% identity with any of SEQ ID NO 1 to 39 the complementary form and RNA form thereof,
- any fragments thereof encoding a protein having an oxidase activity, the complementary form and RNA form thereof, and,
- 30 - any fragments thereof of at least 15 nucleotides, preferably of at least 20 nucleotides, or more preferably of at least 25 nucleotides, the complementary form and RNA form thereof.

[018] Another aspect of the invention relates to a protein having an oxidase activity, of about 85 kDa as shown by polyacrylamide gel electrophoresis and Coomassie blue staining.

5 [019] Another aspect of the invention relates to a protein having an oxidase activity with a molecular weight of about 70 kDa after deglycosylation with PNGaseF.

[020] The invention also relates to an isolated oxidase polypeptide, the amino acid sequence of which
10 comprises or consists of SEQ ID NO 40 or 41, and any fragments thereof having an oxidase activity.

[021] Another aspect of the invention relates to an isolated polypeptide having an oxidase activity, selected from the group consisting of an amino acid sequence having at least
15 70%, advantageously at least 80%, preferably at least 85%, more preferably at least 90% and even more preferably at least 95% identity with SEQ ID NO 40 or 41.

[022] Another aspect of the invention relates to transformed cells, e.g. *A. nidulans* 2024, comprising a gene
20 according to the invention expressed under the control of its own promoter.

[023] Another aspect of the invention relates to transformed cells, e.g. *A. nidulans* or *A. ficuum*, comprising a gene according to the invention expressed under the control of
25 the *gpdA* promoter of *A. nidulans* (glyceraldehyde-phosphate-dehydrogenase promoter), resulting in a several-fold increase of the expression of said gene.

[024] Another object of the invention relates to the use of an oxidase according to the present invention in food
30 and non-food industrial applications, where oxidation of phenolics is required, for example its use in a baking process or its use as colour enhancer, e.g. in tea.

[025] Another object of the invention relates to a bread improving composition comprising an oxidase polypeptide of the invention.

5 **BRIEF DESCRIPTION OF THE DRAWINGS**

[026] Figure 1 represents a SDS-PAGE and zymogram analysis of the purified enzyme with oxidase activity from *Aspergillus ficuum* (maintained as a deposit with DSMZ under the accession number DSM932).

10 [027] Figure 2 represents the enzyme activity in function of the pH.

[028] Figure 3 represents the nucleotide sequence of the gene coding for the enzyme with oxidase activity from *Aspergillus ficuum* (DSM932), and the corresponding amino acid
15 sequence.

[029] Figure 4 represents the absorbance spectra of tea solutions treated or not with an oxidase according to the invention.

[030] Figure 5 represents the effect of increasing
20 amounts of an oxidase isolated from *Aspergillus ficuum* (DSM932) according to the invention on the volume of bread.

[031] Figure 6 represents the effect of increasing amounts of an oxidase isolated from *Aspergillus ficuum* (DSM932) according to the invention on stickyness of bread.

25 [032] Figure 7 represents the effect of increasing amounts of an oxidase isolated from *Aspergillus ficuum* (DSM932) according to the invention on dough consistency.

[033] Figures 8 to 12 represent the nucleotide and amino acid sequences of the invention.

DETAILED DESCRIPTION OF THE INVENTION**Oxidase encoding gene.**

[034] The present invention provides an isolated nucleic acid molecule encoding an oxidase.

5 [035] The nucleic acid molecule consisting of SEQ ID NO 1 has been isolated from *A. ficuum* deposited with DSMZ under the accession number DSM932 (referred to as *A. ficuum* DSM932).

10 [036] Allelic and species variants are also contemplated and are referred to as homologues or homologous sequences.

[037] In the context of the present invention, a homologue or homologous sequence is a nucleotide or an amino acid sequence having at least 60%, advantageously at least 70%, more advantageously at least 80%, preferably at least 90%, and more preferably at least 95%, 96%, 97%, 98% or 99% homology (or identity) with any of SEQ ID NO 1 to 50.

20 [038] It is meant by "homology" or "identity" the value in percentage given when comparing two sequences. Said comparison can be performed using any available software, for example the Clustalw program and the default parameters values (e.g. available at <http://www.ebi.ac.uk/clustalw>).

25 [039] In the context of the present invention the terms "polynucleotide" or "nucleic acid molecule" refer to single-stranded or double stranded molecules and include DNA molecules (e.g. cDNA or genomic DNA), RNA molecules (e.g. mRNA) and analogs wherein nucleotides have been replaced by nucleotide analogs or derivatives.

30 [040] A nucleic acid consisting of or comprising any of SEQ ID NO 1 to 39 or any homologue, its complementary form (or complementary strand), or its RNA form can be isolated from different micro-organisms producing an oxidase according to the invention.

[041] Said micro-organisms can be bacteria or fungi, including yeasts, and can be more specifically other *Aspergillus* species.

[042] Established standards methods can be used to
5 isolate a homologous sequence of the invention.

[043] Examples of such methods include the construction of a gene library from the genomic DNA of the micro-organisms in a suitable vector, followed by screening of this library by direct expression of said homologous sequence.

10 [044] Another method comprises a hybridization step with e.g. a fragment of at least 15 nucleotides, preferably at least 20 nucleotides and more preferably at least 50 nucleotides of a nucleic acid molecule of the invention, e.g. a fragment of SEQ ID NO 37, 38 or 39.

15 [045] Other methods include the amplification of said homologous sequence by molecular techniques, for instance PCR techniques, using oligonucleotide primers that are designed from a nucleic acid sequence of the invention.

[046] Furthermore, there are established methods for
20 obtaining in a relatively short period of time thousands of mutated sequences together with assessed enzymatic activities of their corresponding polypeptide sequences. These methods include e.g. random mutagenesis, high-throughput screening, etc., and are frequently used to demonstrate the eventual
25 effects of single or multiple mutations.

[047] Such mutations (addition(s), deletion(s) and/or substitution(s)) can be silent or not, can be made inside or outside the regions critical to the function of the molecule and still result in an active protein having an oxidase
30 activity more or less similar to the oxidase activity of a polypeptide of SEQ ID NO 40 or 41, i.e. of at least 70%, advantageously of at least 80%, preferably of at least 90%, or more preferably of at least 95% (also referred to as functional equivalents).

[048] Alternatively a nucleic acid molecule of the invention may be prepared synthetically by methods known in the art. A nucleic acid molecule of the invention may include oligonucleotide analogs or derivatives (e.g. inosine or phosphorothioate nucleotides, etc.) so it has, for example, altered base-pairing abilities or increased resistance to nucleases.

[049] A nucleic acid molecule of the invention may or may not include introns interrupting the coding sequence.

[050] A nucleic acid molecule of the invention may be of mixed genomic, synthetic and/or cDNA origin, prepared by methods known in the art comprising the step of ligating fragments from different origins.

[051] A preferred isolated and purified nucleotide sequence of the invention corresponds to SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 or a fragment thereof that encodes a peptide having an oxidase activity.

[052] A fragment of said sequence SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 has preferably more than 600 nucleotides, more preferably more than 900 nucleotides or even more preferably more than 1200 nucleotides and encodes a protein characterized by a oxidase activity similar to the oxidase activity of the complete amino acid sequence of SEQ ID NO 40 or 41 (also referred to as a functional equivalent).

[053] Preferably, said functional equivalent has an oxidase enzymatic activity of more than 70%, or more than 80% of the initial oxidase activity of the complete enzyme defined by its amino acid sequence of SEQ ID NO 40 or 41, and preferably has an oxidase activity of at least 90% compared to the one having the amino acid sequence of SEQ ID NO 40 or 41.

[054] A polynucleotide of the invention is selected from the group consisting of:

- (a) a nucleic acid molecule comprising a nucleotide sequence having at least 60%, advantageously at least

70%, more advantageously at least 80%, preferably at least 85%, more preferably at least 90% and even more preferably at least 95%, 96%, 97%, 98% or 99% identity with any of SEQ ID NO 1 to 39, the complementary form and RNA form thereof,

- (b) any fragments of a nucleic acid molecule of group (a) encoding a protein having an oxidase activity, the complementary form and RNA form thereof, and
- any fragments of a nucleic acid molecule of group (a) or (b) of at least 15 nucleotides, preferably of at least 20, 25, or 30 nucleotides, or more preferably of at least 50 nucleotides, the complementary form and RNA form thereof.

[055] In other words, a nucleic acid molecule according to the invention may comprise or consist of:

- a nucleotide sequence of any of SEQ ID NO 1 to 39, its complementary form or RNA form,
- a nucleotide sequence having at least 60%, advantageously at least 70%, more advantageously at least 80%, preferably at least 85%, more preferably at least 90% and even more preferably at least 95%, 96%, 97%, 98% or 99% identity with any of SEQ ID NO 1 to 39, or with the complementary form or RNA form thereof,
- a fragment of any of SEQ ID NO 1 to 11 or of any of their homologues, or of any of their complementary form or RNA form, wherein said fragment encodes a protein having an oxidase activity, or
- a fragment of at least 15 nucleotides, preferably at least 20 nucleotides, more preferably of at least 25 nucleotides, of any of SEQ ID NO 1 to 39 or of any of their homologues, or of any of their complementary form or RNA form.

[056] Said fragments of any of SEQ ID NO 1 to 11 or of any of their homologues, or of any of their complementary form

or RNA form, encoding a protein having an oxidase activity, consist preferably of at least 600 nucleotides, preferably of at least 900 nucleotides, or more preferably of at least 1200 nucleotides.

5 [057] A nucleic acid molecule according to the invention comprising or consisting of a fragment of at least 15 nucleotides, preferably of at least 20, 25, or 30 nucleotides, more preferably of at least 50 nucleotides, of any of SEQ ID NO 1 to 39 or of any of their homologues, or of
10 any of their complementary form or RNA form, can be used for example for detection or identification purposes, as a primer or a probe.

Oxidase Protein.

15 [058] Also provided is a protein having an oxidase activity, of about 85 kDa as shown by polyacrylamide gel electrophoresis and Coomassie blue staining.

[059] A protein of the invention, having an oxidase activity, has a molecular weight of about 70 kDa after
20 deglycosylation with PNGaseF.

[060] Advantageously the unglycosylated form of the isolated and purified amino acid sequence according to the invention has a molecular weight comprised between about 60 and about 70 kDa, preferably about 63 kDa or about 65.5 kDa.

25 [061] An isolated oxidase of the invention consists of a polypeptide encoded by a nucleic acid of the invention.

[062] An isolated oxidase polypeptide of the invention may comprise or consist of:

- an amino acid sequence of any of SEQ ID NO 40 to 50,
- 30 - a fragment of at least 100 amino acids of SEQ ID NO 40, 41 or 45, or
- an amino acid sequence presenting at least 60% identity with the amino acid sequence of any of SEQ ID 40 to 50,

or with any fragments of at least 100 amino acids of said SEQ ID NO 40, 41 or 45.

[063] More specifically, an isolated oxidase of the invention consists of a polypeptide encoded by a nucleic acid molecule comprising or consisting of:

- Any of SEQ ID NO 1 to 39,
- a nucleotide sequence having at least 60%, advantageously at least 70%, more advantageously at least 80%, preferably at least 85%, more preferably at least 90% and even more preferably at least 95%, 96%, 97%, 98% or 99% identity with any of SEQ ID NO 1 to 39 (also referred to as homologues), or
- any fragments of any of SEQ ID NO 1 to 11, or of their homologues, encoding a protein having an oxidase activity.

[064] An isolated oxidase polypeptide of the invention comprises or consists of the amino acid sequence of any of SEQ ID NO 40 to 50, or any fragments thereof that have retained an oxidase activity.

[065] An isolated oxidase polypeptide of the invention comprises or consists of an amino acid sequence presenting at least 60%, preferably at least 70%, 80% or 85%, more preferably at least 90%, or even more preferably at least 95%, 96%, 97%, 98% or 99% homology (or sequence identity) with the amino acid sequence of SEQ ID NO 40 or 41, or with any fragments of said SEQ ID NO 40 or 41 having an oxidase activity.

[066] A preferred fragment of an oxidase polypeptide of the invention, in particular a preferred fragment of SEQ ID NO 40 or 41, consists or comprises an amino acid sequence of at least 100 amino acids, preferably of at least 200, more preferably of at least 300 amino acids, and even more preferably of at least 400 amino acids.

[067] A preferred fragment of an oxidase polypeptide of the invention has at least 70%, advantageously at least

80%, more advantageously at least 85%, preferably at least 90%, or more preferably at least 95%, 96%, 97%, 98% or 99% of the oxidase activity of an oxidase polypeptide defined by an amino acid sequence of SEQ ID NO 40 or 41.

5 [068] A preferred fragment of an oxidase polypeptide of the invention, in particular a preferred fragment of SEQ ID NO 40 or 41, consists or comprises an amino acid sequence of at least 100 amino acids, preferably of at least 200, more preferably of at least 300 amino acids, and even more
10 preferably of at least 400 amino acids, and has at least 70%, advantageously at least 80%, more advantageously at least 85%, preferably at least 90%, or more preferably at least 95%, 96%, 97%, 98% or 99% of the oxidase activity of an oxidase polypeptide defined by an amino acid sequence of SEQ ID NO 40
15 or 41.

[069] Indeed, an isolated oxidase polypeptide of the invention consisting of or comprising an amino acid sequence of any of SEQ ID NO 40 to 50 can be deleted partially while maintaining its enzymatic activity. Said enzymatic activity
20 can be measured by methods well known in the art.

[070] A protein fragment according to the invention can also be prepared by recombinant techniques.

[071] An isolated oxidase polypeptide according to the invention may also result from the substitution, deletion
25 and/or insertion of one or more amino acids in an amino acid sequence of any of SEQ ID NO 40 to 50.

[072] Said substitution can be conservative, which means that an amino acid is replaced with an amino acid having a similar side chain without affecting significantly the
30 enzymatic activity of said polypeptide compared to an oxidase polypeptide consisting of the amino acid sequence of SEQ ID NO 40 or 41. These families are known in the art and include amino acids with basic side chains (e.g. lysine, arginine and histidine), acidic side chains (e.g. aspartic acid, glutamic

acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched
5 side chains (threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine).

[073] Moreover, said substitution, deletion or insertion may concern non-essential amino acid, also resulting in no significant alteration of the oxidase activity of said
10 polypeptide in comparison with an oxidase polypeptide consisting of the amino acid sequence of SEQ ID NO 40 or 41.

[074] An isolated and purified oxidase enzyme according to the invention is also characterized by an optimum pH around 5.5. More generally the maximum activity is
15 comprised between a pH of about 5 and a pH of about 6.5 (see figure 2).

[075] An isolated oxidase polypeptide according to the invention can also be characterized by the fact that it can use, among others, N,N-dimethyl-p-phenylenediamine and 2,2'-
20 azinobis(3-ethylbenzthiazoline-6-sulfonic acid) as substrates.

[076] Therefore an isolated oxidase polypeptide according to the invention can also be referred to as a laccase.

25 **Expression and/or purification of the enzyme**

[077] Another aspect of the present invention is related to a recombinant nucleotide sequence comprising, operably linked to a nucleotide sequence according to the invention, one or more adjacent regulatory sequence(s). Said
30 adjacent regulatory sequence(s) is/are preferably originating from homologous micro-organisms.

[078] However said adjacent regulatory sequences may also be originating from heterologous micro-organisms.

[079] Said adjacent regulatory sequences are specific sequences such as promoters, enhancers, secretion signal sequences and/or terminators.

[080] Preferred adjacent regulatory sequences of the invention are capable of directing the overexpression of a nucleotide sequence of the invention in a recombinant host cell. A preferred adjacent regulatory sequence according to the invention is the constitutive *gpdA* (glyceraldehyde -3-phosphate dehydrogenase) promoter of *Aspergillus nidulans*.

[081] Another aspect of the invention is related to a vector comprising a nucleic acid molecule of the invention, possibly operably linked to one or more adjacent regulatory sequence(s) originating from homologous or from heterologous micro-organisms.

[082] In the present context "vector" is defined as any biochemical construct which may be used for the introduction of a nucleotide sequence (by transduction, tranfection, transformation, infection, conjugation, etc.) into a cell.

[083] Advantageously, a vector according to the invention is selected from the group consisting of plasmids (including replicative and integrative plasmids), viruses, phagemids, chromosomes, transposons, liposomes, cationic vesicles, or a mixture thereof. Said vector may already comprise one or more adjacent regulatory sequence(s), allowing the expression of said nucleic acid molecule and its transcription into a polypeptide of the invention. Preferably said vector is a plasmid.

[084] The present invention is also related to a transformed host cell, or recombinant host cell, containing (or having incorporated) one or more of the nucleotide sequences and/or vectors according to the invention.

[085] In the present context, a "transformed host cell" or "recombinant cell", also referred to as

"transformant", is a cell having incorporated one or more of the nucleotide sequences and/or vectors according to the invention. The transformed host cell may be a cell in which said vector(s) and/or said nucleotide sequence(s) is/are introduced by means of genetic transformation, preferably by means of homologous recombination, or by any other well known methods used for obtaining a recombinant organism.

[086] Said host cell used for the transformation, may or may not already have (a) nucleotide sequence(s) and/or vector(s) of the invention. Both prokaryotic and eukaryotic cells are included, e.g. bacteria, fungi, yeast, etc.

[087] Preferred host cells are *A. nidulans*, in particular *A. nidulans* 2024, *A. niger*, more specifically *A. niger* N402 or *A. ficuum*, more particularly *A. ficuum* DSM 932.

[088] Said host cell may also be the original cell, e.g. *A. ficuum*, containing already nucleotide sequences of the present invention, and genetically modified to over-express, or express more efficiently, an oxidase polypeptide of the invention (better pH or temperature profile, higher extracellular expression, etc.).

[089] A transformed host cell of the invention may have integrated into its genome an isolated nucleic acid molecule according to the present invention and/or may contain (an) episomal vector(s) comprising an isolated nucleic acid molecule of the invention.

[090] A preferred transformed host cell of the invention is capable of over-expressing (i.e. higher expression than the expression observed in the original or wild-type microorganism) (a) nucleotide sequence(s) and/or vector(s) of the invention, advantageously allowing a high production of polypeptide encoded by said nucleotide sequence(s) and/or said vector(s).

[091] Preferably, said recombinant host cell contains regulatory sequences adjacent to a nucleic acid molecule

according to the invention, that are capable of directing the overexpression of said nucleic acid molecule.

[092] An overexpression of an oxidase of the invention may also result from an increasing number of copies of nucleic acid sequences according to the invention in said recombinant host cell.

[093] For an optimal expression of an oxidase according to the invention, the original production species, e.g. *A. ficuum*, and/or a suitable transformed host cell, e.g. transformed *A. niger* or transformed *A. nidulans*, are in a suitable growth medium and/or expression medium. Some examples of the optimal conditions, such as culture media, temperature and pH conditions, etc., are described in the examples section.

[094] According to the present invention, a polypeptide of the invention with an oxidase activity may be obtained by first culturing the strain in/on a medium suitable for expressing said oxidase, and then may be recovered from the medium by conventional methods including but not limited to centrifugation, microfiltration, ultrafiltration, spray-drying, evaporation or precipitation. Said oxidase according to the invention may be further purified using for example electrophoretic procedures, extraction, or a variety of chromatographic procedures, such as ion exchange chromatography, gel filtration chromatography, affinity chromatography, etc. All these techniques are described in the scientific literature and are well known techniques.

[095] Said oxidase can be extra-cellular or intra-cellular expressed and/or secreted by a micro-organism producing said oxidase or by a recombinant host according to the invention.

[096] Said polypeptide of the invention may be expressed in a modified form, such as a fusion protein, and may include one or more secretion signals and/or one or more

additional heterologous functional regions. Said regions may consist of particularly charged amino acids added to the N_t of said polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling
5 and storage. They may also consist of short peptides added to said polypeptide of the invention to facilitate purification.

Different applications of an oxidase of the invention

[0097] The oxidase enzymes according to the invention
10 may be used in different kinds of industries.

[0098] A polypeptide with oxidase activity according to the present invention, further purified or not purified, is particularly suited as a bread improving agent. Bread improving agents or bread improving compositions are products
15 that are able to improve and/or increase texture, flavour, anti-staling effects, softness, crumb softness upon storage, freshness, dough machinability and/or volume of a dough and/or of a final baked product.

[0099] A polypeptide with oxidase activity according to
20 the invention is preferably used to improve the dough handling and/or increase the specific volume of the final baked product. A polypeptide with oxidase activity according to the invention is advantageously used in a bread improver formula or bread improving composition.

25 [0100] The present invention also relates to a bread improving composition comprising an oxidase polypeptide of the invention.

[0101] The term "baked product" includes any product prepared from a dough and obtained after baking of the dough,
30 and includes in particular yeast raised baked products.

[0102] Dough is obtained from any type of flour or meal (e.g. based on wheat, rye, barley, oat, or maize). Preferably, dough is prepared with wheat and/or with mixes including wheat.

[0103] A bread improving composition according to the invention may also comprised other bread-improving agents such as, but not limited to enzymes, emulsifiers, oxidants, milk powder, fats, sugars, amino acids and/or proteins (gluten, cellulose binding site).

[0104] Examples of such enzymes include, but are not restricted to, alpha-amylases, beta-amylases, maltogenic amylases, xylanases, proteases, glucose oxidases, oxido-reductases, glucanases, cellulases, transglutaminases, isomerases, lipases, phospholipases, pectinases, etc.

[0105] A preferred bread improving composition according to the invention comprises an oxidase polypeptide of the invention and an alpha-amylase, preferably an alpha-amylase from *Aspergillus oryzae*.

[0106] In another aspect of the present invention an oxidase polypeptide of the invention is particularly suited for the improvement or enhancement of the color of tea based foodstuffs.

[0107] An oxidase polypeptide of the invention may be used in food applications such as baking, pastry, cakes, brewing, prevention of wine discoloration, deoxygenation of food items, juice manufacturing, etc., or in feed applications.

[0108] In another aspect of the present invention, an oxidase polypeptide of the invention may be used in industrial applications such as pulp and paper processing, dye transfer inhibition in detergents or phenol polymerization, etc., in environmental applications such as environmental pollutants detoxification, waste water treatment, etc., or in pharmaceuticals applications (transformations of steroids and antibiotics, etc.).

[0109] The effect of an oxidase polypeptide of the invention may be further improved by adding other enzymes. Such enzymes may belong, but are not restricted, to hydrolytic

enzymes families such as glucanase, proteases, cellulases, hemicellulases, and pectinases. Other enzymes are transglutaminases, oxido-reductases, isomerases, etc.

[0110] Depending on the application, an oxidase polypeptide of the invention may be used under several forms. Micro-organisms (recombinant or not) expressing an oxidase polypeptide of the invention, such as yeasts, fungi, archaea bacteria or bacteria, may be used directly in the process.

[0111] An oxidase polypeptide of the invention may be used as a cell extract, a cell-free extract (i.e. portions of the host cell that has been submitted to one or more disruption, centrifugation and/or extraction steps) and/or as a purified protein.

[0112] One or more of said forms may be used in combination with one or more enzymes under any of the above-described forms.

[0113] Said whole cells, cell extracts, cell-free extracts or said purified oxidase polypeptide of the invention may be immobilized by any conventional means on a solid support for instance to allow protection of the oxidase polypeptide of the invention, or to allow continuous hydrolysis of a substrate and/or to allow recycling of the enzymatic preparation.

[0114] Said cells, cell extracts (including crude and partially purified extracts), cell-free extracts and/or said purified oxidase polypeptide of the invention may be mixed with different ingredients, e.g. in the form of a dry powder or a granulate, in particular a non-dusting granulate, or in a form of a liquid, for example with stabilizers such as polyols, sugars, organic acids, sugar alcohols according to well-established methods.

[0115] The invention is described in further details in the following examples, which are intended for illustration

purposes only, and should not be construed as limiting the scope of the invention in any way.

5 EXAMPLES

MATERIALS AND METHODS

EXAMPLE 1: Strains and media.

[0116] The bacterial strains used were the E. coli
10 strains RR1ΔM15 (F'lacIQ lacZΔM15 hsdS20 supE44 ara-14 proA2
rspL20(strR) lacY1 galK2 xyl-5 mtl-1) and MC1061 (hsdR mcrB
araD139 Δ (araABC-leu) 7697 Δ lacX74 galU galK rpsL thi).
Fungal strains were *Aspergillus ficuum* DSM932, *Aspergillus*
nidulans 2024 (biA1 argB3) and *Aspergillus niger* N402 (cspA1
15 derivate of the ATCC strain 9029).

[0117] Bacteria were grown at 37°C in LB (0.5% yeast
extract, 1% Bacto peptone, 1% NaCl) or TB medium (Terrific
Broth, Gibco BRL Life Technologies Inc., Gaithersburg, MD) and
fungi in *Aspergillus* minimal medium (Ponteverco & al, 1953,
20 Adv. Genet., 5: 141) at 28°C and 250 rpm.

EXAMPLE 2: Fermentations

[0118] *Aspergillus* strains were cultivated in 15l
Biostat E fermentors (B. Braun Biotech - working volume 10l).
25 The culture medium composition was the following: Maldex 15:
40 g/l; Salt solution: 50ml/l; Trace elements solution: 1ml/l;
CuSO4 solution (1.6g/l): 1ml/l. The salt solution contained
120g/l NaNO3, 10.40g/l KCl, 10.40 g/l MgSO4.7H2O and 30.40g/l
KH2PO4. The Trace elements solution (pH 6.5) contained 22g/l
30 ZnSO4.7H2O, 11g/l H3BO3, 4.1g/l MnCl2.2H2O, 5g/l FeSO4.7H2O,
1.7g/l CoCl2.6H2O, 1.6g/l CuSO4.5H2O, 1.5g/l Na2MoO4.2H2O and
50g/l ethylenedinitrilotetraacetic acid disodium salt
dihydrate. 1 mg/l biotine was added after sterilization.

[0119] The initial pH of the fermentation was 6.0 and the temperature was fixed at 30°C. The duration of the fermentation was around 60 to 70 hours.

5 **EXAMPLE 3: Isolation of genomic DNA.**

[0120] The preparation of genomic DNA from mycelium of *A. ficuum* was based on the protocol of Blin and Stafford (Nucl. Ac. Res., 1976, 3:2303).

10 [0121] The mycelium was washed with 100% ethanol, dried in a vacuum desiccator and ground to powder under liquid nitrogen. The powder was resuspended in extraction buffer (50 mM Tris-HCl pH 7.4, 10 mM MgCl₂, 50 mM NaCl, 1% SDS) and incubated at 55 °C for 15 min.

15 [0122] Phenol/chloroform (1/1 v/v) was added and the solution was placed on a rocking platform at room temperature for 30 min. The mixture was centrifuged 15 min at 3,000 rpm. The DNA phase was extracted again with phenol/chloroform and then with chloroform.

20 [0123] The DNA was precipitated with the addition of 0.1 volume of 3 M NaAc and 0.5 volume of isopropanol at room temperature.

[0124] After centrifugation the pellet was washed with 70% ethanol, briefly dried and dissolved in TE-buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

25 [0125] To remove residual RNA, 100 µg DNase-free RNase A was added and the solution was incubated for 30 min at 37°C. SDS to a final concentration of 0.5% and predigested proteinase K to a final concentration of 50 µg/ml were added to the DNA solution for 1 hour at 50°C.

30 [0126] Extraction was performed once with an equal volume of phenol/chloroform and once with chloroform. The DNA was precipitated again, centrifuged, washed with 70% ethanol and redissolved in TE-buffer.

EXAMPLE 4: PCR amplification on genomic DNA.

[0127] Based on the amino-terminal sequence (SEQ ID NO 46) and on the sequence of internal peptide (1) (SEQ ID NO 47), a set of degenerated oligonucleotides primers was synthesized.

NH₂-terminal sequence (SEQ ID NO 46):

A V V Q F Q L D L T

forward PCR-primer (SEQ ID NO 37):

5' GTI GTI CAG TTT CAG YTI GAT YTI AC 3'

internal sequence 1 (SEQ ID NO 47):

S E D Q A G D Y T I

R reverse PCR-primer (SEQ ID NO 38)

3' CTY CTR GTY CGI CCI CTG ATG TGI TA 5'

[0128] These two primers were used for PCR amplification. PCR was performed on 100 ng genomic DNA of *A. ficuum* DSM932.

[0129] The DNA was suspended in 100 µl of thermophilic buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100; Promega Corporation, Madison, WI), supplemented with 0.2 mM dNTP, 3 mM MgCl₂, 50 pmoles of each primer and 1.5 units of Taq-DNA-polymerase (Promega Corporation).

[0130] The temperature scheme for the amplification was as follows: 10 min denaturation at 95°C, hot start at 80°C for the Taq-DNA-polymerase, touch-down for two cycles starting at 95°C for 30 sec and 67°C for 2 min. This was repeated with a gradual decline to 65, 63 and 61°C instead of 67°C. The final cycling parameters were: 95°C 30 sec, 60°C 30 sec and 72°C 1 min (35 cycles). The reactions were carried out in a Biometra "Trio-Thermoblock" thermocycler (Biometra, Göttingen, Germany).

EXAMPLE 5: Sequence analysis of the PCR products.

[0131] The PCR products were cloned in the pUC18 vector (Yanisch-Perron & al, 1985, Gene, 33: 103). This plasmid (purified on a Qiagen column (Qiagen Inc., Chatsworth, CA)) was digested with SmaI (New England Biolabs Inc., Beverly, MA), generating blunt ends.

[0132] After extraction with phenol/chloroform, the blunt ends were dephosphorylated with calf intestine alkaline phosphatase (Boehringer) in 50 mM Tris-HCl - 0.1 mM EDTA-buffer (pH 8.5) at 37°C for 30 minutes. Electrophoresis through a 1 % agarose gel was performed and the dephosphorylated vector band was eluted and purified by the GeneClean method (Bio 101, La Jolla, CA).

[0133] The PCR reaction mixtures were initially purified on a Qiagen column. The ends of the PCR DNA fragments were filled in with 0.2 mM dNTP using Pfu-DNA-polymerase (Stratagene, La Jolla, CA) and T4-DNA-polymerase (Boehringer) in Pfu-buffer (20 mM Tris-HCl pH 8.75, 10 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄, 0.1% Triton X-100, 0.1 mg/ml bovine serum albumin; Stratagene) at 37°C for 45 min.

[0134] After a new Qiagen purification, the blunt ends of the PCR fragments were phosphorylated at 37°C for 30 min with 0.2 mM rATP using T4-polynucleotide kinase (Amersham, Buckinghamshire, England) in kinase buffer (20 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM β-mercaptoethanol), supplemented with 6% polyethylene glycol 8,000 and 8 mM MgCl₂.

[0135] After electrophoresis through a 0.8% agarose gel, the 1,150 bp DNA fragments was eluted and purified by the GeneClean method.

[0136] The eluted DNA fragments were ligated in the pUC18 vector using T4-DNA-ligase (Boehringer) in ligase buffer (66 mM Tris-HCl pH 7.5, 5 mM MgCl₂, 1 mM DTE, 1 mM ATP; Boehringer) at 18°C for 16 hours.

[0137] The ligation mixtures were transformed in the *E. coli* strain RR1ΔM15. The colonies obtained were submitted to a DNA extraction following the Birnboim procedure (Birnboim & al, 1979, Nucl. Ac. Res, 7: 1513). The DNA was analyzed by
5 EcoRI - HindIII digestion and electrophoresis through a 1.2% agarose gel.

[0138] The plasmid DNA (Qiagen purified) of a positive clone was sequenced following the ABI Taq DyeDeoxy Terminator Cycle Sequencing protocol (ABI, Foster City, CA). The samples
10 were run on an automated ABI373A sequencing system (ABI). The DNA sequences obtained were converted to ASCII format and transferred to a HIBIO DNASIS software package (Pharmacia LKB Biotechnology, Uppsala, Sweden) for assembly.

15 **EXAMPLE 6: Construction of a genomic DNA library of *A. ficuum* DSM932.**

[0139] Genomic DNA (5 μg) of *A. ficuum* DSM932 was partially digested with the restriction enzyme AluI (7.5 units) for 15 min. The DNA ends obtained were further polished
20 with T4-DNA-polymerase (1 unit) and dNTP nucleotides (final concentration of 100 μM of each) for 5 min at 37°C. The partial digest was then extracted with phenol/chloroform and size-fractionated on a 0.7% agarose gel.

[0140] The genomic DNA fragments with a length of 9,000 to 23,000 bp were eluted from the gel by centrifugal filtration (Zhu & al, 1985, Bio/Technology, 9: 1014; membrane type GV, 0.22 μm, Millipore Intertech, Bedford, MA), extracted with phenol/chloroform and concentrated by ethanol precipitation.

30 [0141] Sfi I adaptors (5'-GTTGGCCTTTT) (SEQ ID NO 52) were ligated to the DNA ends. The ligation reaction was performed in PEG buffer (25 mM Tris-HCl pH 7.5, 5 mM MgCl₂, 2.5 % (w/v) PEG 8,000, 0.5 mM DTT, 0.4 mM ATP), with 24 units

of T4 DNA ligase and 250 pmoles of phosphorylated Sfi I adaptors in a volume of 50 μ l overnight at 12°C.

[0142] After extraction with phenol/chloroform, the SfiI - SfiI genomic DNA fragments were purified and separated again on a 0.7% agarose gel. Portions of the gel containing fragments from 10 to 20 kb length were cut out, membrane-eluted and concentrated by ethanol precipitation. These fragments (100 ng) were finally cloned into the SfiI-digested YCp50SfiI-SfiI vector (an E. coli/S. cerevisiae shuttle vector derived from the plasmid YCp50 (Trash & al, 1985, Proc. Nat. Acad. Sci. USA, 82: 4374) bearing an EcoRI - HindIII fragment, that contains the hIFN β gene flanked by two SfiI sites in inverse direction).

[0143] The ligations were performed in a volume of 20 μ l at 12°C for 4 hours with 8 units T4 DNA ligase (Pharmacia LKB Biotechnology, Uppsala, Sweden), further extracted with phenol/chloroform and finally electroporated in freshly prepared MC1061 cells.

[0144] Bacteria were plated on LB agarose and the resulting colonies were scraped off from the plates in groups of 1,000 clones. The library size was approximately 100,000 clones and the average insert size was $\pm$ 16 kb.

EXAMPLE 7: Screening of the genomic DNA library by colony hybridization.

[0145] The 1,150 bp fragment was isolated from the pUC18 vector by EcoRI-HindIII digestion, agarose gel electrophoresis, elution and purification by the Geneclean method.

[0146] The fragment was randomly labeled with α 32P-dCTP using the procedure of Feinberg and Vogelstein (Anal. Biochem, 1984, 137:.266). 75 ng of the 1,150 bp fragment were boiled for 10 minutes and immediately chilled on ice. The labeling

reaction was performed at 37°C for 30 minutes in a total volume of 60 μ l using 6 units of Klenow polymerase, dGTP, dATP and dTTP (25 μ M of each), a hexanucleotide mixture (Random Primed DNA Labeling Kit from Boehringer) and 45 pmoles α 32P-dCTP (Amersham, Buckinghamshire, England). The reaction mixture was then dialyzed for 90 minutes against bidistilled water using a membrane filter (type VS, 0.025 mm; Millipore Intertech, Bedford, MA).

[0147] Groups of clones of the genomic DNA library of *Aspergillus ficuum* DSM932 were spread out onto LB plates and overnight incubated at 37°C, resulting in 2,000 to 6,000 colonies per plate. These colonies were submitted to colony lifting on nylon membranes (Hybond N, Amersham) and UV-cross-linking (UV StratalinkerTM 1800 from Stratagene), followed by hybridization with the radioactively labeled probe. The prehybridization and hybridization steps were performed at 62°C in 7% SDS-phosphate buffer (1 mM EDTA, 0.5 M NaHPO₄ pH 7.2 and 7 % SDS), the washing steps at 62°C in 5% SDS-phosphate buffer (1 mM EDTA, 40 mM NaHPO₄ pH 7.2, 5 % SDS). Filters were finally exposed to X-ray films.

EXAMPLE 8: Subcloning of the genomic DNA.

[0148] The plasmid DNA ($\pm$ 10 μ g; Qiagen purified) of positive clones, obtained after screening of the genomic DNA library, was sonicated (Vibra Cell VC500; Sonics & Materials, Danbury, CT) on ice in sonication buffer (1 M tetramethyl ammonium chloride, 2 mM EDTA, 50 mM Tris-HCl pH 7.6) for 30 seconds (50% duty cycle).

[0149] After precipitation with isopropanol, the DNA was blunted using 5 units each of T4- and Klenow-DNA polymerase (Boehringer) in T4 DNA polymerase buffer (50 mM Tris-HCl pH 8.5, 15 mM (NH₄)₂SO₄, 7 mM MgCl₂, 0.1 mM EDTA, 10 mM β -mercaptoethanol; Boehringer), supplemented with 1 mM dNTP, at 37°C for 30 min. The fragments were size-fractionated

by electrophoresis on a 1% agarose gel and the DNA of the portion of the gel comprising fragments from 800 to 1,200 bp was eluted and purified using the Geneclean method.

[0150] The blunted fragments were introduced into the dephosphorylated SmaI site of pUC18. Ligation was performed (molar ratio of vector/insert 1/3) using T4 DNA ligase (Pharmacia LKB Biotechnology) in blunt-end buffer (25 mM Tris-HCl pH 7.5, 5 mM MgCl₂, 2.5% (w/v) PEG 8,000, 0.5 mM DTT, 0.4 mM ATP) at 23°C for 4 hours. The ligation mixes were transformed into E. coli MC1061 to generate two libraries of subclones.

EXAMPLE 9: DNA preparation for *Aspergillus* transformation.

[0151] Plasmids were isolated by a modified protocol based on the 'cleared lysate' procedure of Kahn & al. (Meth. Enzymol., 1979, 68: 268) and purified by equilibrium centrifugation in a cesium chloride - ethidium bromide gradient.

[0152] A one liter culture (TB medium) was centrifuged in a GSA Sorvall rotor for 10 min at 5,000 rpm. The pellet was resuspended in 15 ml of lysing buffer (25% sucrose, 50 mM Tris-HCl pH 8, 20 mM EDTA) and 0.6 ml of a lysozyme suspension (10 mg lysozyme/ml H₂O; Boehringer) was added.

[0153] After 30 min incubation on ice 15 ml of 2% Triton X-100 were mixed with the suspension and again incubated on ice for 30 min.

[0154] Then, the suspension was centrifuged in a SS34 Sorvall rotor for 30 min at 20,000 rpm. Cesium chloride (1.1 g cesium chloride/ml supernatant) as well as 1500 µl of ethidium bromide (5 mg/ml) were added. This suspension was ultracentrifuged at 25°C and 55,000 rpm for 16 hours.

[0155] After isolation of the plasmid DNA band, the remaining ethidium bromide was removed by several extractions with an 1/1 volume of isobutanol (centrifugation at 10,000 rpm

for 10 min) and the DNA was further precipitated (several times) with 0.6 volume of isopropanol, washed with 70 % ethanol and finally resuspended in 200 μ l of H₂O.

5 **EXAMPLE 10: Transformation of *Aspergillus nidulans*.**

[0156] The transformation of *A. nidulans* was based on the procedure of Yelton et al. (Proc. Nat. Acad. Sci. USA, 1984, 81:1470) and included firstly the protoplasting of the mycelium by lytic enzymes (20 mg/g mycelium; Sigma Chemical, 10 St. Louis, MO) and secondly the transformation itself using a polyethylene glycol treatment.

[0157] 107 protoplasts were transformed with 1 μ g DNA of pSal23 selection plasmid and 19 μ g DNA of the plasmid of interest. The protoplasts were spread onto *Aspergillus* minimal 15 medium plates [containing 1.2 M sorbitol, 1.5% Noble agar (Difco Laboratories, Detroit, MI) and biotin (1 μ g/ml) but no arginin] included in a top agar (same composition as the plates, but with 0.8% agar). The plates were incubated at 37°C until sporulating colonies appeared ($\pm$ three days). Spores of 20 these transformants were restreaked several times onto the same selection medium to obtain single colonies.

EXAMPLE 11: Small scale genomic DNA preparation.

[0158] 108 spores were inoculated into 20 ml of 25 *Aspergillus* minimal medium supplemented with biotin (1 μ g/ml) and incubated overnight at 37°C and 240 rpm.

[0159] Genomic DNA was isolated using a modified protocol based on the procedure of Raeder and Broda (Lett. Appl. Microbiol., 1985, 1:17). The mycelium was harvested by 30 filtration using a folded filter (Schleicher & Schuell, Dassel, Germany) and washed with H₂O, with 0.5 M EDTA pH 8 and finally with 100% ethanol. The mycelium pellet was dried under vacuum for several hours and then ground to powder under

liquid nitrogen. 100 mg of mycelium were resuspended in 500 μ l of lysing buffer (200 mM Tris-HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5 % SDS) and extracted twice with one volume of phenol/ chloroform/isoamylalcohol (25/24/1 - v/v/v) and once
5 with chloroform/ isoamylalcohol (24/1 - v/v). The DNA was precipitated with 0.6 volume of isopropanol (after addition of 0.1 volume of 3 M potassium acetate pH 4.8), washed with 70% ethanol and resuspended in H₂O with RNase A (50 μ g/ml; Sigma Chemical). After incubation at 37°C for 30 min, the DNA is
10 stored at -20°C.

EXAMPLE 12: Southern blot analysis.

[0160] 10 μ g of *Aspergillus* genomic DNA of were digested with 120 units of Eco RI (New England Biolabs Inc.,
15 Beverly, MA) at 37°C overnight in the appropriate EcoRI buffer (total volume of 200 μ l). A 0.8 % agarose gel was run and stained with ethidium bromide.

[0161] After electrophoresis, the gel was incubated in 0.25 M HCl for 15 min. Transfer of the DNA fragments onto a
20 Hybond N+ filter was performed using the 'alkali blotting' protocol of Amersham.

[0162] The filter was hybridized with the 1,150 bp DNA fragment described above, which was labeled with α 32P-dCTP. The prehybridization and hybridization steps were performed at
25 68°C in 7% SDS-phosphate buffer (1 mM EDTA, 0.5 M NaHPO₄ pH 7.2 and 7% SDS). The filter was washed twice with 5% SDS-phosphate buffer (1 mM EDTA, 40 mM NaHPO₄ pH 7.2, 5% SDS) and once with 1% SDS-phosphate buffer (1 mM EDTA, 40 mM NaHPO₄ pH 7.2, 1% SDS) at 68°C and finally exposed to X-ray film.

30

EXAMPLE 13: Enzymatic assay

[0163] Enzymatic assay was performed in microtiter plates using N,N-dimethyl-p-phenylenediamine as substrate. 100

μ l of substrate solution (0.4 mg of N,N-dimethyl-p-phenylenediamine per ml of 40 mM sodium acetate pH 5.3) were added to 25 μ l sample at the appropriate dilution and incubated at room temperature protected from light. The optical density was measured at 550 nm.

[0164] The activity was expressed arbitrarily as Δ Abs/sec.

[0165] For tea color modification studies, the laccase activity was determined with 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS - Sigma-Aldrich Chemie, Germany) as substrate. After oxidation, its greenish-blue color was measured photometrically at 415 nm. Enzymatic assay were performed in microtiter plates using 75 μ l of 1.66 mM ABTS in 100 mM Citrate buffer pH 4 which were mixed with 25 μ l of supernatant and incubated during 10 min at 50°C.

[0166] 1 laccase unit (LACU) is the amount of enzyme that catalyzes the conversion of 1 μ mole ABTS per minute in these conditions.

EXAMPLE 14: Deglycosylation with PNGase F.

[0167] To a concentrated protein suspension (final volume of 90 μ l) 10 μ l of denaturation buffer (5% SDS, 10% β -mercaptoethanol) was added and the mix was boiled for 10 min at 100°C.

[0168] After addition of 12 μ l of NP40 (10 %) and 12 μ l of 0.5 M sodium phosphate pH 7.5, the sample was divided into two aliquots. 3000 units of PNGase F (peptide N-glycosidase F; New England Biolabs Inc.) were added to the first sample. The other one was used as negative control.

[0169] Incubation was performed overnight at 37°C. After addition of 2 x Laemmli buffer (Nature, 227:680(1970)) and boiling for 5 min, the samples were run on a 12.5% SDS-polyacrylamide gel.

[0170] The protein bands were visualized by Coomassie blue staining.

EXAMPLE 15: Exchange of the *Aspergillus ficuum* DSM932 oxidase gene promoter by the *gpdA* promoter of *A. nidulans*.

[0171] The gene, encoding the oxidase protein, was isolated from the YCp50 SfiI/SfiI vector by a BamHI-SspI digestion and ligated to the plasmid pMa58 (Stanssens & al, 1989, Nucl. Ac. Res., 17: 4411) prepared in the following way: the pMa58 plasmid was digested with Hind III and the sticky ends were filled in with dNTP by T4 DNA polymerase and further digested with BamHI and PvuI. The resulting plasmid was termed pMa58Afic. A BspHI site was then created at the position of the ATG codon of the oxidase gene via site-specific mutagenesis, based on the method of Deng and Nickoloff (Anal. Biochem, 200: 81 (1992); TransformerTM Site-Directed Mutagenesis Kit of Clontech Inc., CA, USA), resulting in plasmid pMac58Aficm.

[0172] Via an XbaI digest on pMac58Aficm, followed by blunting the sticky ends with dNTP by T4 DNA polymerase, and a BspHI digest the oxidase gene was isolated and ligated into the pFGPDGLAT2 vector. The pFGPDGLAT2 plasmid contains the glyceraldehyde -3-P dehydrogenase promoter of *Aspergillus nidulans* that allows a strong constitutive transcription of the genes located downstream of it (Punt & al, 1990, Gene, 93:..101; Punt & al, 1991, J. Biotechnol. 17:..19).

[0173] pFGPDGLAT2 was digested first with HindIII, treated with T4 DNA polymerase and finally digested with NcoI. The BspHI - XbaI fragment of pMac58Aficm was then ligated to this vector to generate the plasmid pFGPDafic.

EXAMPLE 16: PCR analysis of *Aspergillus* transformants.

[0174] Genomic DNA was isolated from the transformants as described. The oligonucleotides used for the PCR reaction were the following : 5' GAA GTG GAA AGG CTG GTG TGC (SEQ ID NO 51), corresponding to a partial sequence of the *gpdA* promoter, and 5' CAA CCC AGG TAC CGT ACT CC (SEQ ID NO 39), corresponding to a partial sequence of the oxidase gene.

[0175] 50 ng of genomic DNA was suspended in 50 μ l of thermophilic buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100; Promega Corporation), supplemented with 0.2 mM dNTP, 3 mM MgCl₂, 25 pmole of each primer and 1.5 units of Taq-DNA-polymerase (Promega Corporation). The temperature scheme for the amplification was as follows: After a 10-min denaturation at 95°C, a hot start at 80°C was used for the Taq-DNA-polymerase. 30 cycles of [95°C 30 sec, 65°C 30 sec, 72°C 2 min] were performed. The reactions were carried out in a Biometra "Trio-Thermoblock" thermocycler (Biometra). The PCR products were analyzed by electrophoresis on a 0.8% agarose gel.

EXAMPLE 17: Purification of an enzyme with oxidase activity from *Aspergillus ficuum* DSM932.**1. Obtention of the desalted cell-free supernatant**

[0176] A 10 l fermentation of the strain *Aspergillus ficuum* DSM932 was performed for 68 hours as described in example 2.

[0177] The supernatant of the culture was separated from the mycelium by centrifugation. It was desalted by passing through a 6 liters Sephadex G-25 column (Amersham Biosciences) equilibrated in 10 mM Tris-HCl, pH 7.0 buffer (buffer A). The same buffer was used to elute the proteins from the column. The final volume of the oxidase containing sample was 25 liters.

2. Chromatography on DEAE Macro Prep.

[0178] The sample of step 1 was applied on a 1l DEAE Macro Prep (Bio-Rad) column equilibrated with buffer A. Flow rate was 145 ml/min. After washing with the same buffer, the bound proteins were eluted by increasing stepwise the NaCl concentration in buffer A to give the following fractions: fraction 1 eluted with 1.5 l buffer A + 50 mM NaCl, fraction 2 eluted with 1.5 l buffer + 60 mM NaCl, fraction 3 eluted with 1.5 l buffer A + 110 mM NaCl, fraction 4 eluted with 1.5 l buffer 1 + 200 mM NaCl and fraction 5 eluted 1.8 l buffer A + 500 mM NaCl. Oxidase activity was detected in fraction 5.

3. Chromatography on S-Sepharose.

[0179] The buffer of 200 ml of fraction 5 from step 2 was exchanged to buffer B (15 mM ammonium acetate, pH 4.0) by passing through a Sepharose G-25 (Amersham Biosciences) column equilibrated in the same buffer.

[0180] The 260 ml eluted from G-25 column were applied on a 20 ml Hiload S-Sepharose HP 16/10 column (Amersham Biosciences) equilibrated in buffer B.

[0181] After washing with buffer B, proteins were eluted with a linear gradient of NaCl in buffer B (250 ml from 0 to 250 mM NaCl at 5 ml/min). 5 ml fractions were collected and tested for oxidase activity.

[0182] Active fractions (10 ml) were pooled and concentrated with Centriprep YM-30 centrifugal filter unit (Millipore). Buffer was exchanged to buffer C (50 mM MES - pH 5.5 with NaOH). The final volume was 400 μ l.

4. Chromatography on Resource Q.

[0183] 200 μ l from step 3 were applied on a 1 ml Resource Q column (Amersham Biosciences) equilibrated in buffer C.

[0184] After washing with buffer C, proteins were eluted with a linear gradient of NaCl in buffer C (15 ml from 0 to 175 mM NaCl at 1 ml/min).

5 5. Chromatography on TSKG3000SW and TSKG2000SW.

[0185] Fractions with oxidase activity from step 4 (3.5ml) were concentrated by centrifugation to 400 μ l on a Centriprep YM-30 centrifugal filter unit (Millipore). Thereafter, 100 μ l were applied on TSKG3000SW and TSKG2000SW
10 columns (Tosohaas) mounted in series and equilibrated in buffer D (20 sodium phosphate, 200 mM NaCl, pH 6.5). The proteins were eluted with buffer D at 0.5 ml/min. Active fractions were pooled (1.5 ml). 1 ml was desalted using a Microcon YM-10 centrifugal filter unit and concentrated to a
15 volume of 200 μ l.

6. SDS-PAGE electrophoresis

[0186] The purified sample from step 5 was loaded on a 10-15% SDS polyacrylamide gel (PhastGel Gradient - 10-15;
20 Amersham Biosciences) and run according to the recommendations of the manufacturer. One half of the gel was stained using the PhastGel Silver staining kit (Amersham Biosciences) and the other half was subjected to a zymogram analysis.

[0187] For this purpose the gel is incubated in a N,N-dimethyl-p-phenylenediamine solution (Sigma Chemicals; 0.4
25 mg/ml of 40mM sodium acetate pH 5.3) at room temperature. Proteins with oxidase activity appear black.

[0188] The results of this analysis are presented on figure 1. It shows that the enzyme with oxidase activity is
30 pure and that it exhibits oxidase activity. The apparent molecular weight of the purified oxidase is about 90 kDa.

EXAMPLE 18: Determination of the amino acid sequence of the enzyme with laccase activity

[0189] General procedures were followed to perform the N-terminal sequencing of the protein after electrophoresis on a 12% SDS-polyacrylamide gel and electroblotting on a PVDF Immobilon-P membrane (Millipore). An automatic 477A Protein Sequencer coupled to a HPLC 120A Analyser (Applied Biosystem) was used.

[0190] For the determination of the sequence of internal fragments, the protein was first digested on the membrane with trypsin. The resulting peptides were separated by reverse phase chromatography on HPLC, and subjected to N-terminal sequencing as above.

[0191] The following sequences have been obtained:

- N-terminal: A V V Q F Q L D L T Y E D V S V A G X V X K A I V L N G X I (SEQ ID NO 48)
- Internal peptide 1: S E D Q A G D Y T I R (SEQ ID NO 47)
- Internal peptide 2: A S Q Y X S Y I Y H S H T R (SEQ ID NO 50)

EXAMPLE 19: cloning of an oxidase encoding gene from *Aspergillus ficuum* DSM932

Partial cloning of an oxidase gene from *A. ficuum* DSM932.

[0192] Based on known partial protein sequences encoded by the oxidase gene, the polymerase chain reaction (PCR) was used to isolate a DNA fragment, which has then be used as a probe to screen a genomic DNA library of *A. ficuum* DSM932. A set of degenerated primers (oligonucleotides) were synthesized based on the amino-terminal sequence and on the sequence of an internal peptide of the purified protein.

[0193] These nucleotide primers were:

- forward PCR-primer 5' GTI GTI CAG TTT CAG YTI GAT YTI AC 3' (SEQ ID NO 37);
- reverse PCR-primer 3' CTY CTR GTY CGI CCI CTG ATG TGI TA 5' (SEQ ID NO 38).

5 [0194] These two primers were used for PCR amplification on the genomic DNA of *A. ficuum* DSM932. The PCR reaction mixtures were analyzed directly by electrophoresis through a 1.3% agarose gel, followed by ethidium bromide staining.

10 [0195] Two weak DNA bands of $\pm$ 550 and 700 bp and one strong DNA band of 1,150 bp were generated.

[0196] Nucleotide sequence analysis revealed the relation between the 1,150 bp PCR product and the purified enzyme with oxidase activity.

15 [0197] For this, the PCR products were cloned in the pUC18 vector as described in example 5. Sequencing with the "universal" forward and reverse oligonucleotides primers (compatible with the insert-flanking regions of the vector) resulted in sequence determination of about 400 nucleotides on
20 both sides of the insert. These nucleotide sequences were translated to peptide sequences. These peptides sequences included the sequence YEDVSVAGKVXXAIVLNG (SEQ ID NO 49), corresponding to a part of the amino terminal sequence determined in example 18 (from amino acid 11 to 28, SEQ ID NO
25 48) and the sequence ASQYXSIIYHSHTR (SEQ ID NO 50) corresponding to the sequence of the internal peptide 2 described in example 18.

30 Cloning of the entire oxidase encoding gene of *A. ficuum* DSM932.

[0198] Using PCR amplification, groups of clones of the genomic DNA library, containing plasmids with the oxidase gene, were found.

[0199] The PCR reaction was performed on plasmid DNA (80 ng), extracted by the Birnboim procedure from 20 pools of the library, each of about 1,000 clones. The resulting PCR products were analyzed by electrophoresis through a 1.2% agarose gel.

[0200] Six groups of clones generated a PCR product of the expected size, 1,150 bp, the same length as obtained from the original PCR amplification on the genomic DNA of *Aspergillus ficuum* DSM932. Three of these positive pools were further screened by colony hybridization using the radioactively labeled 1,150 bp DNA fragment as probe. Five positive signals were obtained after autoradiography detection.

[0201] The positive clones were further purified by a new cycle of colony hybridization, and by PCR amplification of the 1150 bp fragment from the purified plasmids. This finally resulted in the isolation of two single positive clones.

[0202] The DNA of the positive clones was analyzed by *Sfi*I digestion, resulting in the release of the genomic insert from the YCp50 vector.

[0203] One clone showed after electrophoresis on a 0.8 % agarose gel a genomic insert of a length of 11,000 bp and the other one an insert of more than 12,000 bp. Both inserts contained an internal *Sfi*I cleavage site. The plasmid with the 12,000 bp insert was termed pAFLAC and has been deposited as an *E. coli* MC1061 transformant at the LMBP collection with reference number LMBP4366.

[0204] BELDEM S.A., who has registered office at B-5300 Andenne (Belgium) Rue Bourrie, 12, has made on 11 May 2001 (under the expert solution) of the microorganism *Escherichia coli* MC1061(pAFLAC) according to the invention, at the BCCM/LMBP Culture Collection (Laboratorium voor Moleculaire Biologie, Universiteit Gent, 'Fiers-Schell-Van Montagu' building, Technologiepark 927, B-9052 Gent-Zwijnaarde,

Belgium). This deposit has received the accession number LMBP 4366.

Sequence analysis of the *A. ficuum* DSM932 oxidase gene.

5 [0205] Genomic DNA inserts from pAFLAC were sheared and subcloned in the pUC18 plasmid vector. The ligation mixes were transformed into *E. coli* MC1061 to obtain two libraries of subclones. Subclones, containing part of the desired gene, were selected after colony hybridization using the
10 radioactively labeled 1,150 bp PCR fragment as probe.

[0206] Purified (Qiagen) plasmid DNA of 12 positive subclones was further sequenced and the DNA sequences obtained were analyzed.

[0207] This random sequencing gave the nucleotide
15 sequence of the first 1,100 bp of the gene as well as 500 bp of the promoter region. To obtain the entire gene sequence, primer walking was performed to sequence the 3' end of the gene. Remaining ambiguities were resolved using custom primers. Only one of the two positive genomic clones (the one
20 with the genomic DNA insert of more than 12,000 bp) appeared to contain the entire gene.

[0208] The nucleotide sequence and the corresponding amino-acid sequence (SEQ ID NO 1 and SEQ ID NO 40) are shown in figure 3. The gene is 1,923 base pairs. It contains two
25 introns of respectively 85 (starting at base pair 245) and 49 (starting at base pair 808) base pairs and encodes an open reading frame of 596 amino acids with a calculated molecular weight of 65,476 Da.

[0209] A search for similarities between the sequence
30 obtained and sequences in databases was performed using the BLAST program (Basic Local Alignment Search Tool; Altschul & al, 1990, *J. Mol. Biol.*, 215, p.403). The closest homologous gene product was a hypothetical protein from *Aspergillus nidulans* FGSC A4 (AN 0878.2 - accession number EAA65907). The

overall homology was 56.7% as found by analysis with the Clustalw program using the default parameters (<http://www.ebi.ac.uk.clustalw>).

5 **EXAMPLE 20: Heterologous expression of the oxidase gene.**

[0210] The fungal strain *Aspergillus nidulans* 2024, which is auxotrophic for biotin and arginin, was chosen as host strain for the expression of the isolated *Aspergillus ficuum* oxidase gene.

10 [0211] Cotransformation of the positive clone (YCp50 vector with the genomic DNA insert of more than 12,000 bp) with the selection plasmid pSal23 (John & al, 1984, Enzyme Microbiol. Technol., 6:.386), containing the argB-gene, was performed.

15 [0212] Whether the resulting transformants (obtained on plates without arginin) had also integrated the positive clone, was checked using PCR amplification and Southern blot analysis.

[0213] Genomic DNA was isolated from cultures of the transformants. This genomic DNA was submitted to a PCR amplification reaction. DNA of *A. ficuum* DSM932 and of the untransformed *A. nidulans* 2024 were used as positive and negative controls, respectively.

25 [0214] The PCR products were analyzed by electrophoresis on a 1.2% agarose gel with ethidium bromide staining.

[0215] The PCR reaction with the DNA of *A. ficuum* DSM932 or the DNA from some transformants generated as expected a 1,150 bp DNA fragment, indicating the *A. ficuum* gene integration in the genome of these transformants. The PCR reaction on the untransformed *A. nidulans* gave no amplification signal. Southern blot analysis confirmed these results.

[0216] The cotransformants showed one or more signals on the blot, indicating one or more integrated *A. ficuum* gene copies.

[0217] A cotransformation efficiency of 60 % was
5 obtained.

[0218] Another phenomenon, observed on the positive transformants, was the change of the spore color. Non-transformed and pSal23-transformed *A. nidulans* colonies showed dark green spores, while transformants with the oxidase gene
10 integrated gave yellow spores.

Analysis of *Aspergillus nidulans* transformants.

[0219] The transformants were checked whether expression of the integrated *A. ficuum* gene was established
15 and whether the synthesized protein was enzymatically active.

[0220] Transformants were grown in *Aspergillus* minimal medium. *A. ficuum* was used as positive control, and the untransformed and pSal23-transformed *A. nidulans* as negative controls.

[0221] The proteins secreted in the medium were analyzed by electrophoresis on a 12.5% SDS-polyacrylamide gel. The culture medium samples were previously concentrated by deoxycholate-trichloroacetic acid precipitation. Proteins were stained on the gel with Coomassie blue. A very large protein
25 band of 85 kDa, with a size close to the size of the *A. ficuum* oxidase, appeared only in the samples of the transformants of *A. nidulans*. The protein secreted by *A. nidulans* is about 5 kDa smaller than the one secreted by *A. ficuum*. This is due to differences in the glycosylation patterns, as shown below.

[0222] These results show that the isolated *A. ficuum* oxidase gene was expressed in *A. nidulans* under the control of its own promoter. More surprisingly, the expression level was
30

even higher in *A. nidulans* transformants than in the wild-type *A. ficuum* DSM932 strain.

[0223] Zymogram was performed to study the enzymatic activity of the expressed protein. Proteins in the culture medium were concentrated and loaded on a SDS-polyacrylamide gel under semi-denaturing conditions (absence of β -mercaptoethanol; no boiling of the samples before loading). After electrophoresis, the gel was incubated in a N,N-dimethyl-p-phenylenediamine substrate solution. The observed protein bands of *A. ficuum* DSM932 and of the *A. nidulans* transformants were firstly pink-colored and became black after a longer incubation time, confirming that the protein could oxidize the substrate and was enzymatically active. The same results were also obtained in solution in an oxidative assay with the same substrate N,N-dimethyl-p-phenylenediamine. The medium of the untransformed *A. nidulans* showed no activity at all.

[0224] The results obtained above demonstrated that the integrated *A. ficuum* gene in the *A. nidulans* transformants code for an enzymatically active oxidase. Some transformants showed more activity than the original *A. ficuum* strain, due to a higher gene expression.

EXAMPLE 21: Glycosylation of the enzyme with oxidase activity.

[0225] *A. ficuum* DSM932 and one *A. nidulans* transformants were grown in *Aspergillus* minimal medium. The secreted proteins in the medium were concentrated and further submitted to a PNGase F treatment. Then, the deglycosylated samples were loaded on a polyacrylamide gel and a Coomassie blue staining was performed.

[0226] A clear shift of the protein pattern was visible. Deglycosylation with PNGase F decreased the molecular weight of the two oxidases to about 70 kDa. This experiment showed that the observed 5 kDa difference in size of the non-

deglycosylated oxidases of *A. ficuum* and *A. nidulans* transformant was indeed due to differences in glycosylation, since deglycosylation resulted into proteins of the same size.

5 **EXAMPLE 22: Expression of the enzyme with oxidase activity in various *Aspergillus* species under the control of the *gpdA* promoter.**

[0227] The promoter of the isolated *A. ficuum* oxidase gene was replaced by the constitutive *gpdA* (glyceraldehyde-3-phosphate dehydrogenase A) promoter of *A. nidulans*, known as a
10 strong fungal promoter (Deng & al, 1992, Anal. Biochem., 200: p.81).

[0228] This plasmid was transformed into *A. nidulans* 2024, *A. niger* N402 and *A. ficuum* DSM932.

15 [0229] The transformation procedure was based on the cotransformation with the pSal23 (arginine selection).

[0230] To transform *A. niger* and *A. ficuum* another selection procedure was used, based on the acetamidase encoding gene (Kelly & al, 1985, EMBO J., 4: 475). In these
20 cases, the plasmid p3SR2, containing the *amdS* gene (encoding the acetamidase) of *A. nidulans*, was used for the cotransformation, resulting in colonies which could grow on acetamide as sole nitrogen source.

[0231] The transformants obtained (146 for *A. nidulans*,
25 89 for *A. niger* and 326 for *A. ficuum*) were analyzed by PCR amplification with a set of two primers. The upstream primer hybridized with a sequence in the *gpdA* promoter and the downstream primer with a sequence in the oxidase gene. A DNA band of 2281 bp (corresponding to the expected size between
30 the sequences of the oligonucleotide primers in the sequences of the *gpdA* promoter and the oxidase gene) was detected in the PCR mix of some transformants. This demonstrated that these

transformants were cotransformed and thus had integrated not only the selection plasmid but also the plasmid pFGPDafic.

[0232] These cotransformants of *A. niger* and *A. ficuum* exhibited the same spores color change, as mentioned above for the *A. nidulans* transformants (grey to light brown spores instead of black - example 4).

[0233] The transformants were further analyzed by SDS-polyacrylamide gel electrophoresis and by the enzymatic assay with the substrate N,N-dimethyl-p-phenylenediamine. The transformants were grown in *Aspergillus* minimal medium (5 ml in a 50 ml plastic tube) for three days and the culture supernatant was submitted to the enzymatic assay and to electrophoresis.

[0234] The best *A. nidulans* transformants showed an expression level 5.5 times higher than the expression obtained with the original oxidase promoter in *A. nidulans*.

[0235] This observation showed that promoter exchange resulted in a higher expression level of the oxidase in *A. nidulans*.

[0236] The best transformants of *A. ficuum* exhibited also a 4-fold increase in comparison with *A. nidulans*, which was transformed with the oxidase, controlled by its own promoter.

[0237] The *A. niger* transformants secreted about the same amount.

EXAMPLE 23: characterization of the oxidase

1. Obtention of an oxidase sample

[0238] One transformant of *Aspergillus ficuum* obtained in the example 22 was selected for further experiments. A 10 l fermentation was performed as described in example 2.

[0239] The supernatant of the fermentation was desalted in the same conditions as described in example 17 and concentrated by ultrafiltration.

5 2. Determination of the optimum pH

[0240] The optimum pH of the oxidase was determined by assaying its activity at various pHs using a sodium phosphate-citrate buffer. The results of this experiment are shown in figure 3.

10 [0241] The optimum pH lies around pH 5.5.

EXAMPLE 24: Tea color modification by laccase

[0242] 30 ml of a green tea solution, made by pre-incubation of commercial tea (Pickwick Green Tea, Douwe
15 Egberts N.V., Belgium) in warm water (1g per 100 ml) during 15 min, were stirred with various amounts of *Aspergillus ficuum* oxidase (0,1 and 10 U per ml of green tea) at 40°C.

[0243] After 1 hour, tea solutions incubated with oxidase became red-brown, and their absorbance spectra were
20 recorded between 350 and 680 nm and compared with that of the control without enzyme.

[0244] The results on Figure 4 clearly show that the use of the oxidase of *Aspergillus ficuum* enhance the color of the tea solution as compared with an untreated sample.

25

EXAMPLE 25: Effect of the oxidase in baking

[0245] Baking trials were performed to demonstrate the positive effect of the oxidase of the present invention in baking. The positive effect was evaluated by the increase in
30 bread volume as compared to a reference, which does not contain this enzyme.

[0246] The oxidase from example 23 was evaluated in mini baking tests consisting of preparing dough with 100g of flour.

[0247] The procedure described is well established and it will be readily apparent to a person skilled in the art that the same results may be obtained by using other protocols or equipments from other suppliers.

- 5 [0248] The ingredients used are listed in table 1 below:

Table 1

Ingredients (g)	RECIPE
Flour (Surbi -Molens van Deinze)	100
Water	57
Instant yeast (Bruggeman-Belgium)	2
Sodium chloride	1.5
Dextrose	6
Fat (Solix (Puratos - Belgium) 80% / Soy oil 20%)	3
oxidase	see Table 2

- [0249] The ingredients were mixed for 3.5 min in a
10 National mixer. 150 g dough pieces were weighed and rested for 20 min at 25°C in plastic boxes.

[0250] The doughs were reworked and rested for a further 20 min. The final proofing time was 60 min at 36°C. The dough pieces were then baked at 220°C for 24 min.

- 15 [0251] The volume of the bread was measured using the commonly used rapeseed displacement method.

[0252] The results of the baking trials with the oxidase are presented in table 2 below and on figure 5:

Table 2

Oxidase units /100 g flour(*)	Rolls volume increase (%) compared to control without oxidase
1225	2
2450	5
4900	9
12250	8

- 20 (*) See example 13 for the enzymatic assay

[0253] The above results show that the oxidase of the present invention has a positive effect on the volume of bread.

5 **EXAMPLE 26: Effect of the oxidase on rye flour dough stickyness**

[0254] A series of doughs was prepared by mixing the following ingredients in a Farinograph mixer at 30°C for 2min.

10 Table 3

Rye flour Werhahn	200g
Wheat flour Duo Ceres	20g
Water (tap at 37°C)	125g
Salt solution (100g NaCl/1000 ml water)	50 ml
Basic improver RB14120 (Puratos)*	8g
Oxidase	see Table 4

* The basic bread improver RB14120 contains alpha-amylase, xylanase, ascorbic acid and citric acid.

[0255] The dough stickyness was measured 5 min after
15 mixing using a Stable Micro Systems TA-XT2i texture analyzer, equipped with a Chen-Hoseney dough stickyness cel.

[0256] The results are presented on table 4 and on figure 6.

20 Table 4

composition	Stickyness (g)	Standard deviation (g)
Reference	51.5	3.0
1360u/100g Rye flour	46.1	1.2
2720u/100g Rye flour	45.2	3.7
4080u/100g Rye flour	46.7	0.9
5440u/100g Rye flour	44.7	3.5

[0257] From these data, it can be seen that the dough stickyness is significantly reduced by the addition of the oxidase according to the present invention.

5

Example 27: effect of the oxidase on rye flour dough consistency.

[0258] The same doughs as described in example 10 were prepared.

10 [0259] The dough consistency was measured using a Physica UDS 200 Rheometer with the following parameters:

- oscillating mode
- temperature = 30°C
- plate-plate system $\varnothing=30\text{mm}$, $d=2\text{mm}$
- 15 - frequency sweep: $\gamma=0.02\%$, F increases linearly from 1 to 50Hz.

[0260] The results obtained at 10 Hz are presented on Table 5 and figure 7.

20 Table 5 :

composition	G' ($\cdot 10^4$)	G'' ($\cdot 10^4$)
Reference	3.88	1.23
1360u/100g Rye flour	3.52	1.14
2720u/100g Rye flour	4.73	1.63
5440u/100g Rye flour	4.90	1.60

[0261] These data show that the dough consistency is significantly increased by the addition of the oxidase of the present invention. Moreover the dough becomes stiffer and
25 permits a better handling and shaping of the dough.

CLAIMS

1. An isolated oxidase polypeptide comprising:

- an amino acid sequence of any of SEQ ID NO 40 to 50, or
- 5 - a fragment of at least 100 amino acids of SEQ ID NO 40, 41 or 45, or
- an amino acid sequence presenting at least 60% identity with the amino acid sequence of any of SEQ ID 40, 41 or 45, or
- 10 - an amino acid sequence presenting at least 70% identity with any fragments of at least 100 amino acids of SEQ ID NO 40, 41 or 45.

2. An isolated nucleic acid molecule encoding a
15 polypeptide according to claim 1.

3. An isolated nucleic acid molecule comprising:

- a nucleotide sequence of any of SEQ ID NO 1 to 36, its complementary form or RNA form, or
- 20 - a nucleotide sequence having at least 70% identity with any of SEQ ID NO 1 to 36, or with the complementary form or RNA form thereof, or
- a fragment of any of SEQ ID NO 1 to 11 of at least 600 nucleotides, or of any of their complementary form or RNA
25 form, wherein said fragment encodes a protein having an oxidase activity, or
- a fragment of at least 20 nucleotides of any of SEQ ID NO 1 to 36, or of any of their complementary form or RNA form.

30

4. An isolated nucleic acid molecule according to claim 3 wherein said fragments of any of SEQ ID NO 1 to 11, or of any of their complementary form or RNA form, encoding a

protein having an oxidase activity, consist of at least 900 nucleotides.

5 5. A vector comprising a nucleic acid molecule according to any of claims 2 to 4.

6. A vector according to claim 5 wherein said nucleic acid molecule according to any of claims 2 to 4 is operatively linked to one or more regulatory sequences.

10

7. A vector according to claim 5 or 6 wherein said regulatory sequence is the *gpdA* promoter of *Aspergillus nidulans*.

15 8. A transformed host cell having incorporated a nucleic acid molecule according to any of claims 2 to 4.

9. A transformed host cell containing a vector according to any of claims 5 to 7.

20

10. A fusion protein comprising an oxidase polypeptide according to claim 1.

25 11. A bread improving composition comprising an oxidase polypeptide according to claim 1.

12. Use of an oxidase polypeptide according to claim 1 in a baking process or as color enhancer.

Figure 1

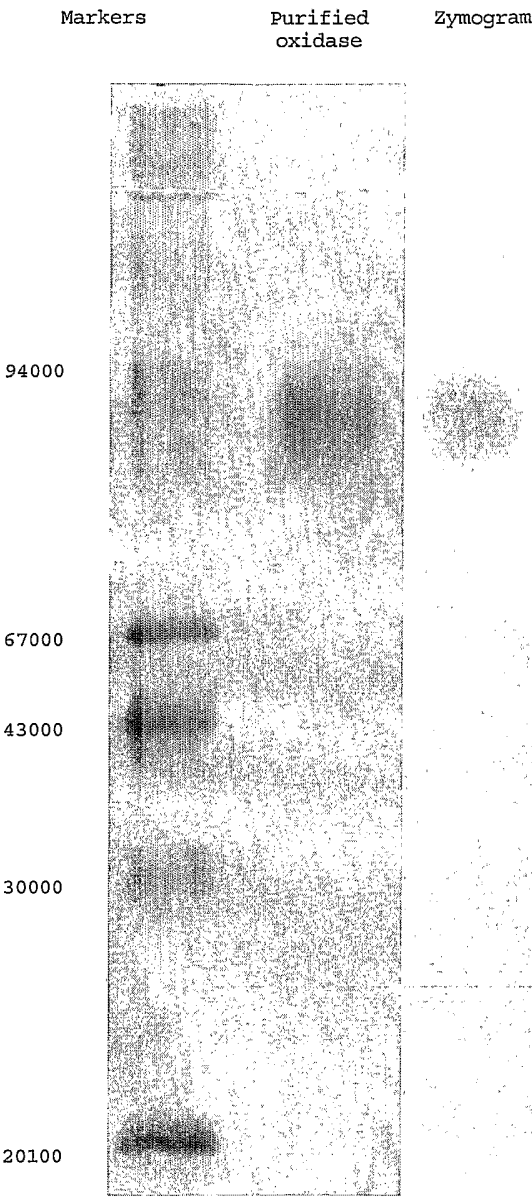


Figure 2

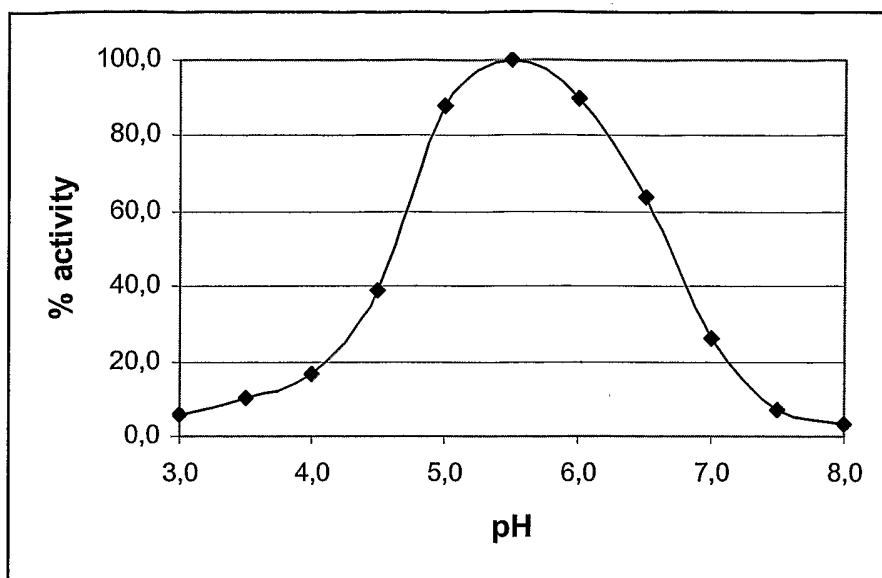


FIGURE 3

5' TAA TTG AAG CTT GCG GTG AGC AAG ACA GTC TGC ACC CTG CTA GAA ATG TTC GTA

9 18 27 36 45 54

TGC CGG TAG GCC CTG CAT ATG ACG GAT CCA GTA TCT TGG CCA TCG GAC GGC ATT

63 72 81 90 99 108

ATG ACA AGC CCT AGT CAT GAA CCT TTC GCT ATA CGT CTC GGG TCC TCC ATC CCT

117 126 135 144 153 162

TGC CTT GAC CTT CGG GCA TCA ATG GCA TCA GGT CAA TGT GCC TCT TGA GTT CTG

171 180 189 198 207 216

TCA TTA TAA AAG CCT CAA ACT TCT CGC AGG ATG AAG GGA ACT CAA CCA CGA CAA

225 234 243 252 261 270

ACC AGT CCG CGA CTC GGC CCT GTT CGA CTG TAT ATA TTC TGT GGA ACT AGG TAG

279 288 297 306 315 324

TTG GTT CCT TGT ATA TTT CTT TAT TTG GTT TTT TGA GCC CCA GCA TGA GTA TAT

333 342 351 360 369 378

M S I S

CTC AAA GCA GGC TGG CCC TGT TGG CCC TTT GCT TCG TGC AAT GGG TGT CCG GCC

387 396 405 414 423 432

Q S R L A L L A L C F V Q W V S G R

GTG TTG TGC AGT TCC AGC TAG ACC TGA CCT ATG AAG ATG TCA GTG TCG CTG GTG

441 450 459 468 477 486

V V Q F Q L D L T Y E D V S V A G D

ATG TAC ACA AGG CTA TTG TGA GTA ATG GAC AGA TTC CTG GGC CTA CGT TGT GGT

495 504 513 522 531 540

V H K A I V S N G Q I P G P T L W L

TGA AGC AGG GTG ATG ATG TCG AAT TTT TGG TCA ACA ACT CGA TGT CAA TCA ATA

549 558 567 576 585 594

K Q G D D V E F L V N N S M S I N T

CTA CGG TTC ATT TTC ATG GTG AGT ACT ACC TCT CGA TTG TAT GAT CCT TTG GCT

603 612 621 630 639 648

T V H F H

TCG ATG TTC AGA ACG CCT TTT CCT ATA GAG CTT TAC TAA AGA TTT GTA GGT ATC

657 666 675 684 693 702

G I

GAG CAA CTA GGC ACC CCG TGG TCA GAT GGT GTA CCT GGA CTC TCG CAG GAG CAG

711 720 729 738 747 756

E Q L G T P W S D G V P G L S Q E Q

ATC AAG CCG GGC GAA CAG TTT CTC TAT AAA TGG AAG GCA AGC CAG TAT GGC TCG

765 774 783 792 801 810

I K P G E Q F L Y K W K A S Q Y G S

FIGURE 3 (bis)

819	828	837	846	855	864
TAC ATT TAT CAC TCG CAC ACA CGG GCT CAG ATT GAT GAT GGC CTC TAT GGG GCT					
Y I Y H S H T R A Q I D D G L Y G A					
873	882	891	900	909	918
ATC TAC ATT GAA CCG GCA GAC TCC GTG GAG AGG CCT TTC CAT TTG ATC GCT GGA					
I Y I E P A D S V E R P F H L I A G					
927	936	945	954	963	972
TCT GAT GCG GAT GAG CAG CAG GCG ATG TTG GCA GCT GAA AAG AAT ACA CGG CCA					
S D A D E Q Q A M L A A E K N T R P					
981	990	999	1008	1017	1026
GTT CTC ATC TCG GAT TGG CGT GCC TTT TCC TCG CAT GAT ATT CTC CAG ATT CAA					
V L I S D W R A F S S H D I L Q I Q					
1035	1044	1053	1062	1071	1080
ACC GAA TCT GGT GTT GAG GCC TAC TGT GCC AAC TCT GTT TTG ATC AAC GGA AAG					
T E S G V E A Y C A N S V L I N G K					
1089	1098	1107	1116	1125	1134
GGC TCG GTG ATT TGC CCG TCT CAG GAA CAC ATC AAT GCT GCC ACG ACA CCG CAG					
G S V I C P S Q E H I N A A T T P Q					
1143	1152	1161	1170	1179	1188
CAA AAG CAG ATC CTG GGC AAC TTG ACT CTG ACT GAT ATG GGG TAT GAA GTC CCA					
Q K Q I L G N L T L T D M G					
1197	1206	1215	1224	1233	1242
AGA CTT GAT GAC GGT TAC AAT GCT GAT CCT GGC TAG ATG CCT GCC TCC CAC GCC					
				C L P P T P	
1251	1260	1269	1278	1287	1296
GGG TGT TGT GGG TCC GTA CCC GTA CGA CCT CAG CAA GAT TCC CAA AGG TTT CTA					
G V V G P Y P Y D L S K I P K G F Y					
1305	1314	1323	1332	1341	1350
CGA AGG ATG CAC ACC TAG TGA AGG GCC CAC AGA GGT TTT CAA GGT CAA CTC CTC					
E G C T P S E G P T E V F K V N S S					
1359	1368	1377	1386	1395	1404
ATC GCG GTA TGC TAG CTT TGA CTT TAT TAG TAT GGC TGG CTC GAC TTC GCT TGT					
S R Y A S F D F I S M A G S T S L V					
1413	1422	1431	1440	1449	1458
GTT CTC CAT CGA TGA GCA TCC GAT GTA TGT TTA CGC GGT CGA TGG CCG TTA TGT					
F S I D E H P M Y V Y A V D G R Y V					
1467	1476	1485	1494	1503	1512
TGA ACC TTT GCT TGT CGA AGC TGT TAC TGT GCC TGT CGC TTC AAG ATA CTC AGT					
E P L L V E A V T V P V A S R Y S V					
1521	1530	1539	1548	1557	1566
TAT GGT GCC ACT CAA GTC GGA AGA CCA GGC AGG AGA TTA TAC TAT CCG CGT TGC					
M V P L K S E D Q A G D Y T I R V A					
1575	1584	1593	1602	1611	1620
CAA TAA CTA TGC GAA CCA GCT CAT CAA CGG GAC CGC CGT CCT GTC GTA TGA CAC					
N N Y A N Q L I N G T A V L S Y D T					

FIGURE 3 (ter)

1629	1638	1647	1656	1665	1674
CGC TAC ACC GAA GCA GAT CGG TAC CTC GCA GCC TTA TAT CAA CGA GGC TGG CGC					
A T P K Q I G T S Q P Y I N E A G A					
1683	1692	1701	1710	1719	1728
TAA TGC CAC TGC CTC CAC GGT CAT TTT GAA CGA AAC CGA CGT GAT ACC CTT CCC					
N A T A S T V I L N E T D V I P F P					
1737	1746	1755	1764	1773	1782
CGT GGT AGC ACC TGC TAA GAA GGC GGA CCG GAC TTA CAT CCT GAA TGT CGA AAA					
V V A P A K K A D R T Y I L N V E N					
1791	1800	1809	1818	1827	1836
TGC CAA TTC ATC CTA CAC CTG GAC CCT GGG CAA CCA GTA CCC CGT GTC GAA TGA					
A N S S Y T W T L G N Q Y P V S N E					
1845	1854	1863	1872	1881	1890
AGA GCT TCG CCC TCC TGT CTT GTT CAA CCT CAG CTC AAT CTC GCA GGC GTA CTC					
E L R P P V L F N L S S I S Q A Y S					
1899	1908	1917	1926	1935	1944
GGC CAT GAC GGA GTA CGG TAC CTG GGT TGA TCT CAT CAT CAA CAT CAC CAC CTC					
A M T E Y G T W V D L I I N I T T S					
1953	1962	1971	1980	1989	1998
GGG CCA GCC TCA ACA CCC CAT CCA CAA GCA CTC CAA CAA GTA CTT CGT GAT CGG					
G Q P Q H P I H K H S N K Y F V I G					
2007	2016	2025	2034	2043	2052
AAC CGG TAA CCA GCC CTT CAT CTG GTC CTC CGT GGA AGA GGC AAT GAA AGA CAT					
T G N Q P F I W S S V E E A M K D I					
2061	2070	2079	2088	2097	2106
ACC GGA GAA TTT CAA CTT CGA AAA CCC CCA GAT GCG GGA CAC TTT CTA CTC GCC					
P E N F N F E N P Q M R D T F Y S P					
2115	2124	2133	2142	2151	2160
AAG CTC AAG CAC AGG GCC GAG CTG GCT GGC AAT GCG CTA CCA TAT CGT GAA CCC					
S S S T G P S W L A M R Y H I V N P					
2169	2178	2187	2196	2205	2214
AGG GCC ATT CCT ACT TCA TTG CCA TCT TCA GAT GCA CCA TGT CGG TGG GTT GGC					
G P F L L H C H L Q M H H V G G L A					
2223	2232	2241	2250	2259	2268
ACT GGC CTT GCT GGA CGG GGT GGA CGC CTG GCC AAC CGA CAT TCC CGA GGG ATA					
L A L L D G V D A W P T D I P E G Y					
2277	2286	2295	2304	2313	2322
TCG GTT GCC AGT GAT GCC AAT CTA GGG ACT TTG ACT CAA TTG TAC AGA GGT GGA					
R L P V M P I *					
2331	2340	2349	2358	2367	2376
CGA GAG TAT CTA CTT GTA ATA TGG CCA TAT AGC GCC TCT CAT ATA GAT CCG GTG					
2385	2394	2403	2412	2421	2430
TAA TAT TAT TTA TTT ACT ACA TAA TGA ACA TGC TTG GTG ACT TAC CAA TTC CCC					

FIGURE 3 (quater)

2439 2448 2457 2466 2475 2484
AGT TAT GGC TCC AAA GTA AAA GTG AGA GCA ATC ACA CCA AAT CAG GCA CAG CAG
2487
TAA 3'

Figure 4

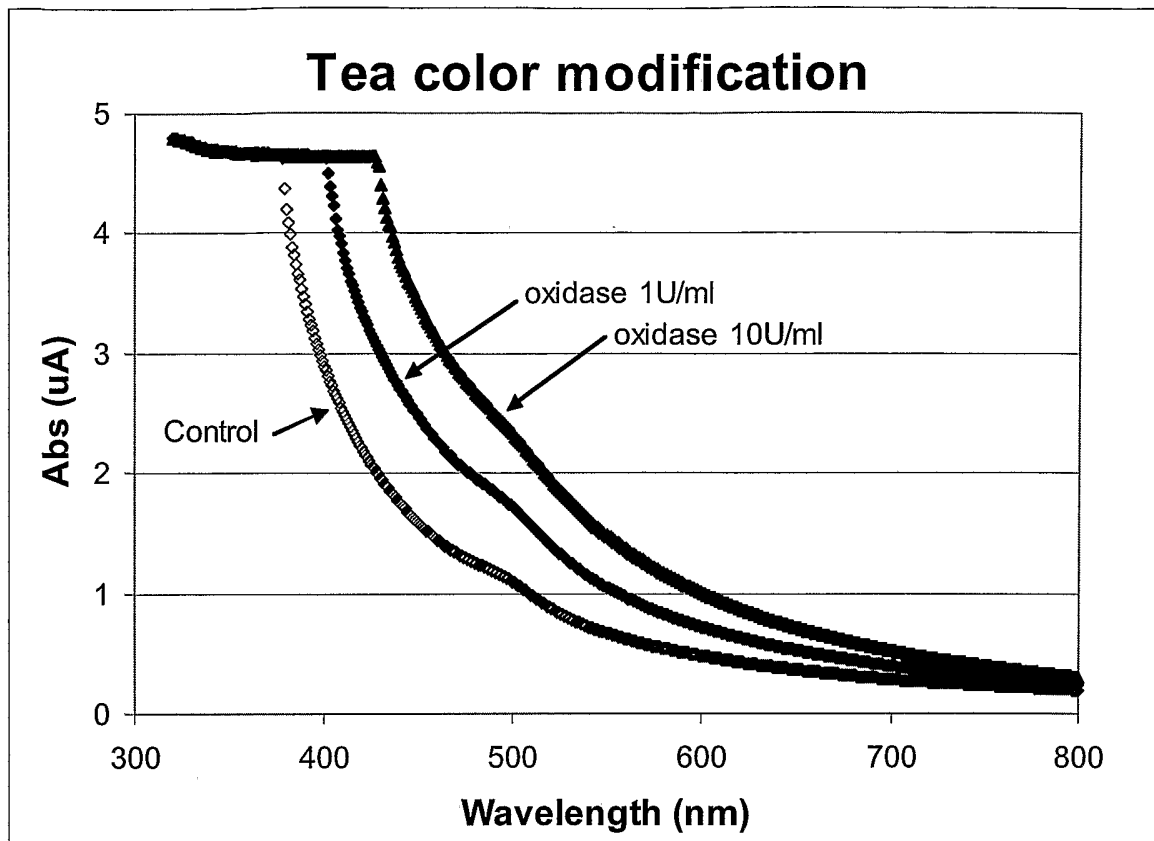


Figure 5

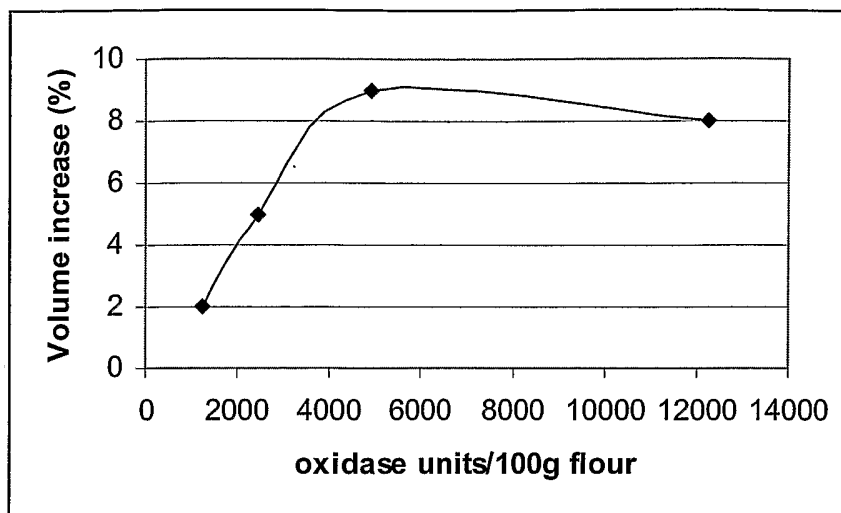


Figure 6

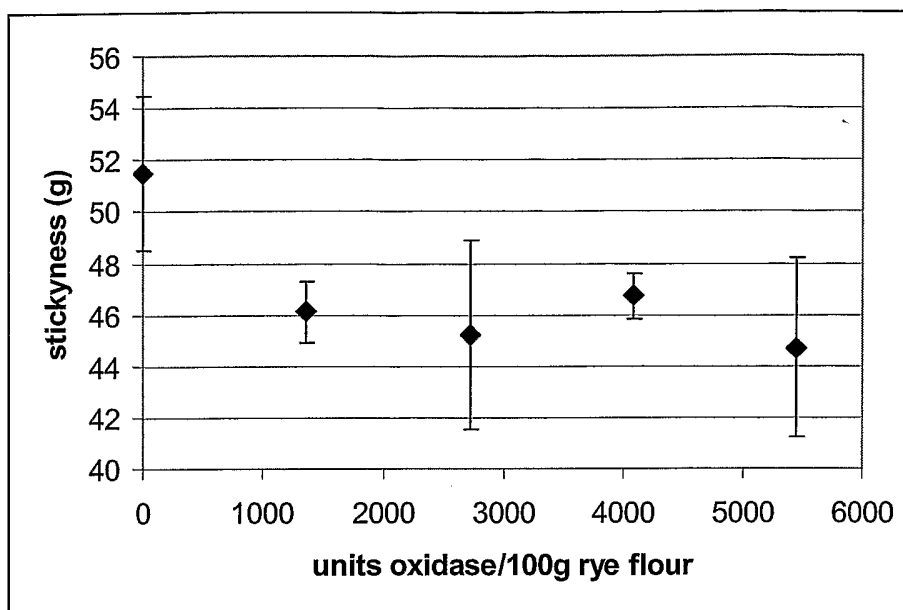


Figure 7

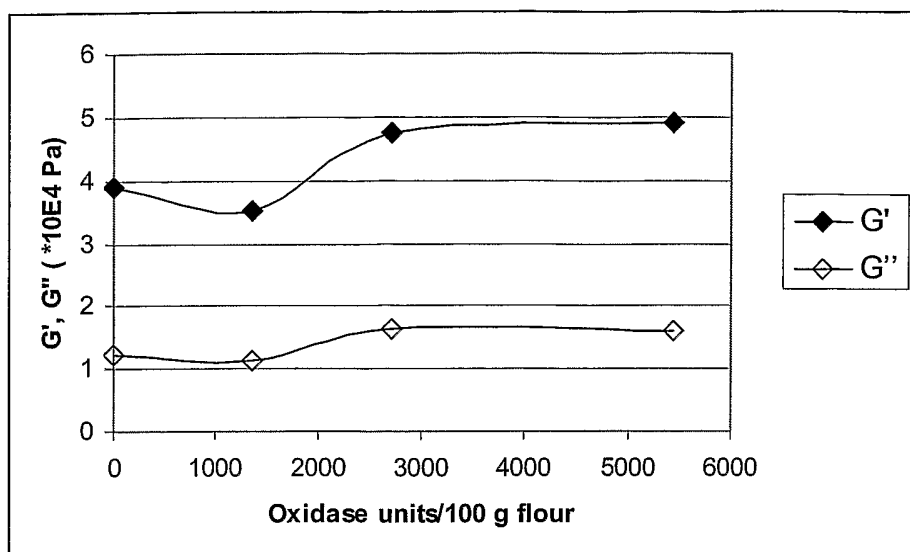


Figure 8

Entire sequenced DNA fragment (SEQ ID NO 1)

TAATTGAAGCTTGCGGTGAGCAAGACAGTCTGCACCCTGCTAGAAATGTTTCGTATGCCGGTAGG
CCCTGCATATGACGGATCCAGTATCTTGGCCATCGGACGGCATTATGACAAGCCCTAGTCATGA
ACCTTTTCGCTATACGTCTCGGGTCCTCCATCCCTTGCCCTTGACCTTCGGGCATCAATGGCATCA
GGTCAATGTGCCTCTTGAGTTCTGTCAATTATAAAAGCCTCAAACCTTCTCGCAGGATGAAGGGAA
CTCAACCACGACAAACCAGTCCGCGACTCGGCCCTGTTTCGACTGTATATATTCTGTGGAAGTAG
GTAGTTGGTTTCCTTGTATATTTCTTTATTTGGTTTTTTTGAGCCCCAGCATGAGTATATCTCAA
GCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGCAGTTCCA
GCTAGACCTGACCTATGAAGATGTCAGTGTGCTGGTGATGTACACAAGGCTATTGTGAGTAAT
GGACAGATTCTTGGGCCTACGTTGTGGTTGAAGCAGGGTGATGATGTGCAATTTTTGGTCAACA
ACTCGATGTCAATCAATACTACGGTTCATTTTCATGGTGAGTACTACCTCTCGATTGTATGATC
CTTTGGCTTCGATGTTTCAAGACGCCTTTTCTATAGAGCTTTACTAAAGATTTGTAGGTATCGA
GCAACTAGGCACCCCGTGGTCAGATGGTGACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGC
GAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACAC
GGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAG
GCCTTTTCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAG
AATACACGGCCAGTTCTCATCTCGGATTGGCGTGCCCTTTTCTCGCATGATATTCTCCAGATTCT
AAACCGAATCTGGTGTTGAGGCCTACTGTGCCAACTCTGTTTTGATCAACGAAAGGGCTCGGT
GATTTGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCAAAAGCAGATCCTGGGC
AACTTGACTCTGACTGATATGGGGTATGAAGTCCCAAGACTTGATGACGGTTACAATGCTGATC
CTGGCTAGATGCCTGCCTCCACGCCGGGTGTTGTGGGTCCGTACCCGTACGACCTCAGCAAGA
TTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGGTTTTCAAGGTCAA
CTCCTCATCGCGGTATGCTAGCTTTGACTTTATTAGTATGGCTGGCTCGACTTCGCTTGTGTTC
TCCATCGATGAGCATCCGATGTATGTTTACGCGGTGATGGCCGTTATGTTGAACCTTTGCTTG
TCGAAGCTGTTACTGTGCCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCAAGTCGGAAGA
CCAGGCAGGAGATTATACTATCCGCGTTGCCAATAACTATGCGAACCAGCTCATCAACGGGACC
GCCGTCTGTGCTATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCTTATATCAACG
AGGCTGGCGCTAATGCCACTGCCTCCACGGTCATTTTGAACGAAACCGACGTGATACCCTTCCC
CGTGGTAGCACCTGCTAAGAAGGCGGACCGGACTTACATCCTGAATGTCGAAAATGCCAATTCA
TCCTACACCTGGACCCTGGGCAACCAGTACCCCGTGTGCAATGAAGAGCTTCGCCCTCCTGTCT
TGTTCAACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGTACGGTACCTGGGTTGA
TCTCATCATCAACATCACCACCTCGGGCCAGCCTCAACACCCCATCCACAAGCACTCCAACAAG
TACTTCGTGATCGGAACCGGTAACCAGCCCTTCATCTGGTCTTCCGTGGAAGAGGCAATGAAAG
ACATACCGGAGAATTTCAACTTCGAAAACCCCCAGATGCGGGACACTTTCTACTCGCCAAGCTC
AAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCGTGAACCCAGGGCCATTCTACTT
CATTGCCATCTTCAGATGCACCATGTCGGTGGGTGGCACTGGCCTTGCTGGACGGGGTGGACG
CCTGGCCAACCGACATTCCCGAGGGATATCGGTTGCCAGTGATGCCAATCTAGGGACTTTGACT
CAATTGTACAGAGGTGGACGAGAGTATCTACTTGTAATATGGCCATATAGCGCCTCTCATATAG
ATCCGGTGTAATATTATTTATTTACTACATAATGAACATGCTTGGTGACTTACCAATCCCCAG
TTATGGCTCCAAAGTAAAAGTGAGAGCAATCACACCAATCAGGCACAGCAGTAA

Figure 9

DNA Sequence corresponding to the coding region including the introns (SEQ ID NO 3)

```
ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCC
GTGTTGTGCAGTTCCAGCTAGACCTGACCTATGAAGATGTCAGTGTGCTGGTGTATGTACACAA
GGCTATTGTGAGTAATGGACAGATTCCCTGGGCCTACGTTGTGGTTGAAGCAGGGTGATGATGTC
GAATTTTTTGGTCAACAACCTCGATGTCAATCAATACTACGGTTTCATTTTCATGGTGAGTACTACC
TCTCGATTGTATGATCCTTTTGGCTTCGATGTTTCAGAACGCCCTTTTCCTATAGAGCTTTACTAAA
GATTTGTAGGTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGA
GCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATT
TATCACTCGCACACACGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCGG
CAGACTCCGTGGAGAGGCCTTTCCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGAT
GTTGGCAGCTGAAAAGAATAACACGGCCAGTTCTCATCTCGGATTGGCGTGCCTTTTCCTCGCAT
GATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCTACTGTGCCAACTCTGTTTTGATCA
ACGGAAGGGCTCGGTGATTTGCCCCTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCA
AAAGCAGATCCTGGGCAACTTGACTCTGACTGATATGGGGTATGAAGTCCCAAGACTTGATGAC
GGTTACAATGCTGATCCTGGCTAGATGCCTGCCTCCACGCCGGGTGTTGTGGGTCCGTACCCG
TACGACCTCAGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCCACAG
AGGTTTTCAAGGTCAACTCCTCATCGCGGTATGCTAGCTTTGACTTTATTAGTATGGCTGGCTC
GACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGTATGTTTACGCGGTCGATGGCCGTTAT
GTTGAACCTTTGCTTGTGCGAAGCTGTTACTGTGCCTGTGCTTCAAGATACTCAGTTATGGTGC
CACTCAAGTCGGAAGACCAGGCAGGAGATTATACTATCCGCGTTGCCAATAACTATGCGAACCA
GCTCATCAACGGGACCGCCGTCTGTGCTATGACACCGCTACACCGAAGCAGATCGGTACCTCG
CAGCCTTATATCAACGAGGCTGGCGCTAATGCCACTGCCTCCACGGTCATTTTGAACGAAACCG
ACGTGATACCCTTCCCCGTGGTAGCACCTGCTAAGAAGGCGGACCGGACTTACATCCTGAATGT
CGAAATGCCAATTCATCCTACACCTGGACCCTGGGCAACCAGTACCCCGTGTGGAATGAAGAG
CTTCGCCCTCCTGTCTTGTTCAACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGT
ACGGTACCTGGGTGATCTCATCATCAACATCACACCTCGGGCCAGCCTCAACACCCCATCCA
CAAGCACTCCAACAAGTACTTCGTGATCGGAACCGGTAACCAGCCCTTCATCTGGTCCTCCGTG
GAAGAGGCAATGAAAGACATACCGGAGAATTTCAACTTCGAAAACCCCCAGATGCGGGACACTT
TCTACTCGCCAAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCGTGAACCC
AGGGCCATTCCTACTTCATTGCCATCTTCAGATGCACCATGTGCGTGGGTGGCACTGGCCTTG
CTGGACGGGGTGGACGCTGGCCAACCGACATTCCCGAGGGATATCGGTTGCCAGTGATGCCAA
TCTAG
```

Figure 10

DNA Sequence of the coding region without the introns (SEQ ID NO 2)

ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCC
GTGTTGTGCAGTTCCAGCTAGACCTGACCTATGAAGATGTCAGTGTCGCTGGTGATGTACACAA
GGCTATTGTGAGTAATGGACAGATTCTTGGGCTACGTTGTGGTTGAAGCAGGGTGATGATGTC
GAATTTTTGGTCAACAACCTCGATGTCAATCAATACTACGGTTCATTTTCATGGTATCGAGCAAC
TAGGCACCCCGTGGTCAGATGGTGACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACA
GTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCT
CAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCTT
TCCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATAC
ACGGCCAGTTCTCATCTCGGATTGGCGTGCCCTTTTCCTCGCATGATATTCTCCAGATTCAAACC
GAATCTGGTGTTGAGGCCTACTGTGCCAACTCTGTTTTGATCAACGGAAAGGGCTCGGTGATTT
GCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCAAAAGCAGATCCTGGGCAACTT
GACTCTGACTGATATGGGATGCCTGCCTCCCACGCCGGGTGTTGTGGGTCCGTACCCGTACGAC
CTCAGCAAGATTCCCAAAGGTTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGGTTT
TCAAGGTCAACTCCTCATCGCGGTATGCTAGCTTTGACTTTATTAGTATGGCTGGCTCGACTTC
GCTTGTGTTCTCCATCGATGAGCATCCGATGTATGTTTACGCGGTCGATGGCCGTTATGTTGAA
CCTTTGCTTGTGCGAAGCTGTTACTGTGCCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCA
AGTCGGAAGACCAGGCAGGAGATTATACTATCCGCGTTGCCAATAACTATGCGAACCAGCTCAT
CAACGGGACCGCCGTCCTGTCGTATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCT
TATATCAACGAGGCTGGCGCTAATGCCACTGCCTCCACGGTCATTTTGAACGAAACCGACGTGA
TACCCTTCCCCGTGGTAGCACCTGCTAAGAAGGCGGACCGGACTTACATCCTGAATGTCGAAAA
TGCCAATTTCATCCTACACCTGGACCCTGGGCAACCAGTACCCCGTGTGCAATGAAGAGCTTCGC
CCTCCTGTCTTGTTCAACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGTACGGTA
CCTGGGTTGATCTCATCATCAACATCACACCTCGGGCCAGCCTCAACACCCCATCCACAAGCA
CTCCAACAAGTACTTCGTGATCGGAACCGGTAACCAGCCCTTCATCTGGTCCTCCGTGGAAGAG
GCAATGAAAGACATAACCGGAGAATTTCAACTTCGAAAACCCCGAGATGCGGGACACTTTCTACT
CGCCAAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCGTGAACCCAGGGCC
ATTCCTACTTCATTGCCATCTTCAGATGCACCATGTCGGTGGGTTGGCACTGGCCTTGCTGGAC
GGGTGGACGCCTGGCCAACCGACATTCCCGAGGGATATCGGTTGCCAGTGATGCCAATCTAG

Figure 11

Protein sequence (SEQ ID NO 40)

MSISQSRLALLALCFVQWVSGRVVQFQLDLTYEDVSVAGDVHKAIVSNGQIPGPTLWLKQGDDV
EFLVNNSMSINTTVHFHFGIEQLGTPWSDGVPGLSSEQIKPGEQFLYKWKASQYGSYIYHSHTRA
QIDDGLYGAIYIEPADSVERPFHLIAGSDADEQQAMLA AEKNTRPVLISDWRAFSSHDILQIQT
ESGVEAYCANSVLINGKGSVICPSQEHINAATTPQQKQILGNLTLTDMGCLPPTPGVVGPYPYD
LSKIPKGFYEGCTPSEGPTEVFKVNSSSRYSASFDFISMAGSTSLVFSIDEHPMYVYAVDGRYVE
PLLVEAVTVPVASRYSVMVPLKSEDQAGDYTIRVANNYANQLINGTAVLSYDTATPKQIGTSQP
YINEAGANATASTVILNETDVI PFPVVAPAKKADRTYILNVENANSSYTWT LGNQYPVSNEELR
PPVLFNLSSISQAYSAMTEYGTWVDLI INITTS GQPQHPIHKHSNKYFVIGTGNQPF IWSSVEE
AMKDI PENFNFENPQMRDTFYSPSSSTGPSWLAMRYHIVNPGPFLLHCHLQMHHVGGLALALLD
GVDAWPTDIPEGYRLPVMPI

Protein sequence without signal sequence (SEQ ID NO 41)

RVVQFQLDLTYEDVSVAGDVHKAIVSNGQIPGPTLWLKQGDDVEFLVNNSMSINTTVHFHFGIEQ
LGTPWSDGVPGLSSEQIKPGEQFLYKWKASQYGSYIYHSHTRAQIDDGLYGAIYIEPADSVERP
FHLIAGSDADEQQAMLA AEKNTRPVLISDWRAFSSHDILQIQTESGVEAYCANSVLINGKGSVI
CPSQEHINAATTPQQKQILGNLTLTDMGCLPPTPGVVGPYPYDLSKIPKGFYEGCTPSEGPTEV
FKVNSSSRYSASFDFISMAGSTSLVFSIDEHPMYVYAVDGRYVEPLLVEAVTVPVASRYSVMVPL
KSEDQAGDYTIRVANNYANQLINGTAVLSYDTATPKQIGTSQPYINEAGANATASTVILNETDV
IPFPVVAPAKKADRTYILNVENANSSYTWT LGNQYPVSNEELRPPVLFNLSSISQAYSAMTEYG
TWVDLI INITTS GQPQHPIHKHSNKYFVIGTGNQPF IWSSVEEAMKDI PENFNFENPQMRDTFY
SPSSSTGPSWLAMRYHIVNPGPFLLHCHLQMHHVGGLALALLDGVDAWPTDIPEGYRLPVMPI

Figure 12 (a)

SEQ ID No	SEQUENCE
01	TAATTGAAGCTTTCGGGTGAGCAAGACAGTCTGCACCCCTGCTAGAAATGTTTCGTATGCCGGTAGGCCCTGCATATGACGGATCCAGTATCTTTGGCCA TCGGACGGCATTATGACAAGCCCTAGTCTCATGAACCTTTTCGTATACGTCTTCGGGTCTCCATCCCTTGCCCTTGACCTTCGGGCATCAATGGCATCG GGTCAATGTGCCCTCTTGAGCTTCTGTCAATTATAAAGCCTCAAACTTCTCGCAGGATGAAGGGAATCAACCAGCAAAACCGATCCGGCAGCTCGC CCTTTCGACATGTATAATATTCTGTGAAGACTAGGTAGTTGGTTCCCTTGATATATTCTTTTGGTTTTCGAGCCCCAGCATGAGTATATCTCAAA GCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTTGTGCAGTTCCAGCTAGACCTGACCTATGAAGATGTCAAGTGTCTG CTGGTGATGTACAAGGCTATTGTGAGTAATTGGACAGATTCCTGGGCCCTACGTTGTGTGTTGAAGCAGGGTGATGATGTCAAAATTTTTGGTCAACA CTCGATGTCAATCAATACCTACGGTTCACTGTTTCATGGTGAGTACCTACCTCGATTGATTCCTTTGGCTTCGATGTTTCAGAACGCCCTTTTCCT ATACAGCTTTTACTAAAGATTTGTAGGTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGC GAACAGTTTCTCTATAAATGGAAGGCCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACCGGGCTCAGATTGATGTGGCCTCTATGGGGCT ATCTACATTTGAAACCGGCAGACTCCGTGGAGAGGCCCTTTCATTTGATCTGCTGGATCTGATCGGGATGAGCAGGCGAGTGTGGCAGCTGAAAG AATACACGGCCAGTTCTCATCTCGGATTGGCGTGCCCTTTTCCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGTAGGCCCTACTGTGCC AATCTCTGTTTGTATCAACGGAAGGGCTCGGTGATTTGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCTGGGC AACTTGACTCTGACTGATATGGGGTATGAAGTCCCAAGACTTGAAGCAGGTTACAATGCTGATCCTGGCTAGATGCCCTGCCCTCCACGCCGGGTGT TGTGGGTCCGTACCCCGTACGACCTCAGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGGCCACAGAGGTTTTCAAGGTCAA CTCCTCATCGCGGATGCTAGCTTTGACTTTTATGATGTGGCTGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGTATGTTTACGC GTCCTAGCTGGCGTTATGTGTGAACCTTTGCTTGTCTGAAGCTGTACTGTGCTGCTGCGCTTCAAGATACAGTATGGTGCCACTCAAGTCGGAAGA CCAGGCAGAGGATTTATATCTTCGCGTGTGCCAATAACTATGCGAACAGCTCATCAACGGGACCGCGCTCTGTCGTATGACACCGCTACACCGAA CGAGATCGGTACCTCGCAGCTTATATCAACGAGGCTGGCGCTAATGCCATCGCTCCCTCAGGTCATTTTGAACGAAACCGACGTGATACCTTCCC CGGTGATCACTGCTTAAGAAGGCCGACCGGACTTACATCTGAATGTGAAATGCCAAATTCATCTACACCTGGACCTGGGGCAACCGTACCC CGTGTCTGAATGAAGAGCTTCGCCCTTCTGTCTTGTTCACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACCGGAGTACGGTACTGGGTGA TCTCATCATCAACATCACCACCTCGGCCAGCCTCAACACCCCTCCACAGCATCTCAAACAAGTATCTCGTGATCGGAACCGGTACCAAGCCCTT CATCTGGTCTCCGTGGAAGAGGCAATGAAGACATACCGGAGAATTTCAACTTCGAAAAACCCAGATGCGGGACATTTTCTACTCGCCAGGCTC AAGCAGAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCTGTAAGCCAGGGCCATCTCTATCTTATGCCATCTTCAGATGACCATGTCTGGTGG GTTGGCAGCTGGCCTTGTCTGACGGGTGAGCGCTGGCCAACCGACATCTCCGAGGATATCGGTTGCCAGTATGCCAATCTAGGAGCTTTGACT CAATTGTACAGAGGTGAGCAGAGATCTTACTTGTAAATATGGCCATATAGCGCCTCTCATATAGATCCGGTGTAAATATTATTATTACTACATAA TGAACATGCTTGGTGACTTACCAATTCCCGAGTTATGGCTCAAAGTAAAGTGAGAGCAATCACACCAATCAGGCACAGCAGTAA
02	ATGAGTATATCTCAAAGCAGGCTGGCCCTTGTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGTCAGTTCAGCTAGACCTGACCTAT GAAGATGTCAGTGTTCGCTGGTGATGTACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTTGTGGTTGAAGCAGGGTGATGATGTC GAAATTTTTGGTCAACCACTCGATGTCATTAATACTACGGTTCAATTTCTATGTTGATCGAGCAACTAGGCACCCCGTGTGTCAGATGTTGACTCGGA TCTTCGACAGGAGCAGATCAAGCGGGCAGACGTTTCTCTATAAATGGAAGCAGCAAGTATGCTGTCATTCATCTCGCACACCGGGCT CAGTGTGATGATGGCCCTCTATGGCGCATCTACATTTGAACCGGCAGACTCCGTTGGAGAGGCCCTTTCCATTTGATCTGCTGGAATCTGATGCGGATGAG CAGCAGGGCGATGTTGGCAGCTGAAAGAATAACACGGCCAGTTCTCATCTCGGATTCGGGCTGCCCTTTTCTCGCATGATATTCTCCAGATTCAAACC GAATCTGGTGTGTGAGCGCTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTGGGTGTTTGGCCCTTCAGGAACACATCAATGCTGCCACG ACACCGCAGCAAAAGCAGATCTGGGCACTTTGACTCTGACTGATATGGGATGCGCTGCCCTCCACCGCCGGGTGTTGTGGGTCCGTACCCGTACGAC CTCAGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGGTTTCAAGGTCAACTCTCATCTCGCGGTATGCTAGC TTTGATTTATTAGTATGCTGGCTGCATCTGCTTGTGTTCTCATCATGATGAGCATCCGATGTATGTTTACCGCGTGCATGGCCGTTATGTTGAA CCTTGTCTTGTGCAAGCTGTTTACTGTGCCCTGTGCTTCAAGATACTCAGTTATGGTGGCACTCAAGTCGGAAGACCAGGCAGGAGATTATACTATC CGCGTTGGCCAAATTAATCTGCAACAGCAGCTCATCAACGGGACCGCGCTCTGTCGTATGACACCGTATCAACCGAAGCAGATCGGTACTCTCGACGCT TATATCAACGAGGCTGGCGTATGATGCCATCGCTCCACGGTCAATTTGAACGAAACCGACGTCGATACCTTTCCCGTGGTGACCTGCTGAAGAAG CGCGACGACTTACATCTCTGAATGTGCAAAATGCCAATTCATCTACCTTGACCCCTGGGCAACCAGTACCCCGTGTGCAATGAAGAGCTTTCCGC CCTCCTGTCTTGTTCACCTCAGCTCAATCTCGCAGCGCTACTCGGCATGACGGAATACGGTATACCTGGTGTGATCTCATCATCAACATCAACACC TCGGGCCAGCCTCAACACCCCTACCAAGCACTCCCAAGACTCTGTCGATGCGAACCGGTAAACCGGCTTCATCTGCTTCCGTGGTGAAGAG GCAATGAAAGACATACCGGAGAATTTCAACTTCGAAACCCCGAGATGCGGGACACTTTTCTACTCGCAAGCTCAAGCACAGGGCCGAGCTGGCTG GCAATGCGCATACCATATCTGTAACCCAGGCGCAATCTCACTTCAATGGCATCTTCAGATGACCACTATCTCGGTGGGTGGCAGTGGCCTTGTCTGGAC GGGTGTCGACGCTGGCCCAACCGACATCTCCGAGGGATATCGGTTGCCAGTGTGCCAATCTAG
03	ATGAGTATATCTCAAAGCAGGCTGGCCCTTGTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGTCAGTTCAGCTAGACCTGACCTAT GAAGATGTCAGTGTTCGCTGGTGATGTACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTTGTGGTTGAAGCAGGGTGATGATGTC GAAATTTTTGGTCAACCACTCGATGTCATTAATACTACGGTTCAATTTCTATGTTGATGATACCTCTCGATTGATGATCCTTTTGGCTCGATGTT CAGAACGCCTTTTCTTATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGA CGCATCAAGCCGGGCCAAGCACTTTCTCTATAAATGGAAGGCCAGGTCATGGCTCGTATCAATTTATCATCTCGCACACCGGGCTCAGATTGATGA TGGCCTCTATGGGGCTATCTACATTTGAACCGGCAGACTCCGTGGAGAGGCCCTTTCATTTGATCGCTGATCTGATGCGGATGAGCAGGCGAT GTTGGCAGCTGAAAGAATAACACGGCCAGTTCATCTCGGATTGGCGTGCCCTTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGT TGAGCCCTTACTGTGCCAATCTGTTTGTATCAACGGAAGGGCTCGGTGATTTGGCCGTCTCAGGAACACATCAATGCTGCCACGACCCGACGA AAAGCAGATCTTGGGCAACTTGACTTGTGACTGATATGGGGTATGAAGTCCCAAGACTTGTATGACGGTTACAATGCTGATCCTGGCTAGATGCTGTC CTCCACGCGCGGGTGTGTGGGTCCGTACCCGTACGACCTCAGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGGCCACAG AGGTTTTCAGGTCAGCTCCTCATCGCGGTATGCTAGCTTTGATTTATGATGCTGGCTGCATCTTCGCTTGTGTTCTCCATCGATGAGCATCT CGATGTATGTTTACGCGGTGATGCGCCTTTATGTTGAACCTTTGCTTGTGCAAGCTGTACTGTGCTGCTGCTTCAGATACATCAAGTATGCTGTC CACTCAAGTCGGAAGACCAGGCAGGAGATTATACTATCCGCGTTGCCAATAACTATGCGAACCCAGCTCATCAACGGGACCGCGCTCTGTCGTATG ACACCGCTACACCGAAGCAGATCGGTACTCGCAGCCTTATATCAACGAGGCTGGCGCTAATGCCATGCTCCCTCAGCGTCTTTGAACGAAACCC ACGTTGATACCTTCCCGTGGTGAAGCACTGTCTGAAGAAGCGGCACTTACATCTGAATGTGAAATGCCAAATTCATCTTGAACACCTGGACCC TGGGCAACCAGTACCCCGTGTGCAATGAAGAGCTTCGCCCTCTGCTTGTTCATCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGT ACGCTACCTGGGTTGATCTCATCATCAACATCAACCTCGGGCCAGCCTCAACACCCCTCAACAGCACTCAACAGCACTTCTGATCTGGA CCGTTAACCGAGCTTTCATCTGCTGCTCGTGGGAAGGGCAATGAAGACATCCGAGGAATTTCACTTCGAAAAACCCCGACTGCGGACACTT TCTACTCGCAAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCTGTAACCCAGGCGCAATCTTACTTCAATGCCATCTTCAGA TGCAACATGTCGGTGGGTGGCAGTGGCCTTGTCTGGACGGGGTGGACGCTGGCCAACCGACATTCCTCGAGGGATATCGGTTGCCAGTGTGCCAA TCTAG

Figure 12 (b)

04	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGCAGTTCAGCTAGACCTGACCTAT GAAGATGTCTAGTGTCTGCTGGTGATGTACACAAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTC GAATTTTGTGTCACAACTCGATGTCAATCAATACACGGTTCATTTTCATGGTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTT CAGAACCGCTTTTCTATAGAGCTTTACTAAAGATTGTAGGTATCGAGCACTAGGCAACCCCGTGGTCAGATGGTGATCTCTGGACTCTCGCAGGA GCAGATCAAGCCGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTATCACTCGCACACACGGGCTCAGATTGATGA TGGCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCGCTGGATCTGATCGGGATGAGCAGCAGGCGAT GTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCCCTTTTCTCGCATGATATCTCCAGATTCAAACCGAATCTGGTGT TGAGGCTTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTCGGTGATTGGCCGCTCAGGAACACATCAATGCTGCCACACACCGCAGCA AAAGCAGATCTGGGCACTTGACTCTGACTGATATGGGATGCTGCTCCACGCGGGTGTGTGGGTCCGTACCCGTACGACCTCAGCAAGAT TCCCAAGGTTTCTACGAAGGATGACACCTAGTGAAGGGCCACAGAGGTTTCAAGGTCAACTCCTCATCGCGGTATGCTAGCTTTGACTTTAT TAGTATGGCTGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGATATGTTTACGCGGTGATGGCCGTTATGTTGAACCTTTGCTTGT CGAAGCTGTTACTGTGCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCAAGTCGGAAGACACGAGCAGGAGATTATACTATCCGCTTGCCAA TAACTATCGCAACAGCTCATCAACGGGACCGCGCTCTGTGCTATGACACCGCTACCGAAGCAGATCGGTACCTCGCAGCCTTATATCAACGA GGCTGGCGCTTACCAAGCTCGCTTCCACGGTCAATTTGAACGGAACCGCAGTATACCCCTTCCCGTGGTAGACCTTCTAGAAGGCGGACCGGAC TTACATCTGAATGTGAAAATGCCAATTCTCTACCTTGACCTGGGCAACAGTACCCCGTGTGCAATGAAGAGCTTCGCCCTCTGTCTT GTTCAACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGTACGGTACCTGGGTTGATCTCATCAACATCACCACCTCGGGCCAGCC TGACACCCCATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGGATCGGAACCGGTAAACAGCCCTTCTATCTGGTCTCCGTGGAAGAGGCAATGAAAG CATACCGGAGATTTCACCTTCAAAAACCCCGAGATGCGGGACACTTTCTACTCTGCCAAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTA CCATATCGTGAACCCAGGGCCATTCTACTTCAATTTGCCATCTTCAGATGCACCATGTCGGTGGGTGGCACTGGCCCTTGCTGGACGGGTGGACGC CTGGCCAGCCGACATTTCCGAGGGATATCGGTTGCCAGTATGCCAATCTAG
05	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGCAGTTCAGCTAGACCTGACCTAT GAAGATGTCTAGTGTCTGCTGGTGATGTACACAAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTC GAATTTTGTGTCACAACTCGATGTCAATCAATACACGGTTCATTTTCATGGTATCGAGCACTAGGCAACCCCGTGGTCAGATGGTGATACCTGGA CTCTCGCAGGAGCAGATCAAGCCGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTATCACTCGCACACACGGGCT CAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCGCTGGATCTGATCGGGATGAG CAGCAGCCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGGATTCGCAATGGCTGCTTTTCTCGCATGATATTTCTCCAGATTCAAAC GAATCTGGTGTGAGGCCCTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTCGGTGATTGGCCGCTCAGGAACACATCAATGCTGCCACG ACACCGCAGCAAAAGCAGATCTGGGCAACTTGACTCTGACTGATATGGGGTATGAAGTCCCAAGACTTGATGACGGTTACAATGCTGATCCTGGC TAGATGCTTGCCTCCGACGCGGGGTGTTGGGTCCGTACCCGTACGACCTCAGCAAGATTCCCAAAGGTTTTCACGAAGGATGCACACCTAGTGA AGGGCCACAGAGGTTTCAAGGTCAACTCTCATCGCGGTATGCTAGCTTTGACTTTATGATGCTGGCTCGACTTCGCTTGTGTTCTCCAT CGATGAGCATCCGATGTATGTTTACGCGGTGATGGCCGTTATGTTGAACCTTTGCTTGTGCAAGCTGTTACTGTGCTGTGCTTCAAGATCTC AGTTATGGTGCCACTCAAGTCGGAAGACACGAGCAGGAGATTATACTATCCGCTTGTGCAATTAACATATGCGCAACAGCTCATCAACGGGACCGCGT CCTGTGATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCTTATATCAACGAGGCTGGCGCTAATGCCACTGCTCCACGGTCATTTT GAACGAAACCGACGTGATACCTTCCCGTGGTAGACCTGCTAAGAAGGCGGACCGGACTTACATCTGATGTGCAAAATGCCAATTCATCCTA CACCTGGACCTCGGCAACAGTACCCCGTGTGCAATGAAGAGCTTCGCCCTCTGCTTGTGTTCAACCTCAGCTCATCTCGCAGGCTGCTGCTG CATGACGGAGTACGGTACCTGGGTGATCTCATCAACATCAACACCTCGGGCCAGCCTCAACACCCATCCACAAGCACTCCAACAAGTACTT CGTGATCGGAACCGGTAAACAGCCCTTCATCTGGTCTCCGTGGAGAGGCAATGAAAGACATACCGGAGAAATTCACACTCGAAAACCCCGAGAT GGGGGACATTTCTACTCGCAAGCTCAAGCACAGGGCCGAGTGGCTGAATGCGCTACCATATCGTGAACCCAGGCGCATCTCTACTTATGCT CCATCTTCAGATGCACCATGTCGGTGGGTGGCACTGGCCTTGCTGACGCGGTGGACGCTGGCCAAACCGACATTCCCGAGGGATATCGGTTGCC AGTGATGCCAATCTAG
06	TAATTTGAAGCTTGGCTGAGCAAGACAGTCTGCACCTGCTAGAAATGTTGATGCGCGTAGGCCCTGCATATGACGGATCCAGTATCTTGGCCA TCGGACGGCATTATGACAAGCCCTAGTCATGAACCTTTGCTATACGCTCTCGGTCCTCCATCCCTTGCCCTTGACCTTCGGGCATCAATGGCATCA GGTCAATGTGCTCTTGAGTCTGTCTATTATAAAGCCCTCAAACTTCTCGCAGGATGAAGGGAATCAACACAGCAAAACAGTCCCGCATCTCGGC CCTGTTCCGACTGATATATCTGTGGAACTAGGTAGTTGGTTCTTGTATATTTCTTTATTTGGTTTTTTGAGCCCCAGCATGAGTATATCTCAAA GCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGCAGTTCAGCTAGACCTGACCTATGAAGATGTCAGTGTGCG CTGGTGATGTACACAAGGCTATGTGAGTAATGGACAGATTCTCGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTCGAATTTTGGTCAACA ACTCGATGTCAATCAATACACTACGGTTCAATTTTCATGGTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCAGAACGCTTTTCTCT ATAGAGCTTTACTAAAGATTGTAGGTATCGAGCACTAGGCAACCCGTGGTTCAGATGGTGATCTGGACTCTCGCAGGAGCAGATCAAGCCGGC GAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCTCTATGGGGCT ATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCGCTGGATCTGATGCGGATGAGCAGGCGATGTTGGCAGCTGAAAAG AATACACGGCCAGTTCTCATCTCGGATTGGCGTGCTTTTCTCTCGATGATATTTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCC AACTCTGTTTGTATCAACGGAAGGGCTCGGTGATTGGCCGCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCTGGGC AACTTGACTCTGACTGATATGGGGTATGAAGTCCCAAGACTTGATGACCGCTTACAATGCTGATCCTGGCTAGATGCTGCTCCACGCGGTGTG TGTGGTCCGTACCCGTACGACCTCAGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGTTTCAAGGTCAA CTCCTCATCGCGGTATGCTAGCTTTGACTTTATGATGCTGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGTATGTTTACGC GGTCTGATGGCCGTATGTTGAACCTTTGCTTGTGCAAGCTGTTACTGTGCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCAAGTCCGAAGA CCAGGAGGAGATTATATCTATCCGCTTGCCAAATAACTATGCGAAGCAGTCAATCAACGGGACCGCGCTCTGTGCTATGACACCGCTACACCGAA GCAGATCGGTACCTCGCAGCCTTATATCAACGAGGCTGGCGCTAATGCCACTGCTCCACGGTCAATTTTGAACGAAACCGACGTGATACCTTCCG CGTGGTAGCCTGCTAAGAAGGCGGACCGGACTTACATCTGGAATGTGCAAAATGCCAATTATCTACCTACACCTGAGCCCTGGCAACAGTACCT CGTGTGCAATGAAGAGCTTCGCCCTCTGTCTTGTTCACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGTACGGTACCTGGGTGA TCTCATCATCAACATCACCACCTCGGGCCAGCCTCAACACCCCATCCACAAGCACTCCAACAAGTACTTCGTGATCGGAACCGGTAAACAGCCCTT CATCTGGTCTCCGTGGAAGAGGCAATGAAAGACATACCGGAGAAATTCAACTTCGAAAACCCCGAGATGCGGGACACTTTCTACTCGCCAAGCTC AAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCGTGAACCCAGGGCCATTCTACTTCAATGGCATCTTCAGATGCACCATGTCGGTGG GTTGGCACTGGCCTTGCTGGACGGGTGGACGCTGGCCAAACCGACATTCCCGAGGGATATCGGTTGCCAGTATGCCAATCTAG

Figure 12 (c)

07	TAATTGAAGCTTGCGGTGAGCAAGACAGTCTGCACCCCTGCTAGAAATGTTCTGATGCCGGTAGGCCCTGCATATGACGGATCCAGTATCTTGGCCA TCGGACGGCATTATGACAAGCCCTAGTCAAGAACTTTTCGCTATACGTCTCGGGTCCCTCCATCCCTTGCCCTTGACCTTCGGGCATCAATGGCATCA GGTCAATGTGCCTCTTGAGTTCCTGTCATTATAAAGCCTCAAACCTTCGCGAGGATGAAGGGAACTCAACACGACAAACAGTCCCGGACTCGGC CCTGTTTCGACTGTATATATTTCTGTGGAACTAGGTAGTTGGTTCCCTTGATATTTCTTTATTTGGTTTTTTGAGCCCCAGCATGAGTATATCTCAAA GCAGGCTGGCCCTGTTGGCCCTTTTGCTTCGTGCAATGGGTGTTCCGGCCGTGTTTACGCGGTTCCAGCTAGACCTGACCTATGAAGATGTCAGTGTG CTGGTGATGTACACAAGGCTATTGTGAGTAATGGACAGATTCTCGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTCGAATTTTGGTCAACA ACTCGATGTCAATCAATACTACGGTTTCATTTTCATGGTATCGAGCACTAGGCACCCCGTGGTCCAGATGGTGTACCTGGGACTCTCGCAGGAGCAGA TCAAGCCGGCGAACAATTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTATCACTCGCACACACGGGCTCAGATTGATGATGGCC TCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGG CAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCCCTTTCCCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTTGAGG CCTACTGTGCCAATCTGTTTTGATCAACGGAAGGGCTCGGTGATTCGATGAGTATGAGTTCGCGGTCAGATGGCCGTTATGTTGAACCTTTGCTTGCAGAG AGATCTCGGGCAACTTGACTCTGACTGATATGGGATGCCTGCCTCCACGCGCGGTGTTGTGGGTCCGTACCCGTACGACCTCAGCAAGATTCCCA AAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGGTTTTCAAGGTCAACTCTCATCGCGGTATGCTAGCTTTGACTTTTATAGTA TGGCTGGCTCGATTCGCTTGTGTTCTCCATCGATGAGCATCCGATGATTTGACCGGTCAGATGGCCGTTATGTTGAACCTTTGCTTGCAGAG CTGTTACTGTGCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCAAGTCGGAAGACCAGGCAGGAGATTATCTATCCGCGTTGCCAATAACT ATGCGAACCCAGCTCATCAACGGGACCGCGCTCTGTGCTATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCTTATATCAACGAGGCTG GCGCTTAATGCCACTCGCTCCACGGTCATTTTGAACGAAACCGACGTTGATACCCCTTCCCGGTGCTAGCACCTGCTAAGAGAGGCGGACCGGATCTACA TCTGATGTGCGAAATGCCAATTCATCTTACACTGGACCTTGGGCAACAGTACCCCGTGTGCAATGAAGAGCTTCGCGCTCTGTCTTGTTC ACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGTACGGTACCTGGGTTGATCTCATCATCAACATCAACCTCGGGCCAGCCTCAAC ACCCATCCACAGCACTCCAAACAGTACTCTGATCGGAACCGGATGATACCCCTTCCCGGTGCTAGCACCTGCTAAGAGAGGCGGACCGGATCTACA CGGAGATTTCAACTTCGAAACCCCGAGATGCGGGACACTTTCTACTCGCAAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCAT TCGTGAACCCAGGGCCATTCTACTTCATTGCCATCTTCAGATGCACCATGTGCGTGGTGGCACTGGCCCTTGTCTGACGGGGTGAGCGCTGGC CAACCGACATTCGCGAGGGATATCGGTTGCCAGTGATGCCAATCTAG
08	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGCAGTTCAGCTAGACCTGACCTAT GAAGATGTGAGTGTGCTGGTGTATGATACACAAGGCTATTTGTGAGTAATGGACAGATTCTCGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTC GAATTTTGGTCAACAACCTCGATGTCAATCAATACTACGGTTTCATTTTCATGGTGTATCGAGCACTAGGCACCTGAGTGGTGTGATGCTGGA CTCTCGCAGGAGCAGATCAAGCCGGCGCAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTATCACTCGCACACACGGGCT CAGATTGATGATGGCCTCTATGCGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCGCTGGATCTGATGCGGATGAG CAGCAGGCTGATGTTGGCAGCTGAAAGAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCCCTTTCCCTCGCATGATATCTCCAGATCAACAT GAATCTGGTGTGAGGCTACTGTGCCAATCTGTTTTGATCAACGGAAGGGCTCGGTGATTTGCCCGTCTCAGGAACACATCAATGCTGCCACG ACACCGCAGCAAAAGCAGATCTCGGCCAATTGACTCTGACTGATATGGGATGCCTGCCTCCACGCGCGGTGTTGTGGGTCCGTACCCGTACGAC CTCAGCAAGATTTCCAAAGGTTTCTACGAAGGATGCACACCTGATGAAGGGCCACAGAGGTTTTCAAGGTCAACTCTCATCGCGGTATGCTAGC TTTGACTTTTATTAGTATGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGATGTTTACGCGGTGATGCGCGTTATGTTGA CCTTTGCTTGTGCAAGCTGTTACTGTGCTGCTCTCAAGATACTCAGTTATGGTGCCACTCAAGTCGGAAGACCAGGCAGGAGATTATCTATC CGCGTTGCCAATATGCTGCAACACAGCTCATCAACGGGACCGCGTCTGTGCTGATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCT TATATCAACGAGGCTGCGCTAATGCCACTGCCTCCACGGTCATTTTGAACGAAACCGACGTTATACCCCTTCCCGGTGCTAGCACCTGCTAAGAG CGGAGCCGACTTACATCTGAATGCGAAATGCCAATTCATCTTACACTGGACCTTGGGCAACAGTACCCCGTGTGCAATGAAGAGCTTTCGC CCTCTGCTTGTGTTCAACTCAGCTCAATCTCGAGGCGTATCGGCCATGACGGAGTACGGTACCTGGGTTGATCTCATCATCAACATCAACCT TCGGGCCAGCCTCAACACCCATCCACAAGCACTCCAAACAGTACTTCGTGATCGGAACCGGTAACAGCCCTTCTATCTGGTCTCGGTGGAAGAG GCAATGAAGAGCATACCGGAGAAATTTCAACTTCGAAACCCCGAGATGCGGGACACTTTCTACTCGCAAGCTCAAGCACAGGGCCGAGCTGGCTG GCAATGCGCTACCATATCTGTGAACCCAGGCGCATCTTCTACTTCATGCGCATCTTCAGATGCAACATGTCGGTGGGTGGCACTGGCTTGTGTCG GGGTGGACCGCTGGCCAACCGACATTCGCGAGGATATCGGTTGGCAGTGATGCCAATCTAGGGAGCTTTGACTCAATGTGACAGAGGTGGACGAG AGTATCTACTTGTAAATGCGCATATAGCGCTCTCATATAGATCCGGTGTAATATTATTTATTTACTACATAATGAACATGCTTGGTGACTTACC AATTTCCCACTTATGGCTCCAAAGTAAAGTGAGAGCAATCACACCAATTCAGGCACAGCAGTAA
09	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGCAGTTCAGCTAGACCTGACCTAT GAAGATGTGAGTGTGCTGGTGTATGATACACAAGGCTATTTGTGAGTAATGGACAGATTCTCGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTC GAATTTTGGTCAACAACCTCGATGTCAATCAATACTACGGTTTCATTTTCATGGTGTATGACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTT CAGAACGCTTTTCTATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCACTAGGCACCCCGTGGTCCAGATGGTGTACCTGGACTCTCGCAGGA GCAGATCAAGCCGGCGCAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTATCACTCGCACACACGGGCTCAGATTGATGA TGGCTTCTATGGGCTATCTACATTGAACCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGAT GTTGGCAGCTGAAAGAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCCCTTTCCCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGT TGAGGCTACTGTGCCAATCTGTTTTGATCAACGGAAGGGCTCGGTGATTTGCCCGTCTCAGGAACACATCAATGCTGCCAGCACACCGCAGCA AAAGCAGATCTCTGGGCACTTGACTCTGACTGATATGGGTTATGAAGTCCCAAGACTGATGACGGTTCAATGCTGATCTCGGCTAGATCCCTGC CTCCACGCGCGGTGTTGTGGGTCCGTACCCGTACGACCTCAGCAAGATTCCCAAGGTTTCTACGAAGGATGCACCTAGTGAAGGGCCACAG AGGTTTCAAGGTCAACTCTCATCGCGGTATGCTAGCTTTGACTTTTATGATATGGCTGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATC CGATGATGTTTTACGCGGTGATGCGCGTTATGTTGAACCTTGTGTTGCGAAGCTGTTACTGTGCTGTGCTTCAAGATACTCAGTTATGGTGC CACTCAAGTCGGAAGACCAGGCAGGAGATTATCTATCCGCGTTGCCAATAACTATGCGAACCAGCTCATCAACGGGACCGCGCTCTGTGCTATG ACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCTTATATCAACGAGGCTGGCGCTAATGCCACTGCCTCCACGGTCAATTTTGAACGAAACCG ACGTGATACCCCTTCCCGTGGTAGCACCTGCTAAGAGGGCGGACCGGACTTACATCTGAAATGTCGAAATGCCAATTCATCTACCTGAGACCC TGGGCAACAGTACCCCGTGTGCAATGAAGAGCTTCGCGCTCTGTCTTGTTCACCTCAGCTCAATCTCGCAGGCTACTCGGCCATGACGGAGT ACGGTACCTGGGTTGATCTCATCATCAACATCAACCTCGGGCCAGCCTCAACACCCATCCACAAGCACTCCAAACAGTACTTCGTGATCGGAA CCGGTAACAGCCCTTCTATCTGGTCTCCGTGGAGAGGCAATGAAGACATACCGGAGAAATTCAACTTCGAAACCCCGAGATGCGGGACACTT TCTACTCGCAAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCATATCGTGAACCCAGGGCCATTCTACTTCATTGCCATCTTCAGA TGCACCATGTCGGTGGGTTGGCACTGGCCCTTGTGAGCGGGGTGAGCCCTGGCCAACCGACATTCGCGAGGGATATCGGTTGCCAGTGATGCCAA TCTAGGCACTTTGACTCAATGTACAGAGGTGAGCAGAGTATCTAGTGAATATGCGCATATAGCGCTCTCATATAGTCCGCTGTAATGTAATTA TTTATTTACTACATAATGAACATGCTTGGTGACTTACCAATTCCCAGTTATGGCTCCAAAGTAAAGTGAGAGCAATCACACCAATCAGGCACA GCAGTAA

Figure 12 (d)

10	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTGTTGTGTCAGTTCCAGCTAGACCTGACCTAT GAAGATGTGTCAGTGTGCTGGTGATGTACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGC GAATTTTGTGTCACAACCTCGATGTCAATCAATACACGGTTCATTTTCATGATCGAGCACTAGGCAACCCCGTGGTCAGATGGTGTACCTGGA CTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCT CAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCCGGCAGACTCCGTGGAGAGGCCCTTCCATTTGATCCGTGGATCTGATGGGGATGAG CAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGGATTGGCGTGCCTTTTCTCGCATGATATTCTCCAGATTCAAACC GAATCTGGTGTGAGGCTACTGTGCCAATCTGTTTGTGATCAACGGAAAGGGCTCGGTGATTGCGCGTCTCAGGAACACATCAATGCTGCCACG ACACCCGAGCAAAAGCAGATCCTGGGCAACTTGACTCTGACTGATGGGATGCCCTGCCCTCCACGCGGGGTGTTGTTGGGTCCGTACCCGTACGAC CTCAGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGGTTTCAAGGTCAACTCTCATCGCGGTATGCTAGC TTGACTTTATAGTATGGCTGGCTCGACTTCGCTTGTGTCTCCATCGATGAGCATCCGATGATGTTTACGCGGTTCGATGGCCGTATGTTGAA CCTTTGCTTGTGCAAGCTGTTACTGTGCTGTGCTTCAAGATACTCAGTATGTTGTTGCTCCACTCAAGTCGGAAGACCAGGCGAGGATTACTACT CGCGTGTGCCAATAATATGCGAACCAGCTCATCAACGGGACCGCGTCTGTGCTATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCT TATATCAACGAGGCTGGCGCTAATGCCACTGCCTCCACGGTCAATTTGAACGAAACCGAGTATACCTTCCCGTGGTAGCAGCTGCTAAGAAAG GCAATGAAAGACATACCGGAGAAATTTCAACTTCGAAAACCCCGCAGATGCGGGACACTTTCTACTCGCCAACTCAAGCACAGGCGCAGCTGGCTG GCAATCGCTACCATATCGTGAACCCAGGGCCATTCTACTTCAATGCGCATCTTCAGATGACCATGTCGGTGGGTGGCAGCTGGCCTTGTGTCGAC GGGTGGACCGCTGGCCAAACCGACATTCCCGAGGGATATCGGTTGGCAGTATGCCAATCTAGGGACTTTGACTCAATTTGACAGAGGTGGACGAG AGTATCTACTTGTAAATATGGCCATATAGCGCTCTCATATAGATCCGGTGTAAATATTTATTATTACTACATAATGAACATGCTTGGTGACTTACC AATTTCCCGAGTTATGGCTCCAAAGTAAAGTGAGAGCAATCACACCAATCAGGCACAGCAGTAA
11	GTATCGAGCACTAGGCAACCCGTGGTCAGATGGTGTACTGGAATCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAG CAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCGGACACTCG TGGAGAGGCCCTTTCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGG ATTGGCGTGCTTTTCTTCGTCATGATATTCTCAGATTCAAACCGAATCTGGTGTGAGGCTTACTGTGCCAATCTGTTTGTATCAACGGAAGG GCTCGGTGATTTTCCCGCTCAGGAACACATCAATGCTGCCAGACACCGGACAGATCCTGGGCAACTCTGACTCTGACTGATGATGGGATGAGG ATGAAGTCCCAAGACTTGATGACGCTTACAATGCTGATCCTGGCTAGATGCCCTGCCCTCCACGCGGGGTGTTGTGGGTCCGTACCCGTACGACCTC AGCAAGATTCCCAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGCCACAGAGGTTTCAAGGTCAACTCTCATCGCGGTATGCTAGCTTT GACTTTATAGTATGGCTGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGATGTTTACGCGGTTCGATGGCCGTATGTTGAACTT TTGCTTGTGCAAGCTGTTACTGTGCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCAAGTCGGAAGACCAGGCGAGGATTATACTATCCGC GTTGCCAATAAATATGCGAACCAGCTCATCAACGGGACCGCGCTCTGTGCTATGACACCGCTACACCGAAGCAGATCGGTACCTCGCAGCCTTAT ATCAACGGCTCGCGCTTAATGCCACTGCCCTCCACGGTCAATTTGAAACGAAACCGACGTCGATACCTTCCCGTGGTAGCAGCTGCTAAGAAAGGCG GACCGGACTTACATCTGAATGTCGAAAATGCCAATTCATCTACACTGGAACCTTGGGCAACAGTACCCGTGTGCAATGAAGAGCTTCGCCCT CCTGTCTTGTTCACCTCAGCTCAATCTCGCAGGCGTACTCGGCCATGACGGAGTACGGTACCTGGGTGATCTCATCATCAACATCACCACTCG GCTGCTCAACACCCCATCCACAAGCACTCCACAAGTACTCTGTCGATGAGCATCCGATGATGTTTACGCGGTTCGATGGCCGTATGTTGAACTT ATGAAGACATACCGGAGAAATTTCAACTTCGAAAACCCCGAGATGCGGGACACTTTCTACTCGCCAAAGCTCAAGCACAGGCGCAGCTGGCTGGCA ATGCGCTACCATATCGTGAACCCAGGGCCATTCTACTTTCATTGGCCATCTTCAGATGACCAATGTGCGGTGGGTGGCAGCTGGCCCTGCTGGACGGG GTGGACGCTGGCCCAACCGACATTCCGAGGGATATCGGTTCCGAGTATGCCCAATCTAG
12	Sequence>TAATTGAAGCTTGCCTGAGCAAGACAGTCTGCACCCCTGCTAGAAATGTTTCGTATGCCGGTAGGCCCTGCATATGACGGATCCAGTA TCTTGGCCATCGGACGGCATTTATGACAAGCCCTAGTCAATGAACCTTTCGCTATACGTCCTCGGGTCTCCATCCCTTGCCTTGACCTTCGGGCATCA ATGGCATCAGGTCAGTGTGCTTCTGAGTTCTGTCAATTATAAAGCCTCAAACCTTCTCGCAGGATGAAGGGAACCTCAACCACGACAAACAGTCCGCGACTCGG CGACTCGGCCCTGTTGCTGATGATATATTCTGTGGAACAGTGTGTTGCTTCTGTATATTTCTTTATTTGGTTTTTTGTAGCCCCAGCATGAGTA TATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCTGTCGAATGGGTGTCCGGCCGTGTTGTGCACTTCCAGCTAGACCTGACCTATGAGATG TCAGTGTGCTGGTGTATGACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGTTGTTGAAGCAGGGTGATGATGTCGAATTTT TGGTCAACAACCTCGATGTCAATCAATACTACGGTTCATTTTCATG
13	TAATTGAAGCTTGCCTGAGCAAGACAGTCTGCACCCCTGCTAGAAATGTTTCGTATGCCGGTAGGCCCTGCATATGACGGATCCAGTATCTTGGCCA TCGGACGGCATTTATGACAAGCCCTAGTCAATGAACCTTTCGCTATACGTCCTCGGGTCCCTCCATCCCTTGCCTTGACCTTCGGGCATCAATGGCATCA GGTCAATGTGCTCTTGTGATTTCTGTCAATTATAAAGCCTCAAACCTTCTCGCAGGATGAAGGGAACCTCAACCACGACAAACAGTCCGCGACTCGGC CCTGTTTCGACTGTATATATTCTGTGGAACTAGGTAGTTGGTTCTTGTATATTTCTTTATTTGGTTTTTTGTAGCCCCAGCATGAGTATCTCAAA GCAGGCTGGCCCTGTTGGCCCTTTGCTTCTGTGCAATGGGTGTCCGGCCGTGTTGTGCACTTCCAGCTAGACCTGACCTATGAAGATGTCAAGTGTG CTGGTGTATGACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGTTGTTGAAGCAGGGTGATGATGTCGAATTTTGGTCAACA ACTCGATGTCAATCAATACTACGGTTCATTTTCATGTTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTTCAGAACGCCCTTTCTCT ATAGAGCTTTACTAAGATTTGTAG
14	TAATTGAAGCTTGCCTGAGCAAGACAGTCTGCACCCCTGCTAGAAATGTTTCGTATGCCGGTAGGCCCTGCATATGACGGATCCAGTATCTTGGCCA TCGGACGGCATTTATGACAAGCCCTAGTCAATGAACCTTTCGCTATACGTCCTCGGGTCCCTCCATCCCTTGCCTTGACCTTCGGGCATCAATGGCATCA GGTCAATGTGCTCTTGTGATTTCTGTCAATTATAAAGCCTCAAACCTTCTCGCAGGATGAAGGGAACCTCAACCACGACAAACAGTCCGCGACTCGGC CCTGTTTCGACTGTATATATTCTGTGGAACTAGGTAGTTGGTTCTTGTATATTTCTTTATTTGGTTTTTTGTAGCCCCAGCATGAGTATATCTCAAA GCAGGCTGGCCCTGTTGGCCCTTTGCTTCTGTGCAATGGGTGTCCGGCCGTGTTGTGCACTTCCAGCTAGACCTGACCTATGAAGATGTCAAGTGTG CTGGTGTATGACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGTTGTTGAAGCAGGGTGATGATGTCGAATTTTGGTCAACA ACTCGATGTCAATCAATACTACGGTTCATTTTCATGTTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTTCAGAACGCCCTTTCTCT ATAGAGCTTTACTAAGATTTGTAGTATCGAGCACTAGGCAACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGG GAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTTTCACTCTCGCACACACGGGCTCAGATTGATGATGGCCCTCTATGGGGCT ATCTACATTGAACCGGACAGCTCCGTGGAGAGGCTTTCCATTGTATGCTGCTGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAG AATACACGGCCAGTTCTCATCTCGGATGGCGTGCCTTTCTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCTTACTGTGCC AACTCTGTTTGTATCAACGGAAGGCTCGGTGATTTGCCGCTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCCTGGGC AACTTGACTCTGACTGATATGGG

Figure 12 (e)

15	TAATTGAAGCTTGGCGTGAGCAAGACAGTCTGCACCCTGCTAGAAATGTTTCGTATGCCGGTAGGCCCTGCATATGACGGATCCAGTATCTTGGCCA TCGGACCGGCATTATGACAAGCCCTAGTCATGAACCTTTTCGCTATACGTCTCGGGTCCCTCCATCCCTTGCCTTGCCTTCCGGCATCAATGGCATCA GGTCAATGTGCCTCTTGAGTTCTGTCTATTATAAAGCCCTCAAATCTCTCGCAGGATGAAGGGAACCTCAACCACGACAACCCAGTCCGCGACTCGGC CCTGTTTCGACTGTATATATTCTGTGGAACCTAGGTAGTTGGTTTCCTGTATATTTCTTTATTTGGTTTTTTCGAGCCCCAGCATGAGTATATCTCAA GCAGGCTGGCCCTGTTGGCCCTTTCGCTTCGTGCAATGGGTGTCGGCCGCTGTTGTGTCAGTTCCAGCTAGACCTGACCTATGAAGATGTCAGTGTCTG CTGGTGATGTACACAAGGCTATTGTGAGTAATGGACAGATTCTGGGCCCTACGTTGTGGTTGAAGCAGGGTGATGATGTCGAATTTTGGTCAACA ACTCGATGTCAATCAACTACTACGGTTCAATTTTCATGGTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTGAGAACGCCCTTTCTCT ATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCAACTAGGCACCCCGTGGTTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGC GAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCTCTATGGGGCT ATCTACATTGAACCGGCAGACTCCGCTGGAGAGGCTTTCATTTTCATTCGCTGGATCTGTATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAG AATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCCTTTTCCTCGCATGATATTTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCC AACTCTGTTTGTATCAACGGAAGGGCTCGGTGATTTGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCAAAAGCAGATCTGGGC AACTGACTCTGACTGATATGGGGTATAGTCCCAAGACTTGATGACGGTTAATGCTGATCCTGGCTAG
16	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTTGTGTCAGTTCCAGCTAGACCTGACCTAT
17	GAAGATGTGAGTGTGCTGCTGGTGTATGACACAAGGCTATTTGTGAGTAATGGACAGATTCTCGGGCCCTACGTTGTGGTTGAAGCAGGGGTGATGATGTC GAATTTTGGTCAACAACCTCGATGTCATCACTACGCTTCATTTTCATG
18	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTTGTGTCAGTTCCAGCTAGACCTGACCTAT GAAGATGTGAGTGTGCTGCTGGTGTATGACACAAGGCTATTTGTGAGTAATGGACAGATTCTCGGGCCCTACGTTGTGGTTGAAGCAGGGGTGATGATGTC GAATTTTGGTCAACAACCTCGATGTCATCACTACGCTTCATTTTCATGGTGTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTT CAGAACGCCCTTTTCTATAGAGCTTTACTAAAGATTTGTAG
19	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTTGTGTCAGTTCCAGCTAGACCTGACCTAT GAAGATGTGAGTGTGCTGCTGGTGTATGACACAAGGCTATTTGTGAGTAATGGACAGATTCTCGGGCCCTACGTTGTGGTTGAAGCAGGGGTGATGATGTC GAATTTTGGTCAACAACCTCGATGTCATCACTACGCTTCATTTTCATGGTGTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTT CAGAACGCCCTTTTCTATAGAGCTTTACTAAAGATTTGTAG
20	ATGAGTATATCTCAAAGCAGGCTGGCCCTGTTGGCCCTTTGCTTCGTGCAATGGGTGTCCGGCCGTTGTGTCAGTTCCAGCTAGACCTGACCTAT GAAGATGTGAGTGTGCTGCTGGTGTATGACACAAGGCTATTTGTGAGTAATGGACAGATTCTCGGGCCCTACGTTGTGGTTGAAGCAGGGGTGATGATGTC GAATTTTGGTCAACAACCTCGATGTCATCACTACGCTTCATTTTCATGGTGTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTT CAGAACGCCCTTTTCTATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCAACTAGGCACCCCGTGGTTCAGATGGTGTACCTGGACTCTCGCAGGA GCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGA TGGCCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGCTGGAGAGGCTTTTCCATTGTATCGCTGGATCTGATGCGGATGAGCAGCAGGCGAT GTTGGCAGCTGAAAAGAAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCCTTTTCTTCGATGATATTCTCCAGATTCAAACCGAATCTGGTGT TGAGGCCCTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTCGGTGATTTGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCA AAAGCAGATCCTGGGCAACTTGACTCTGACTGATATGGGTATGAAGTCCCAAGACTTGATGACGGTTACAATGCTGATCCTGGCTAG
21	GTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTTCAGAACGCCCTTTTCTATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCAA CTAGGCACCCCGTGGTTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTAT GGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTTGGAGAGGCCCT TTCCATTTGATCGCTGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCC TTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTCGGTGATT TGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCAAAAGCAGATCCTGGGCAACTTGACTCTGACTGATATGG
22	GTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTTCAGAACGCCCTTTTCTATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCAA CTAGGCACCCCGTGGTTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTAT GGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTTGGAGAGGCCCT TTCCATTTGATCGCTGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCC TTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTCGGTGATT TGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCAAAAGCAGATCCTGGGCAACTTGACTCTGACTGATATGG
23	GTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTTCAGAACGCCCTTTTCTATAGAGCTTTACTAAAGATTTGTAGGTATCGAGCAA CTAGGCACCCCGTGGTTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTAT GGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCCTCTATGGGGCTATCTACATTGAACCGGCAGACTCCGTTGGAGAGGCCCT TTCCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAAATACACGGCCAGTTCTCATCTCGGATTTGGCGTGCC TTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAACTCTGTTTGTATCAACGGAAGGGCTCGGTGATT TGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCCGAGCAAAAGCAGATCCTGGGCAACTTGACTCTGACTGATATGGGGTATGAAGTCCCA AGACTTGATGACGGTTACAATGCTGATCCTGGCTAG

Figure 12 (f)

24	GTGAGTACTACCTCTCGATTGTATGATCCTTTGGCTTCGATGTTTCAAGACGCTTTTCTATAGAGCTTTACTAAAGATTGTAGGTATCGAGCAA CTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGGCAAGCCAGTAT GGCTCGTACATTTTATCCTCGCACACACGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCCGGCAGACTCCGTGGAGAGGCCCT TTCCATTTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGGATTGGCGTGGCC TTTTCTTCGCTGATATTTCTCCAGATTCAAACCGAATCTGGTGTAGGGCTTACTGTGCCAATCTGTGTTTGTATCAACGGAAAGGGCTCGGTGATT TGCCCGTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCCTGGGCACTTGACTCTGACTGATATGGGGTATGAAGTCCCA AGACTTGATGACGGTTACAATGCTGATCCTGGCTAGATGCCCTGCCACGCGGGGTGTTGTGGGTCCGTACCCGTACGACCTCAGCAAGATTCC CAAAGGTTTCTACGAAGGATGCACACCTAGTGAAGGGGCCACAGAGGTTTCAAGGTCAACTCCTCATCGCGGTATGCTAGCTTTGACTTTATTAG TATGGCTGGCTCGACTTCGCTTGTGTTCTCCATCGATGAGCATCCGATGTATGTTTACGCGGTGATGGCCGTTATGTTGAACCTTTGCTTGTGCA AGCTGTACTGTGCTGTGCTTCAAGATACTCAGTTATGGTGCCACTCAAGTCGGAAGACCAGGCAGGAGATTATATCTATCCGCGTTGCCAATAA CTATGCGAACCCAGCTCATCAACGGGACCCCGTCTGCTGATGACACCTGACACCCGAAGCAGATCGGTACCTCGCAGCCCTTATATCAACGAGGC TGGCGCTAATGCCACTGCTCCACGGTCATTTGAACGAAACCGACGTGATACCTTCCCGGTGGTAGCACCTGCTAAGAAGGCGGACCGGACTTA CATCCTGAATGTCGAAATGCCAATTCATCCTACACCTGGACCTGGGCAACCCAGTACCCCGTGTGCAATGAAGAGCTTCGCCCTCTGTCTTGT CAACCTGAAACCCAGGGCCATTCTACTTTCATTTGCCATCTTCAGATGCAACCATCTCGGTGGGTGGCAGTGGCTTGCATCATCAACGGGCTCGGTGATT ACACCCCATCCACAGCACTCCAAAGTACTTCGTGATCGGAACCGGTAAACAGCCCTTCATCTGTCTCCGTGGAAGAGGCAATGAAGACAT ACCGGAGAAATTTCAACTTCGAAACCCCGAGATGCGGGACACTTTCTACTCGCCAGCTCAAGCACAGGGCCGAGCTGGCTGGCAATGCGCTACCA TATCCTGAAACCCAGGGCCATTCTACTTTCATTTGCCATCTTCAGATGCAACCATCTCGGTGGGTGGCAGTGGCTTGCATCATCAACGGGCTCGGTG GCCAACCGACATTTCCGAGGGATATCGGTTGCCAGTGATGCCAATCTAGGGCACTTTGACTCAATTGTACAGAGGTGGACGAGAGTATCTACTTGTA ATATGGCCATATAGCGCTCTCATATAGATCCGGTGTAATATTTATTTTACTACATAATGAACATGCTTGGTGACTTACCAATTTCCCGAGTTAT GGCTCCAAAGTAAAGTGAGAGCAATCACACCAATCAGGCACAGCAGTAA
25	GTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGG CAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCCGGCAGACTCCG TGGAGAGGCCCTTTCCATTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGG ATTGGCGTGCTTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAATCTGTGTTTGTATCAACGGAAAGG GCTCGGTGATTGCCCCGTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCCTGGGCACTTGACTCTGACTGATATGGG
26	GTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGG CAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCCGGCAGACTCCG TGGAGAGGCCCTTTCCATTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGG ATTGGCGTGCTTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAATCTGTGTTTGTATCAACGGAAAGG GCTCGGTGATTGCCCCGTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCCTGGGCACTTGACTCTGACTGATATGGG
27	GTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGG CAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCCGGCAGACTCCG TGGAGAGGCCCTTTCCATTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGG ATTGGCGTGCTTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAATCTGTGTTTGTATCAACGGAAAGG GCTCGGTGATTGCCCCGTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCCTGGGCACTTGACTCTGACTGATATGGG
28	GTATCGAGCAACTAGGCACCCCGTGGTCAGATGGTGTACCTGGACTCTCGCAGGAGCAGATCAAGCCGGGCGAACAGTTTCTCTATAAATGGAAGG CAAGCCAGTATGGCTCGTACATTTATCACTCGCACACACGGGCTCAGATTGATGATGGCCTCTATGGGGCTATCTACATTGAACCCGGCAGACTCCG TGGAGAGGCCCTTTCCATTGATCGCTGGATCTGATGCGGATGAGCAGCAGGCGATGTTGGCAGCTGAAAAGAATACACGGCCAGTTCTCATCTCGG ATTGGCGTGCTTTTCTCGCATGATATTCTCCAGATTCAAACCGAATCTGGTGTGAGGCCCTACTGTGCCAATCTGTGTTTGTATCAACGGAAAGG GCTCGGTGATTGCCCCGTCTCAGGAACACATCAATGCTGCCACGACACCGCAGCAAAAGCAGATCCTGGGCACTTGACTCTGACTGATATGGG

[illegible]

Figure 12 (h)

41	RVVQFQLDLTYEDVSVAGDVHKAIVSNGQIPGPTLWLKQGDDVEFLVNNSMSINTTVHFGHIEQLGTPWSDGVPGLSQEQIKPGEQFLYKWKASQY GSYIYHSHTRAQIDDDGLYGAIYIEPADSVERPPHFIAGSDADEQQAMLAEEKNTRPVLISDWRAFSSHDILQIQTESGVEAYCANSVLINGKGSVI CPSQEHINAATTPQQKQILGNLTLTDMGCLPPTPGVVGPPYPYDLSKIPKGFYEGCTPSEGPTEVFKNSSSRYSASFDFISMAGSTSLVFSIDHBM YVYAVDGRYVEPLLVEAVTVPVASRYSVMPVKSEDAQAGDYTIRVANNYANQLINGTAVLSYDTATPKQIGTSQPYINEAGANATASTVILNETDV IPFPVAPAKKADRTYILNVENANSSYTTLGNQYPVSNEELRPPVLFNLSSISQAYSAMTEYGTWVDLIINITTSGQPQHPHHSNKYFVIGTG NQFFIWSSVEEAMKDIPENFNENPQMRDTFYSPSSSTGFSWLAMRYHIVNPGPFLHCHLQMHVGGGLALLDGVDAWPTDIEGYRLPVMPI
42	MSISQSRLLALLCFVQWVSG
43	MSISQSRLLALLCFVQWVSGRVVQFQLDLTYEDVSVAGDVHKAIVSNGQIPGPTLWLKQGDDVEFLVNNSMSINTTVHFGH
44	GIEQLGTPWSDGVPGLSQEQIKPGEQFLYKWKASQYGSYIYHSHTRAQIDDDGLYGAIYIEPADSVERPPHFIAGSDADEQQAMLAEEKNTRPVLIS DWRAFSSHDILQIQTESGVEAYCANSVLINGKGSVICPSQEHINAATTPQQKQILGNLTLTDMG
45	CLPPTPGVVGPPYPYDLSKIPKGFYEGCTPSEGPTEVFKNSSSRYSASFDFISMAGSTSLVFSIDHBMVYVAVDGRYVEPLLVEAVTVPVASRYSV MVPLKSEDAQAGDYTIRVANNYANQLINGTAVLSYDTATPKQIGTSQPYINEAGANATASTVILNETDVI PFPVAPAKKADRTYILNVENANSSYT WTLGNQYPVSNEELRPPVLFNLSSISQAYSAMTEYGTWVDLI INITTSGQPQHPHHSNKYFVIGTG NQFFIWSSVEEAMKDIPENFNENPQMR DTFYSPSSSTGFSWLAMRYHIVNPGPFLHCHLQMHVGGGLALLDGVDAWPTDIEGYRLPVMPI
46	AVVQFQLDLT
47	SEDAQAGDYTIR
48	AVVQFQLDLTYEDVSVAGXVXKAIVLNGXI
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50	ASQYXSYYHSHTR
51	GAAGTGGAAAGGCTGGTGTC
52	GTTGGCCTTTT

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<212> DNA
<213> *Aspergillus ficuum*

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<210> 3
<211> 1925
<212> DNA
<213> Aspergillus ficuum

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<210> 4
 <211> 1876
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 5
 <211> 1840
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 6
 <211> 2293
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 7
 <211> 2159
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 8

<211> 1985

<212> DNA

<213> *Aspergillus ficuum*

<400> 8

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<210> 9
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 <213> *Aspergillus ficuum*

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<210> 10
 <211> 1985
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 11
<211> 1596
<212> DNA
<213> Aspergillus ficuum

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<210> 12
<211> 612
<212> DNA
<213> Aspergillus ficuum

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<210> 13
<211> 697
<212> DNA
<213> Aspergillus ficuum

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<210> 14
<211> 1175
<212> DNA
<213> Aspergillus ficuum

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<210> 15
<211> 1224
<212> DNA
<213> Aspergillus ficuum

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<210> 16
 <211> 63
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 17
 <211> 244
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 18
 <211> 329
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 19
 <211> 807
 <212> DNA
 <213> *Aspergillus ficuum*

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 cgaatctggt gttgaggcct actgtgccaa ctctgttttg atcaacggaa agggctcgg 720
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<210> 20
 <211> 856
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 21
 <211> 563
 <212> DNA
 <213> *Aspergillus ficuum*

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aacttgactc tgactgatat ggg 563

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<210> 22
 <211> 612
 <212> DNA
 <213> *Aspergillus ficuum*

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gtacctggac tctcgcagga gcagatcaag ccgggcgaac agttttctcta taaatggaag 180
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<210> 23
 <211> 1681
 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 24
 <211> 1875
 <212> DNA
 <213> *Aspergillus ficuum*

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 gtacctggac tctcgcagga gcagatcaag ccgggccaac agtttctcta taaatggaag 180
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<210> 25
 <211> 478
 <212> DNA
 <213> *Aspergillus ficuum*

<400> 25
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 atcactcgca cacacgggct cagattgatg atggcctcta tggggctatc tacattgaac 180
 cggcagactc cgtggagagg cctttccatt tgatcgctgg atctgatgcg gatgagcagc 240
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 actctgtttt gatcaacgga aagggtctcg tgatttgccc gtctcaggaa cacatcaatg 420
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<210> 26
 <211> 527
 <212> DNA
 <213> *Aspergillus ficuum*

<400> 26
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 atcactcgca cacacgggct cagattgatg atggcctcta tggggctatc tacattgaac 180
 cggcagactc cgtggagagg cctttccatt tgatcgctgg atctgatgcg gatgagcagc 240
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<210> 27
 <211> 1596
 <212> DNA
 <213> *Aspergillus ficuum*

<400> 27
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<210> 28
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 <212> DNA
 <213> *Aspergillus ficuum*

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<210> 29
<211> 1118
<212> DNA
<213> Aspergillus ficuum

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<210> 30
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<212> DNA
<213> Aspergillus ficuum

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tgataccctt ccccggtgga gcacctgcta agaaggcgga ccggacttac atcctgaatg   600
tcgaaaatgc caattcatcc tacacctgga ccctgggcaa ccagtacccc gtgtcgaatg   660
aagagcttcg ccctcctgtc ttgttcaacc tcagctcaat ctcgcaggcg tactcggcca   720
tgacggagta cgggtacctg gttgatctca tcatcaacat caccacctcg ggccagcctc   780
aacaccccat ccacaagcac tccaacaagt acttcgtgat cggaaccggg aaccagccct   840
tcatctgggc ctccgtggaa gaggcaatga aagacatacc ggagaatttc aacttcgaaa   900
acccccagat gcgggacact ttctactcgc caagctcaag cacagggccg agctggctgg   960

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caatgcgcta ccataatcgtg aacccagggc cattcctact tcattgccat cttcagatgc 1020
accatgtcgg tgggttggca ctggccttgc tggacggggt ggacgcctgg ccaaccgaca 1080
ttcccagagg atatacggtt ccagtgatgc caatctaggg actttgactc aattgtacag 1140
aggtggacga gagtatctac ttgtaatatg gccatatagc gcctctcata tagatccggt 1200
gtaatatatt ttatttacta cataatgaac atgcttggtg acttaccaat tccccagtta 1260
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<210> 31
<211> 1069
<212> DNA
<213> Aspergillus ficuum

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ctcctcatcg cggtatgcta gctttgactt tattagtatg gctggctcga cttcgcttgt 180
gttctccatc gatgagcatc cgatgtatgt ttacgcgggtc gatggccggt atgttgaacc 240
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caagtcggaa gaccaggcag gagattatac tatccgcgtt gccaataact atgcgaacca 360
gctcatcaac gggaccgccg tcctgtcgta tgacaccgct acaccgaagc agatcgggtac 420
ctcgagcct tatatcaacg aggttggcgc taatgccact gcctccacgg tcattttgaa 480
cgaaaccgac gtgataccct tccccgtggt agcacctgct aagaaggcgg accggactta 540
catcctgaat gtcgaaaatg ccaattcatc ctacacctgg accctgggca accagtaccc 600
cgtgtcgaat gaagagcttc gccctcctgt cttgttcaac ctcagctcaa tctcgaggc 660
gtactcggcc atgacggagt acggtacctg gggtgatctc atcatcaaca tcaccacctc 720
gggccagcct caacacccca tccacaagca ctccaacaag tacttcgtga tcggaaccgg 780
taaccagccc ttcattctggt cctccgtgga agaggcaatg aaagacatac cggagaattt 840
caacttcgaa aacccccaga tgcgggacac tttctactcg ccaagctcaa gcacagggcc 900
gagctggctg gcaatgcgct accatatact gaaccaggg ccattcctac ttatttgcca 960
tcttcagatg caccatgtcg gtgggttggc actggccttg ctggacgggg tggacgcctg 1020
gccaaccgac attccccgagg gatatacggtt gccagtgatg ccaatctag 1069

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<210> 32
<211> 1263
<212> DNA
<213> Aspergillus ficuum

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caaaggtttc tacgaaggat gcacacctag tgaaggggccc acagagggtt tcaagggtcaa 120
ctcctcatcg cggtatgcta gctttgactt tattagtatg gctggctcga cttcgcttgt 180

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gttctccatc gatgagcatc cgatgtatgt ttacgcggtc gatggccgtt atgttgaacc 240
tttgcttgtc gaagctgtta ctgtgcctgt cgcttcaaga tactcagtta tggtgccact 300
caagtcggaa gaccaggcag gagattatac tatccgcgtt gccaataact atgcgaacca 360
gctcatcaac gggaccgccg tcctgtcgtg tgacaccgct acaccgaagc agatcgggtac 420
ctcgcagcct tatatcaacg aggctggcgc taatgccact gcctccacgg tcattttgaa 480
cgaaaccgac gtgataccct tccccgtggg agcacctgct aagaaggcgg accggactta 540
catcctgaat gtcgaaaatg ccaattcatc ctacacctgg accctgggca accagtaccc 600
cgtgtcgaat gaagagcttc gccctcctgt cttgttcaac ctgagctcaa tctcgcaggc 660
gtactcggcc atgacggagt acggtacctg ggttgatctc atcatcaaca tcaccacctc 720
gggccagcct caacacccca tccacaagca ctccaacaag tacttcgtga tcggaaccgg 780
taaccagccc ttcattctggg cctccgtgga agaggcaatg aaagacatac cggagaatth 840
caacttcgaa aacccccaga tgcgggacac tttctactcg ccaagctcaa gcacagggcc 900
gagctggctg gcaatgcgct accatatcgt gaaccagggg ccatttcctac ttcattgcc 960
tcttcagatg caccatgtcg gtgggttggc actggccttg ctggacgggg tggacgcctg 1020
gccaaccgac attcccggag gatatcggtt gccagtgatg ccaatctagg gactttgact 1080
caattgtaca gaggtggacg agagtatcta cttgtaatat ggccatatag cgcctctcat 1140
atagatccgg tgtaatatta tttatttact acataatgaa catgcttggt gacttaccaa 1200
ttccccagtt atgggtccaa agtaaaagtg agagcaatca caccaaatca ggcacagcag 1260
taa 1263

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<210> 33
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 <212> DNA
 <213> *Aspergillus ficuum*

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<400> 33
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taggccctgc atatgacgga tccagtatct tggccatcgg acggcattat gacaagccct 120
agtcatgaac ctttcgctat acgtctcggg tcctccatcc cttgccttga ctttcgggca 180
tcaatggcat caggtcaatg tgcctcttga gttctgtcat tataaaagcc tcaaacttct 240
cgcaggatga agggaaactca accacgacaa accagtccgc gactcggccc tgttcgactg 300
tatatatctt gtggaactag gtagttgggt ccttgatatat ttctttatth ggthttttga 360
gccccagc 368

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<210> 34
 <211> 85
 <212> DNA
 <213> *Aspergillus ficuum*

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<400> 34
gtgagtacta cctctcgatt gtatgatcct ttggcttcga tgttcagaac gccttttcct 60

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atagagcttt actaaagatt tgtag 85

<210> 35
 <211> 49
 <212> DNA
 <213> Aspergillus ficuum

<400> 35
 gtatgaagtc ccaagacttg atgacgggta caatgctgat cctggctag 49

<210> 36
 <211> 194
 <212> DNA
 <213> Aspergillus ficuum

<400> 36
 ggactttgac tcaattgtac agaggtggac gagagtatct acttgtaata tggccatata 60
 ggcctctca tatagatccg gtgtaatatt atttatttac tacataatga acatgcttgg 120
 tgacttacca attccccagt tatggctcca aagtaaaagt gagagcaatc acaccaaatc 180
 aggcacagca gtaa 194

<210> 37
 <211> 26
 <212> DNA
 <213> Aspergillus ficuum

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 <223> I

<220>
 <221> misc_feature
 <222> (3)..(3)
 <223> n is a, c, g, or t

<220>
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 <222> (6)..(6)
 <223> I

<220>
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 <223> n is a, c, g, or t

<220>
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 <222> (18)..(18)
 <223> I

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 <223> n is a, c, g, or t

<220>
 <221> modified_base
 <222> (24)..(24)
 <223> I

<220>
 <221> misc_feature
 <222> (24)..(24)
 <223> n is a, c, g, or t

 <400> 37
 gtngtncagt ttcagytnga tytnac

26

<210> 38
 <211> 29
 <212> DNA
 <213> Aspergillus ficuum

<220>
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 <222> (12)..(12)
 <223> I

<220>
 <221> misc_feature
 <222> (12)..(12)
 <223> n is a, c, g, or t

<220>
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 <222> (15)..(15)
 <223> I

<220>
 <221> misc_feature
 <222> (15)..(15)
 <223> n is a, c, g, or t

<220>
 <221> modified_base
 <222> (18)..(18)
 <223> I

<220>
 <221> misc_feature
 <222> (18)..(18)
 <223> n is a, c, g, or t

<220>
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 <222> (27)..(27)
 <223> I

<220>
 <221> misc_feature
 <222> (27)..(27)
 <223> n is a, c, g, or t

<400> 38
 ctyctrgtyc gncncncnt gatgtgnta

29

<210> 39
 <211> 20
 <212> DNA
 <213> Aspergillus ficuum

<400> 39
 caacccagg accgtactcc

20

<210> 40
 <211> 596
 <212> PRT
 <213> Aspergillus ficuum

<400> 40

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

Gln Trp Val Ser Gly Arg Val Val Gln Phe Gln Leu Asp Leu Thr Tyr
 20 25 30

Glu Asp Val Ser Val Ala Gly Asp Val His Lys Ala Ile Val Ser Asn
 35 40 45

Gly Gln Ile Pro Gly Pro Thr Leu Trp Leu Lys Gln Gly Asp Asp Val
 50 55 60

Glu Phe Leu Val Asn Asn Ser Met Ser Ile Asn Thr Thr Val His Phe
 65 70 75 80

His Gly Ile Glu Gln Leu Gly Thr Pro Trp Ser Asp Gly Val Pro Gly
 85 90 95

Leu Ser Gln Glu Gln Ile Lys Pro Gly Glu Gln Phe Leu Tyr Lys Trp
 100 105 110

Lys Ala Ser Gln Tyr Gly Ser Tyr Ile Tyr His Ser His Thr Arg Ala
 115 120 125

Gln Ile Asp Asp Gly Leu Tyr Gly Ala Ile Tyr Ile Glu Pro Ala Asp
 130 135 140

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

Gln Gln Ala Met Leu Ala Ala Glu Lys Asn Thr Arg Pro Val Leu Ile
 165 170 175

Ser Asp Trp Arg Ala Phe Ser Ser His Asp Ile Leu Gln Ile Gln Thr
 180 185 190

Glu Ser Gly Val Glu Ala Tyr Cys Ala Asn Ser Val Leu Ile Asn Gly
 195 200 205

Lys Gly Ser Val Ile Cys Pro Ser Gln Glu His Ile Asn Ala Ala Thr
 210 215 220

Thr Pro Gln Gln Lys Gln Ile Leu Gly Asn Leu Thr Leu Thr Asp Met
 225 230 235 240

Gly Cys Leu Pro Pro Thr Pro Gly Val Val Gly Pro Tyr Pro Tyr Asp
 Page 28

245 250 255
 Leu Ser Lys Ile Pro Lys Gly Phe Tyr Glu Gly Cys Thr Pro Ser Glu
 260 265 270
 Gly Pro Thr Glu Val Phe Lys Val Asn Ser Ser Ser Arg Tyr Ala Ser
 275 280 285
 Phe Asp Phe Ile Ser Met Ala Gly Ser Thr Ser Leu Val Phe Ser Ile
 290 295 300
 Asp Glu His Pro Met Tyr Val Tyr Ala Val Asp Gly Arg Tyr Val Glu
 305 310 315 320
 Pro Leu Leu Val Glu Ala Val Thr Val Pro Val Ala Ser Arg Tyr Ser
 325 330 335
 Val Met Val Pro Leu Lys Ser Glu Asp Gln Ala Gly Asp Tyr Thr Ile
 340 345 350
 Arg Val Ala Asn Asn Tyr Ala Asn Gln Leu Ile Asn Gly Thr Ala Val
 355 360 365
 Leu Ser Tyr Asp Thr Ala Thr Pro Lys Gln Ile Gly Thr Ser Gln Pro
 370 375 380
 Tyr Ile Asn Glu Ala Gly Ala Asn Ala Thr Ala Ser Thr Val Ile Leu
 385 390 395 400
 Asn Glu Thr Asp Val Ile Pro Phe Pro Val Val Ala Pro Ala Lys Lys
 405 410 415
 Ala Asp Arg Thr Tyr Ile Leu Asn Val Glu Asn Ala Asn Ser Ser Tyr
 420 425 430
 Thr Trp Thr Leu Gly Asn Gln Tyr Pro Val Ser Asn Glu Glu Leu Arg
 435 440 445
 Pro Pro Val Leu Phe Asn Leu Ser Ser Ile Ser Gln Ala Tyr Ser Ala
 450 455 460
 Met Thr Glu Tyr Gly Thr Trp Val Asp Leu Ile Ile Asn Ile Thr Thr
 465 470 475 480
 Ser Gly Gln Pro Gln His Pro Ile His Lys His Ser Asn Lys Tyr Phe
 485 490 495
 Val Ile Gly Thr Gly Asn Gln Pro Phe Ile Trp Ser Ser Val Glu Glu
 500 505 510
 Ala Met Lys Asp Ile Pro Glu Asn Phe Asn Phe Glu Asn Pro Gln Met
 Page 29

515

520

525

Arg Asp Thr Phe Tyr Ser Pro Ser Ser Ser Thr Gly Pro Ser Trp Leu
 530 535 540

Ala Met Arg Tyr His Ile Val Asn Pro Gly Pro Phe Leu Leu His Cys
 545 550 555 560

His Leu Gln Met His His Val Gly Gly Leu Ala Leu Ala Leu Leu Asp
 565 570 575

Gly Val Asp Ala Trp Pro Thr Asp Ile Pro Glu Gly Tyr Arg Leu Pro
 580 585 590

Val Met Pro Ile
 595

<210> 41
 <211> 575
 <212> PRT
 <213> Aspergillus ficuum

<400> 41

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

Pro Thr Leu Trp Leu Lys Gln Gly Asp Asp Val Glu Phe Leu Val Asn
 35 40 45

Asn Ser Met Ser Ile Asn Thr Thr Val His Phe His Gly Ile Glu Gln
 50 55 60

Leu Gly Thr Pro Trp Ser Asp Gly Val Pro Gly Leu Ser Gln Glu Gln
 65 70 75 80

Ile Lys Pro Gly Glu Gln Phe Leu Tyr Lys Trp Lys Ala Ser Gln Tyr
 85 90 95

Gly Ser Tyr Ile Tyr His Ser His Thr Arg Ala Gln Ile Asp Asp Gly
 100 105 110

Leu Tyr Gly Ala Ile Tyr Ile Glu Pro Ala Asp Ser Val Glu Arg Pro
 115 120 125

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

Ala Ala Glu Lys Asn Thr Arg Pro Val Leu Ile Ser Asp Trp Arg Ala
 145 150 155 160

Phe Ser Ser His Asp Ile Leu Gln Ile Gln Thr Glu Ser Gly Val Glu
 165 170 175
 Ala Tyr Cys Ala Asn Ser Val Leu Ile Asn Gly Lys Gly Ser Val Ile
 180 185 190
 Cys Pro Ser Gln Glu His Ile Asn Ala Ala Thr Thr Pro Gln Gln Lys
 195 200 205
 Gln Ile Leu Gly Asn Leu Thr Leu Thr Asp Met Gly Cys Leu Pro Pro
 210 215 220
 Thr Pro Gly Val Val Gly Pro Tyr Pro Tyr Asp Leu Ser Lys Ile Pro
 225 230 235 240
 Lys Gly Phe Tyr Glu Gly Cys Thr Pro Ser Glu Gly Pro Thr Glu Val
 245 250 255
 Phe Lys Val Asn Ser Ser Ser Arg Tyr Ala Ser Phe Asp Phe Ile Ser
 260 265 270
 Met Ala Gly Ser Thr Ser Leu Val Phe Ser Ile Asp Glu His Pro Met
 275 280 285
 Tyr Val Tyr Ala Val Asp Gly Arg Tyr Val Glu Pro Leu Leu Val Glu
 290 295 300
 Ala Val Thr Val Pro Val Ala Ser Arg Tyr Ser Val Met Val Pro Leu
 305 310 315 320
 Lys Ser Glu Asp Gln Ala Gly Asp Tyr Thr Ile Arg Val Ala Asn Asn
 325 330 335
 Tyr Ala Asn Gln Leu Ile Asn Gly Thr Ala Val Leu Ser Tyr Asp Thr
 340 345 350
 Ala Thr Pro Lys Gln Ile Gly Thr Ser Gln Pro Tyr Ile Asn Glu Ala
 355 360 365
 Gly Ala Asn Ala Thr Ala Ser Thr Val Ile Leu Asn Glu Thr Asp Val
 370 375 380
 Ile Pro Phe Pro Val Val Ala Pro Ala Lys Lys Ala Asp Arg Thr Tyr
 385 390 395 400
 Ile Leu Asn Val Glu Asn Ala Asn Ser Ser Tyr Thr Trp Thr Leu Gly
 405 410 415
 Asn Gln Tyr Pro Val Ser Asn Glu Glu Leu Arg Pro Pro Val Leu Phe
 420 425 430

Asn Leu Ser Ser Ile Ser Gln Ala Tyr Ser Ala Met Thr Glu Tyr Gly
435 440 445

Thr Trp Val Asp Leu Ile Ile Asn Ile Thr Thr Ser Gly Gln Pro Gln
450 455 460

His Pro Ile His Lys His Ser Asn Lys Tyr Phe Val Ile Gly Thr Gly
465 470 475 480

Asn Gln Pro Phe Ile Trp Ser Ser Val Glu Glu Ala Met Lys Asp Ile
485 490 495

Pro Glu Asn Phe Asn Phe Glu Asn Pro Gln Met Arg Asp Thr Phe Tyr
500 505 510

Ser Pro Ser Ser Ser Thr Gly Pro Ser Trp Leu Ala Met Arg Tyr His
515 520 525

Ile Val Asn Pro Gly Pro Phe Leu Leu His Cys His Leu Gln Met His
530 535 540

His Val Gly Gly Leu Ala Leu Ala Leu Leu Asp Gly Val Asp Ala Trp
545 550 555 560

Pro Thr Asp Ile Pro Glu Gly Tyr Arg Leu Pro Val Met Pro Ile
565 570 575

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<211> 21
<212> PRT
<213> Aspergillus ficuum

<400> 42

Met Ser Ile Ser Gln Ser Arg Leu Ala Leu Leu Ala Leu Cys Phe Val
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Gln Trp Val Ser Gly
20

<210> 43
<211> 81
<212> PRT
<213> Aspergillus ficuum

<400> 43

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Gln Trp Val Ser Gly Arg Val Val Gln Phe Gln Leu Asp Leu Thr Tyr
20 25 30

Glu Asp Val Ser Val Ala Gly Asp Val His Lys Ala Ile Val Ser Asn
35 40 45

Gly Gln Ile Pro Gly Pro Thr Leu Trp Leu Lys Gln Gly Asp Asp Val
50 55 60

Glu Phe Leu Val Asn Asn Ser Met Ser Ile Asn Thr Thr Val His Phe
65 70 75 80

His

<210> 44
<211> 160
<212> PRT
<213> Aspergillus ficuum

<400> 44

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1 5 10 15

Ser Gln Glu Gln Ile Lys Pro Gly Glu Gln Phe Leu Tyr Lys Trp Lys
20 25 30

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

Ile Asp Asp Gly Leu Tyr Gly Ala Ile Tyr Ile Glu Pro Ala Asp Ser
50 55 60

Val Glu Arg Pro Phe His Leu Ile Ala Gly Ser Asp Ala Asp Glu Gln
65 70 75 80

Gln Ala Met Leu Ala Ala Glu Lys Asn Thr Arg Pro Val Leu Ile Ser
85 90 95

Asp Trp Arg Ala Phe Ser Ser His Asp Ile Leu Gln Ile Gln Thr Glu
100 105 110

Ser Gly Val Glu Ala Tyr Cys Ala Asn Ser Val Leu Ile Asn Gly Lys
115 120 125

Gly Ser Val Ile Cys Pro Ser Gln Glu His Ile Asn Ala Ala Thr Thr
130 135 140

Pro Gln Gln Lys Gln Ile Leu Gly Asn Leu Thr Leu Thr Asp Met Gly
145 150 155 160

<210> 45
<211> 355
<212> PRT
<213> Aspergillus ficuum

<400> 45

Cys Leu Pro Pro Thr Pro Gly Val Val Gly Pro Tyr Pro Tyr Asp Leu
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20 25 30

Pro Thr Glu Val Phe Lys Val Asn Ser Ser Ser Arg Tyr Ala Ser Phe
35 40 45

Asp Phe Ile Ser Met Ala Gly Ser Thr Ser Leu Val Phe Ser Ile Asp
50 55 60

Glu His Pro Met Tyr Val Tyr Ala Val Asp Gly Arg Tyr Val Glu Pro
65 70 75 80

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

Met Val Pro Leu Lys Ser Glu Asp Gln Ala Gly Asp Tyr Thr Ile Arg
100 105 110

Val Ala Asn Asn Tyr Ala Asn Gln Leu Ile Asn Gly Thr Ala Val Leu
115 120 125

Ser Tyr Asp Thr Ala Thr Pro Lys Gln Ile Gly Thr Ser Gln Pro Tyr
130 135 140

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

Glu Thr Asp Val Ile Pro Phe Pro Val Val Ala Pro Ala Lys Lys Ala
165 170 175

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

Trp Thr Leu Gly Asn Gln Tyr Pro Val Ser Asn Glu Glu Leu Arg Pro
195 200 205

Pro Val Leu Phe Asn Leu Ser Ser Ile Ser Gln Ala Tyr Ser Ala Met
210 215 220

Thr Glu Tyr Gly Thr Trp Val Asp Leu Ile Ile Asn Ile Thr Thr Ser
225 230 235 240

Gly Gln Pro Gln His Pro Ile His Lys His Ser Asn Lys Tyr Phe Val
245 250 255

Ile Gly Thr Gly Asn Gln Pro Phe Ile Trp Ser Ser Val Glu Glu Ala
260 265 270

Met Lys Asp Ile Pro Glu Asn Phe Asn Phe Glu Asn Pro Gln Met Arg
 275 280 285

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

Met Arg Tyr His Ile Val Asn Pro Gly Pro Phe Leu Leu His Cys His
 305 310 315 320

Leu Gln Met His His Val Gly Gly Leu Ala Leu Ala Leu Leu Asp Gly
 325 330 335

Val Asp Ala Trp Pro Thr Asp Ile Pro Glu Gly Tyr Arg Leu Pro Val
 340 345 350

Met Pro Ile
 355

<210> 46
 <211> 10
 <212> PRT
 <213> Aspergillus

<400> 46

Ala Val Val Gln Phe Gln Leu Asp Leu Thr
 1 5 10

<210> 47
 <211> 11
 <212> PRT
 <213> Aspergillus

<400> 47

Ser Glu Asp Gln Ala Gly Asp Tyr Thr Ile Arg
 1 5 10

<210> 48
 <211> 30
 <212> PRT
 <213> Aspergillus

<220>
 <221> misc_feature
 <222> (19)..(19)
 <223> Xaa can be any naturally occurring amino acid

<220>
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 <223> Xaa can be any naturally occurring amino acid

<220>
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 <222> (29)..(29)
 <223> Xaa can be any naturally occurring amino acid

<400> 48

Ala Val Val Gln Phe Gln Leu Asp Leu Thr Tyr Glu Asp Val Ser Val
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Ala Gly Xaa Val Xaa Lys Ala Ile Val Leu Asn Gly Xaa Ile
 20 25 30

<210> 49
 <211> 18
 <212> PRT
 <213> Aspergillus

<220>
 <221> misc_feature
 <222> (11)..(12)
 <223> Xaa can be any naturally occurring amino acid

<400> 49

Tyr Glu Asp Val Ser Val Ala Gly Lys Val Xaa Xaa Ala Ile Val Leu
 1 5 10 15

Asn Gly

<210> 50
 <211> 14
 <212> PRT
 <213> Aspergillus

<220>
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 <222> (5)..(5)
 <223> Xaa can be any naturally occurring amino acid

<400> 50

Ala Ser Gln Tyr Xaa Ser Tyr Ile Tyr His Ser His Thr Arg
 1 5 10

<210> 51
 <211> 21
 <212> DNA
 <213> Aspergillus nidulans

<400> 51
 gaagtggaaa ggctggtgtg c

21

<210> 52
 <211> 11
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> sfi I adaptor

<400> 52
 gttggccttt t

11

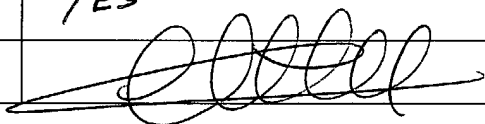
Original (for **SUBMISSION**)

(This sheet is not part of and does not count as a sheet of the international application)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT-SAFE [EASY mode] Version 3.50 (Build 0002.162)
0-2	International Application No.	PCT/BE2005/000138
0-3	Applicant's or agent's file reference	BPPURA030BWO

1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	38
1-2	line	28
1-3	Identification of deposit	
1-3-1	Name of depositary institution	BCCM Belgian Coordinated Collections of Microorganisms
1-3-2	Address of depositary institution	Prime Minister's Services, Federal Office for Scientific, Technical and Cultural Affairs, Rue de la Science 8, B-1000 Brussels, Belgium
1-3-3	Date of deposit	11 May 2001 (11.05.2001)
1-3-4	Accession Number	BCCM LMBP 4366
1-5	Designated States for Which Indications are Made	all designations

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	YES
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	
0-5-1	Authorized officer	