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(21) International Application Number: PCT/DK97/00284 (22) International Filing Date: 30 June 1997 (30.06.97) (30) Priority Data: 0714/96 28 June 1996 (28.06.96) DK 0919/96 30 August 1996 (30.08.96) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): CHRISTENSEN, Tove [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). FUGLSANG, Claus, Crone [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). HALKIER, Torben [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). JOHANSEN, Charlotte [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). (74) Common Representative: NOVO NORDISK A/S; Novo Allé, DK-2880 Bagsværd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: A RECOMBINANT ENZYME WITH DEXTRANASE ACTIVITY (57) Abstract <p>The present invention relates to a cloned DNA sequence encoding an enzyme with dextranase activity, a recombinant expression vector comprising said DNA sequence, a filamentous fungus host cell, a method for producing said recombinant dextranase, and the isolated and purified enzyme. The invention also relates to compositions comprising the recombinant enzyme, oral care compositions and products and the use for removing of dental plaque.</p>		

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Title: A recombinant enzyme with dextranase activity

FIELD OF THE INVENTION

The present invention relates to a cloned DNA sequence
5 encoding an enzyme with dextranase activity, a recombinant
expression vector comprising said DNA sequence, a filamentous
fungus host cell, a method for producing said recombinant
dextranase, and the isolated and purified enzyme.

The invention also relates to compositions comprising the
10 recombinant enzyme, oral care compositions and products and the
use for removing of dental plaque.

BACKGROUND OF THE INVENTION

Dextranases are α -1,6-glucanases (E.C. 3.2.1.11), also
15 known as 1,6- α -D-glucan 6-glucanohydrolases, which degrade the
 α -1,6-glycosidic linkages in dextran.

Dextranases are known to be useful for a number of appli-
cations including the use as ingredient in dentifrice for
prevent dental caries, plaque and/or tartar and for hydrolysis
20 of raw sugar juice or syrup of sugar canes and sugar beets.

Several micro-organisms are known to be capable of pro-
ducing dextranases, among them fungi of the genera *Penicillium*,
Paecilomyces, *Aspergillus*, *Fusarium*, *Spicaria*, *Verticillium*,
Helminthosporium and *Chaetomium*; bacteria of the genera
25 *Lactobacillus*, *Streptococcus*, *Cellvibrio*, *Cytophaga*,
Brevibacterium, *Pseudomonas*, *Corynebacterium*, *Arthrobacter* and
Flavobacterium, and yeasts such as *Lipomyces starkeyi*.

A commercially available dextranase, sold as an industrial
enzyme for breaking down raw sugar juice, is Dextranase 50L
30 from Novo Nordisk produced by fermentation of a strain of
Paecilomyces sp..

Below are summarised prior art documents concerning
dextranase and applications thereof.

35 Prior art documents

EP 663 443 (Centro de Ingenieria Genetica y Biotecnologia) describes a dextranase derived from *Penicillium minioluteum*. The dextranase can be expressed heterologously in the yeast *Pichia pastoris*. Said recombinant enzyme has an optimum
5 temperature in the range from 55°C to 60°C, a N-glycosylation percentage between 13 and 15% and a half-life time of about 7.6 hours at 50°C.

BRIEF DESCRIPTION OF THE DRAWING

- 10 Figure 1 shows plasmid pCaHj483
Figure 2 shows plasmid pToC343
Figure 3 shows plasmid pToC325
Figure 4 shows the pH-profile of recombinant and wild-type *Paecilomyces lilacinus* dextranase
15 Figure 5 shows the temperature profile of recombinant and wild-type *Paecilomyces lilacinus* dextranase
Figure 6 shows the temperature stability of recombinant and wild-type *Paecilomyces lilacinus* dextranase
Figure 7 shows the indirect Malthus standard curve for a mix
20 culture of *S. mutans*, *A. viscosus* and *F. nucleatum* grown in BHI at 37°C.
Figure 8 shows the expression plasmid pJW111. The SP387 promoter and terminator are labelled. Restriction enzyme sites are indicated as well as their relative position within the
25 plasmid. The bar, beta-lactamase (ampR) and dextranase genes are represented by arrows showing the direction of transcription.

SUMMARY OF THE INVENTION

- 30 The object of the invention is to provide a recombinant dextranase from *Paecilomyces lilacinus* by heterologous production in a filamentous fungi host cell.

The present inventors have as the first cloned the complete DNA sequence encoding an enzyme with dextranase
35 activity from *Paecilomyces lilacinus* and produced it heterologously in a filamentous fungi host cell. Said enzyme has previously only been produced homologously in *Paecilomyces lilacinus*. Consequently, according to prior art the enzyme

product comprising said enzyme with dextranase activity is produced together with a mixture of other enzyme activities. It is advantageous to be able to produce a recombinant dextranase heterologously in a suitable host, as it is possible to provide
5 a single component dextranase. Further, it facilitates providing an isolated and purified enzyme of the invention in industrial scale.

In the context of the present invention the term "heterologous" production means expression of a recombinant
10 enzyme in a host organism different from the original donor organism.

The term "homologous" production means expression of the wild-type enzyme by the original organism.

The complete DNA sequence, shown in SEQ ID no. 1, encoding
15 the dextranase of the invention, comprised in the plasmid pToc325, has been transformed into the bacteria strain *Escherichia coli* DH5 α . The strain is deposited at DSM under the number DSM 10706. This will be described further below.

By a database alignment search it was found that the DNA
20 sequence shown in SEQ ID no. 1 is novel. The highest degree of homology found was 59% to the above mentioned *Penicillium minioluteum* (MUCL no. 38929) deposited on 22nd of August 1994 under the provisions of the Budapest Treaty with the Mycothèque de l'Université Catholique de Louvain (MUCL). The DNA and amino
25 acid sequences are disclosed in EP 0 663 443.

In the first aspect the invention relates to a DNA construct comprising a DNA sequence encoding an enzyme exhibiting dextranase activity, which DNA sequence comprises

a) the dextranase encoding part of the DNA sequence shown in
30 SEQ ID no. 1, and/or the DNA sequence obtainable from *E. coli* DSM 10706, or

b) an analogue of the DNA sequence shown defined in a), which
i) is at least 80% homologous with the DNA sequence shown
in SEQ ID no. 1 and/or the DNA sequence obtainable from *E.*
35 *coli* DSM 10706, or
ii) hybridizes with the same oligonucleotide probe as the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from *E. coli* DSM 10706, or

iii) encodes a polypeptide which is 80% homologous with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from *E. coli* DSM 10706, or

5 iv) encodes a polypeptide which is immunologically reactive with an antibody raised against a purified dextranase encoded by the DNA sequence shown in SEQ ID no. 1 derived from *Paecilomyces lilacinus* and/or obtainable from *E. coli*, DSM 10706.

10 In the present context, the "analogue" of the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from *E. coli* DSM 10706, is intended to indicate any DNA sequence encoding an enzyme exhibiting dextranase activity, which has at least one of the properties i)-iv).

15 The analogous DNA sequence

- may be isolated from another or related (e.g. the same) organism producing the enzyme exhibiting dextranase activity on the basis of a partial sequence of the DNA sequences shown in SEQ ID no. 1 and/or obtainable from *E. coli*, DSM 10706, e.g. using the procedures described herein, and thus, e.g. be an allelic or species variant of the DNA sequence shown herein,
- may be constructed on the basis of any partial DNA sequence of the DNA sequence shown in SEQ ID no. 1, e.g. by introduction of nucleotide substitutions which do not give rise to another amino acid sequence of the dextranase encoded by the DNA sequence, but which correspond to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions which may give rise to a different amino acid sequence. However, in the latter case amino acid changes are preferably of a minor nature, that is conservative amino acid substitutions that do not significantly affect the folding or activity of the polypeptide, small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain. See in general Ford et al., (1991), Protein Expression and Purification 2, 95-107.

Examples of conservative substitutions are within the group of basic amino acids (such as arginine, lysine, histidine), acidic amino acids (such as glutamic acid and aspartic acid), polar amino acids (such as glutamine and asparagine), hydrophobic amino acids (such as leucine, isoleucine, valine), aromatic amino acids (such as phenylalanine, tryptophan, tyrosine) and small amino acids (such as glycine, alanine, serine, threonine, methionine).

It will be apparent to persons skilled in the art that such substitutions can be made outside the regions critical to the function of the molecule and still result in an active enzyme. Amino acids essential to the activity of the polypeptide encoded by the DNA construct of the invention, and therefore preferably not subject to substitution, may be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, (1989), *Science* **244**, 1081-1085). In the latter technique mutations are introduced at every residue in the molecule, and the resulting mutant molecules are tested for biological (i.e. dextranase) activity to identify amino acid residues that are critical to the activity of the molecule. Sites of substrate-enzyme interaction can also be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labelling. See, for example, de Vos et al. (1992), *Science* **255**, 306-312; Smith et al., (1992), *J. Mol. Biol.*, **224**, 899-904; Wlodaver et al., (1992), *FEBS Lett.*, **309**, 59-64.

It will be understood that any partial DNA sequence within the protein coding part of the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence transformed into the deposited strain *E. coli* DSM 10706 may be used for isolating the entire DNA sequence encoding the recombinant dextranase of the invention. The amino acid sequence (as deduced from the DNA sequence shown in SEQ ID no. 1) is shown in SEQ ID no. 2.

The homology referred to in i) above is determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known

in the art such as GAP provided in the GCG program package (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, p. 443-453). Using GAP (version 8) with the following settings for DNA sequence comparison: GAP creation
5 penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the DNA sequence exhibits a degree of identity preferably of at least 80%, such as at least 90%, preferably at least 95%, especially at least 99%, with the coding region of the DNA sequence shown in SEQ ID No. 1 or the DNA sequence
10 obtainable from the plasmid in *E. coli* DSM 10706.

The hybridization referred to in ii) above is intended to indicate that the analogous DNA sequence hybridizes to the same probe as the DNA sequence encoding the dextranase under certain specified conditions which are described in detail in the
15 Materials and Methods section hereinafter.

Normally, the analogous DNA sequence is highly homologous to the DNA sequence such as at least 80% homologous to the DNA sequence shown in SEQ ID no. 1 or the DNA sequence obtainable from the plasmid in *E. coli* DSM 10706 encoding a dextranase of
20 the invention, such as at least 90%, preferably at least 95%, such as at least 99% homologous to said DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from the plasmid in *E. coli* DSM 10706.

The homology referred to in iii) above is determined as
25 the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular
30 Biology, 48, p. 443-453). Using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the DNA sequence exhibits a degree of identity preferably of at least 80%, such as at least 90%, preferably at least 95%, especially
35 at least 99%, with the coding region of the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from the plasmid in *E. coli* DSM 10706.

The term "derived from" in connection with property iv) above is intended not only to indicate a dextranase produced by a strain of DSM 10706, but also a dextranase encoded by a DNA sequence isolated from strain DSM 10706 and produced in a host
5 organism transformed with said DNA sequence. The immunological reactivity may be determined by the method described in the "Materials and Methods" section below.

In further aspects the invention relates to an expression vector harbouring a DNA construct of the invention, a cell
10 comprising the DNA construct or expression vector and a method of producing an enzyme exhibiting dextranase activity which method comprises culturing said cell under conditions permitting the production of the enzyme, and recovering the enzyme from the culture.

15 It is also an object of the invention to provide an enzyme preparation enriched with the recombinant dextranase of the invention.

Further, the invention provides an oral care composition which further comprises an enzyme exhibiting an enzyme activity
20 selected from the group of mutanases, oxidases, peroxidases, haloperoxidases, laccases, proteases, endoglucosidases, lipases, amylases, anti-microbial enzymes, and mixtures thereof.

Finally the invention relates to the use of the recombinant dextranase of the invention. The enzyme of the
25 invention or a composition of the invention comprising such an enzyme may be used for preventing dental caries, plaque or/and tartar.

DETAILED DESCRIPTION OF THE INVENTION

30 Cloning

The DNA sequence coding for an enzyme exhibiting dextranase activity of the invention may conveniently be isolated from DNA from a suitable source, such as any of the below mentioned organisms, by use of synthetic oligonucleotide probes
35 prepared on the basis of a DNA sequence disclosed herein.

For instance, a suitable oligonucleotide probe may be prepared on the basis of the nucleotide sequences shown in SEQ ID no. 1 and/or the nucleotide sequence obtainable from the

plasmid in *E. coli* DSM 10706, or the amino acid sequence shown in SEQ ID no. 2 or any suitable sub-sequence thereof.

According to this method primers able to code for these peptides, being a part of SEQ ID No. 2, are designed. Fragments of the gene to be cloned is then PCR amplified by the use of these primers. These fragments are used as probes for cloning the complete gene.

A more detailed description of the screening method is given in Example 1 and 2 below.

Alternatively, the DNA sequence of the invention encoding an enzyme exhibiting dextranase activity may be isolated by a general method involving

- cloning, in suitable vectors, a DNA library from *Paecilomyces lilacinus*,
- transforming suitable host cells with said vectors,
- culturing the host cells under suitable conditions to express any enzyme of interest encoded by a clone in the DNA library,
- screening for positive clones by determining any dextranase activity of the enzyme produced by such clones, and
- isolating the DNA coding an enzyme from such clones.

The general method is further disclosed in WO 93/11249 the contents of which are hereby incorporated by reference.

The DNA sequence coding for the recombinant dextranase of the invention may for instance be isolated by screening a cDNA library of the donor organism, and selecting for clones expressing the appropriate enzyme activity (i.e. dextranase activity as defined by the ability of the enzyme to hydrolyse AZCL-Dextran). The appropriate DNA sequence may then be isolated from the clone by standard procedures.

Depositing of the dextranase sequence

The complete full length DNA sequence obtained from a strain of *Paecilomyces lilacinus* encoding the dextranase of the invention has been transformed into a strain of the bacteria *E. coli* DH5 α , comprised in the plasmid pUC19. Said bacteria has been deposited by the inventors according to the Budapest Treaty on the International Recognition of the Deposit of

Microorganisms for the Purposes of Patent Procedure at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Mascheroder Weg 1b, D-38124 Braunschweig Federal Republic of Germany, (DSM).

5

Deposit date : June 7, 1996
Depositor's ref. : NN49245 = ToC 1065
DSM designation : *E. coli* DSM no. 10706

10 Being an International Depository Authority under the Budapest Treaty, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., affords permanence of the deposit in accordance with the rules and regulations of said treaty, vide in particular Rule 9. Access to the deposit will be available
15 during the pendency of this patent application to one determined by the Commissioner of the United States Patent and Trademark Office to be entitled thereto under 37 C.F.R. Par. 1.14 and 35 U.S.C. Par. 122. Also, the above mentioned deposits fulfil the requirements of European patent applications
20 relating to micro-organisms according to Rule 28 EPC.

The above mentioned deposit represents a substantially pure culture of the isolated bacteria. The deposit is available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny are
25 filed. However, it should be understood that the availability of the deposited strain does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

The DNA sequence encoding the enzyme exhibiting dextranase
30 activity can for instance be isolated from the above mentioned deposited strain by standard methods.

Microbial Sources

It is expected that a DNA sequence coding for a homologous
35 enzyme, i.e. an analogous DNA sequence, is obtainable from other micro-organisms, such as the following filamentous fungi, yeasts or bacteria. For instance, the DNA sequence may be derived from a strain of *Paecilomyces*, such as *Paecilomyces*

lilacinus, or a strain of *Penicillium*, such as *Penicillium lilacinum* or *Penicillium minioluteum*, *Aspergillus*, *Fusarium*, *Spicaria*, *Verticillium*, *Helminthosporium* or *Chaetomium*; bacteria of the genera *Lactobacillus*, *Streptococcus*,
5 *Cellvibrio*, *Cytophaga*, *Brevibacterium*, *Pseudomonas*, *Corynebacterium*, *Arthrobacter* and *Flavobacterium*; yeasts, such as *Lipomyces starkeyi*.

Production of the dextranase

10 The DNA sequence encoding the dextranase may subsequently be inserted into a recombinant expression vector. This may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced.

15 Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into
20 the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding the dextranase should be operably connected to a suitable promoter and terminator sequence. The promoter may be any DNA sequence which
25 shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. The procedures used to ligate the DNA sequences coding for the dextranase, the promoter and the terminator, respectively, and to insert them into suitable
30 vectors are well known to persons skilled in the art (cf., for instance, Sambrook et al., (1989), *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor, NY).

The host cell which is transformed with the DNA sequence encoding the dextranase is preferably a filamentous fungus
35 cell. In particular, the cell may belong to a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or a strain of *Fusarium*, such as a strain of *Fusarium oxysporium*, *Fusarium graminearum* (in the perfect state named

Gibberella zeae, previously *Sphaeria zeae*, synonym with *Gibberella roseum* and *Gibberella roseum* f. sp. *cerealis*), or *Fusarium sulphureum* (in the prefect state named *Gibberella puricaris*, synonym with *Fusarium trichothecioides*, *Fusarium*
5 *bactridioides*, *Fusarium sambucium*, *Fusarium roseum*, and *Fusarium roseum* var. *graminearum*), *Fusarium cerealis* (synonym with *Fusarium crockwellense*), or *Fusarium venenatum*.

The host cell may advantageously be a *F. graminearum* described in WO 96/00787 (from Novo Nordisk A/S), e.g. the
10 strain deposited as *Fusarium graminearum* ATCC 20334. The strain ATCC 20334 was previously wrongly classified as *Fusarium graminearum* (Yoder, W. and Christianson, L. 1997). RAPD-based and classical taxonomic analyses have now revealed that the true identity of the Quorn fungus, ATCC 20334, is *Fusarium*
15 *venenatum* Nirenburg sp. nov.

In the Examples below expression of dextranase is illustrated using *A. oryzae* and *F. venenatum* as host cells.

In a preferred embodiment of the invention the host cell is a protease deficient of protease minus strain.

20 This may for instance be the protease deficient strain *Aspergillus oryzae* JaL125 having the alkaline protease gene named "alp" deleted. This strain is described in PCT/DK97/00135 (from Novo Nordisk A/S).

Filamentous fungi cells may be transformed by a process
25 involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of *Aspergillus* as a host micro-organism is described in EP 238 023 (Novo Nordisk A/S), the contents of which are hereby incorporated by reference.

30

A method of producing an enzyme of the invention

In a still further aspect, the present invention relates to a method of producing an enzyme according to the invention, wherein a suitable host cell transformed with a DNA sequence
35 encoding the enzyme is cultured under conditions permitting the production of the enzyme, and the resulting enzyme is recovered from the culture.

The medium used to culture the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed dextranase may conveniently be secreted into the culture medium and may be recovered there
5 from by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography,
10 or the like.

The Enzyme

The invention also relates to an isolated recombinant enzyme with dextranase activity having essentially an amino
15 acid sequence as shown in SEQ ID no. 2 or a fragment of the same. Mass spectrometry showed that the average mass of the recombinant dextranase is about 65.3 kD.

The pH optimum of the recombinant dextranase was found to lie in the range from 3.5 to 5.5 which equals the pH optimum of
20 the wild-type dextranase (see Figure 4). The temperature optimum of both the recombinant and wild-type dextranase was found to be around 60°C at pH 5.5 (see Figure 2). Further, the recombinant and wild-type dextranases were stable at 60°C at pH 5.5 and pH 7. At 70°C only little residual activity was
25 observed.

Oral care composition

In a still further aspect, the present invention relates to an oral care composition useful as ingredient in oral care
30 products.

An oral care composition of the invention may suitably comprise an amount of the recombinant *Paecilomyces lilacinus* dextranase equivalent to an enzyme activity, calculated as enzyme activity units in the final oral care product, in the range from
35 0.001 KDU to 1000 KDU/ml, preferably from 0.01 KDU/ml to 500 KDU/ml, especially from 0.1 KDU/ml to 100 KDU/ml.

It is also contemplated according to the invention to include other enzyme activities than dextranase activity in the

oral care composition. Contemplated enzyme activities include activities from the group of enzymes comprising mutanases, oxidases, such as glucose oxidase, L-amino acid oxidase, peroxidases, such as e.g. the *Coprinus sp.* peroxidases described
5 in WO 95/10602 (from Novo Nordisk A/S) or lactoperoxidase or, haloperoxidases, laccases, proteases, such as papain, acidic protease (e.g. the acidic proteases described in WO 95/02044 (Novo Nordisk A/S)), endoglucosidases, lipases, amylases, including amyloglucosidases, such as AMG (from Novo Nordisk A/S),
10 anti-microbial enzymes, and mixtures thereof.

Oral care products

The oral care product may have any suitable physical form (i.e. powder, paste, gel, liquid, ointment, tablet etc.). An
15 "oral care product" can be defined as a product which can be used for maintaining or improving the oral hygiene in the mouth of humans and animals, by preventing dental caries, preventing the formation of dental plaque and tartar, removing dental plaque and tartar, preventing and/or treating dental diseases etc.

20 At least in the context of the present invention oral care products do also encompass products for cleaning dentures, artificial teeth and the like.

Examples of such oral care products include toothpaste, dental cream, gel or tooth powder, odontic, mouth washes, pre- or
25 post brushing rinse formulations, chewing gum, lozenges, and candy.

Toothpastes and tooth gels typically include abrasive polishing materials, foaming agents, flavouring agents, humectants, binders, thickeners, sweetening agents, white-
30 ning/bleaching/ stain removing agents, water, and optionally enzymes.

Mouth washes, including plaque removing liquids, typically comprise a water/alcohol solution, flavour, humectant, sweetener, foaming agent, colorant, and optionally enzymes.

35

Abrasives

Abrasive polishing material might also be incorporated into the dentifrice product of the invention. According to the inven-

tion said abrasive polishing material includes alumina and hydrates thereof, such as alpha alumina trihydrate, magnesium trisilicate, magnesium carbonate, kaolin, aluminosilicates, such as calcined aluminum silicate and aluminum silicate, calcium carbonate, zirconium silicate, and also powdered plastics, such as polyvinyl chloride, polyamides, polymethyl methacrylate, polystyrene, phenol-formaldehyde resins, melamine-formaldehyde resins, urea-formaldehyde resins, epoxy resins, powdered polyethylene, silica xerogels, hydrogels and aerogels and the like. Also suitable as abrasive agents are calcium pyrophosphate, water-insoluble alkali metaphosphates, dicalcium phosphate and/or its dihydrate, dicalcium orthophosphate, tricalcium phosphate, particulate hydroxyapatite and the like. It is also possible to employ mixtures of these substances.

Dependent on the oral care product the abrasive product may be present in from 0 to 70% by weight, preferably from 1% to 70%. For toothpastes the abrasive material content typically lies in the range of from 10% to 70% by weight of the final toothpaste product.

Humectants are employed to prevent loss of water from e.g. toothpastes. Suitable humectants for use in oral care products according to the invention include the following compounds and mixtures thereof: glycerol, polyol, sorbitol, polyethylene glycols (PEG), propylene glycol, 1,3-propanediol, 1,4-butanediol, hydrogenated partially hydrolysed polysaccharides and the like. Humectants are in general present in from 0% to 80%, preferably 5 to 70% by weight in toothpaste.

Silica, starch, tragacanth gum, xanthan gum, extracts of Irish moss, alginates, pectin, cellulose derivatives, such as hydroxyethyl cellulose, sodium carboxymethyl cellulose and hydroxypropyl cellulose, polyacrylic acid and its salts, polyvinylpyrrolidone, can be mentioned as examples of suitable thickeners and binders, which helps stabilizing the dentifrice product. Thickeners may be present in toothpaste creams and gels in an amount of from 0.1 to 20% by weight, and binders to the extent of from 0.01 to 10% by weight of the final product.

Foaming agents

As foaming agent soap, anionic, cationic, non-ionic, amphoteric and/or zwitterionic surfactants can be used. These may be present at levels of from 0% to 15%, preferably from 0.1 to 13%, more preferably from 0.25 to 10% by weight of the final
5 product.

Surfactants

Surfactants are only suitable to the extent that they do not exert an inactivation effect on the present enzymes. Surfactants
10 include fatty alcohol sulphates, salts of sulphonated mono-glycerides or fatty acids having 10 to 20 carbon atoms, fatty acid-albumen condensation products, salts of fatty acids amides and taurines and/or salts of fatty acid esters of isethionic acid.

15

Sweetening agents

Suitable sweeteners include saccharin.

Flavouring agents

20 Flavours, such as spearmint, are usually present in low amounts, such as from 0.01% to about 5% by weight, especially from 0.1% to 5%.

Whitening/bleaching agents

25 Whitening/bleaching agents include H_2O_2 and may be added in amounts less than 5%, preferably from 0.25 to 4%, calculated on the basis of the weight of the final product.

The whitening/bleaching agents may be an enzyme, such as an oxidoreductase. Examples of suitable teeth bleaching enzymes are
30 described in WO 97/06775 (from Novo Nordisk A/S).

Water

Water is usually added in an amount giving e.g. toothpaste a flowable form.

35

Additional agents

Further water-soluble anti-bacterial agents, such as chlorhexidine digluconate, hexetidine, alexidine, quaternary

ammonium anti-bacterial compounds and water-soluble sources of certain metal ions such as zinc, copper, silver and stannous (e.g. zinc, copper and stannous chloride, and silver nitrate) may also be included.

5 Also contemplated according to the invention is the addition of compounds which can be used as fluoride source, dyes/colorants, preservatives, vitamins, pH-adjusting agents, anti-carries agents, desensitizing agents etc.

10 Enzymes

Other essential components used in oral care products and in oral care products of the invention are enzymes. Enzymes are biological catalysts of chemical reactions in living systems. Enzymes combine with the substrates on which they act forming an
15 intermediate enzyme-substrate complex. This complex is then converted to a reaction product and a liberated enzyme which continue its specific enzymatic function.

Enzymes provide several benefits when used for cleansing of the oral cavity. Proteases break down salivary proteins, which
20 are adsorbed onto the tooth surface and form the pellicle, the first layer of resulting plaque. Proteases along with lipases destroy bacteria by lysing proteins and lipids which form the structural components of bacterial cell walls and membranes.

Dextranase breaks down the organic skeletal structure produced by
25 bacteria that forms a matrix for bacterial adhesion. Proteases and amylases, not only prevents plaque formation, but also prevents the development of calculus by breaking-up the carbohydrate-protein complex that binds calcium, preventing mineralization.

30 A toothpaste produced from an oral care composition of the invention (in weight % of the final toothpaste composition) may typically comprise the following ingredients:

	Abrasive material	10 to 70%
	Humectant	0 to 80%
	Thickener	0.1 to 20%
	Binder	0.01 to 10%
5	Sweetener	0.1% to 5%
	Foaming agent	0 to 15%
	Whitener	0 to 5%
	Enzymes	0.0001% to 20%

In a specific embodiment of the invention the oral care
 10 product is toothpaste having a pH in the range from 6.0 to about
 8.0 comprising

- a) 10% to 70% Abrasive material
- b) 0 to 80% Humectant
- c) 0.1 to 20% Thickener
- 15 d) 0.01 to 10% Binder
- e) 0.1% to 5% Sweetener
- f) 0 to 15% Foaming agent
- g) 0 to 5% Whitener
- i) 0.0001% to 20% Enzymes.

20 Said enzymes referred to under i) include the recombinant
 dextranase of the invention, and optionally other types of en-
 zymes mentioned above known to be used in toothpastes and the
 like.

A mouth wash produced from an oral care composition of the
 25 invention (in weight % of the final mouth wash composition) may
 typically comprise the following ingredients:

- 0-20% Humectant
- 0-2% Surfactant
- 0-5% Enzymes
- 30 0-20% Ethanol
- 0-2% Other ingredients (e.g. flavour, sweetener
 active ingredients such as florides).
- 0-70% Water

The mouth wash composition may be buffered with an
 35 appropriate buffer e.g. sodium citrate or phosphate in the pH-
 range 6-7.5.

The mouth wash may be in none-diluted form (i.e. must be
 diluted before use).

Method of Manufacture

The oral care composition and products of the present invention can be made using methods which are common in the oral
5 product area.

Use

According to the present invention the recombinant dextranase or compositions comprising a such are useful for a
10 number of applications including the use in oral care products for humans and/or animals for preventing the formation of dental plaque or removing dental plaque; the use for hydrolysis of sugar juice or syrup; the use in food, feed and/or pet food products.

15

MATERIALS AND METHODS

Materials

Micro-organisms

20 *E. coli* DSM no. 10706 deposited according to the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purposes of Patent Procedure at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Mascheroder Weg 1b, D-38124 Braunschweig Federal Republic of
25 Germany, (DSM).

A. oryzae JaL 125: *Aspergillus oryzae* IFO 4177 available from Institute for Fermentation, Osaka; 17-25 Juso Hammachi 2-Chome Yodogawa-ku, Osaka, Japan, having the alkaline protease gene named "alp" (described by Murakami K et al., (1991), Agric. Biol.
30 Chem. 55, p. 2807-2811) deleted by a one step gene replacement method (described by G. May in "Applied Molecular Genetics of Filamentous Fungi" (1992), p. 1-25. Eds. J. R. Kinghorn and G. Turner; Blackie Academic and Professional), using the *A. oryzae* pyrG gene as marker.

35 *Fusarium* CC1-3: A morphological mutant of *Fusarium* A3/5 (ATCC 20334) (Wiebe et al., 1992, Mycological Research 96: 555-562; Wiebe et al., 1991, Mycological Research 95: 1284-1288; Wiebe et al., 1991, Mycological Research 96: 555-562) is a

highly branched, colonial variant. Strain ATTC 20334 is the strain referred to in WO 96/00787 as *Fusarium graminearum* ATTC 20334. Strain ATTC 20334 was previously wrongly classified as *F. graminearum* (Yoder and Christianson, (1997). RAPD-based and
5 classical taxonomic analyses reveal the true identity of the Quorn fungus, ATCC 20334, to be *Fusarium venenatum* Nirenburg sp. nov.

E. coli DH5 α

E. coli strain JM101

10

Plasmids and Vectors:

pCaHj483 (Figure 1)

pToC343 (Figure 2)

pToC325 (Figure 3)

15 pICAMG/Term (EP 238 023)

pUC19 (Yanish-Perron et al., (1985), Gene 33, p. 103-119)

pJW111: Expression plasmid (figure 8) built and amplified in *Escherichia coli* strain JM101.

pDM181: The *Fusarium* expression plasmid (Jones et al. (1996))

20 is a single vector system that encodes the SP387 promoter and terminator, as well as the *bar* gene (Thompson et al., (1987), EMBO, 6 (9): 2519-2514) which confers Basta resistance. Two restriction enzyme sites, those for *SwaI* and *PacI*, have been introduced between the SP387 regulatory sequences to facilitate
25 cloning.

Enzymes:

Dextranase L50 produced by *Paecilomyces lilacinus* (Novo Nordisk A/S).

30 lysyl-specific protease from *Achromobacter*

NOVOZYM 234TM (Novo Nordisk A/S)

Media, Substrates and Solutions:

YPG medium: 1% yeast extract, 2% bactopectone, and 2% glucose

35 YPD: 10 g yeast extract, 20 g peptone, H₂O to 900 ml. Autoclaved, 100 ml 20% glucose (sterile filtered) added.

Dextran 500 (Pharmacia)

AZCL-dextran (MegaZyme).

pCRII (Invitrogen TA Cloning Kit)

Britton-Robinson Buffer

DAPI: 4',6-diamidino-2-phenylindole (Sigma D-9542)

5 BHI: Brain Heart Infusion broth

Vogel's/Basta medium: Vogel's salts (Vogel, H.J. (1964), Am. Nat. 98:436-446), 25 mM NaNO₃, 5 mg/ml Basta (Hoechst), 25 g/L sucrose, 25 g/L noble agar, pH 6.0.

M400Da medium: 50 g of maltodextrin, 2.0 g of MgSO₄-7H₂O, 2.0 g
10 of KH₂PO₄, 4.0 g of citric acid, 8.0 g of yeast extract, 2.0 g
of urea, and 0.5 ml of trace metals solution per liter. The
medium is adjusted to pH 6.0 with 5 N NaOH. The trace metals
solution is comprised of 14.3 g of ZnSO₄-7H₂O, 2.5 g of CuSO₄-
5H₂O, 0.5 g of NiCl₂-6H₂O, 13.8 g of FeSO₄-7H₂O, 8.5 g of MnSO₄-
15 H₂O, and 3.0 g of citric acid per liter.

Sporulation medium: 12.1 g/L NaNO₃, 25 g/L succinic
acid(disodium salt), 1 g/L glucose and 1X Vogel's salts
adjusted to pH 6.0.

STC: 0.8 M sorbitol, 25 mM Tris pH 8.0, 50mM CaCl₂

20 SPTC: 40% PEG4000, 0.8M sorbitol, 25mM Tris pH 8.0, 50mM CaCl₂.

Equipment:

10 kDa cut-off ultra-filtration cassette (Alpha Miniset from
Filtron).

25 Phenyl-sepharose FF (high sub) column (Pharmacia)

Seitz EK1 filter plate

Q-sepharose FF column (Pharmacia)

Applied Biosystems 473A protein sequencer

2 liter Kieler fermenter

30 Olympus model BX50 microscope

Malthus Flexi M2060 (Malthus Instrument Limited)

Primers:

8001 GA(A/G)AA(T/C)TA(T/C)GC(T/C/G/A)TA(T/C)ATGGC (SEQ ID No
35 15)

8002 GCCAT(G/A)TA(G/A/T/C)GC(A/G)TA(G/A)TT(T/C)TC (SEQ ID No
16)

8003 TGGAC(A/G/T/C)CA(A/G)TT(T/C)CA(A/G)TA(T/C)GC (SEQ ID No 17)
8004 GC(A/G)TA(T/C)TG(A/G)AA(T/C)TG(A/G/T/C)GTCC (SEQ ID No 18)
8005 AA(T/C)TGGCA(A/G)AT(T/C/A)GG(A/G/T/C)GG (SEQ ID No 19)
5 8006 CC(A/G/T/C)CC(T/A/G)TA(T/C)TGCCA(A/G)TT (SEQ ID No 20)
8864 CGCGGATCCACCATGCGTTGGCCTGGTAATTTTC (SEQ ID No 23)
8867 CCGCTCGAGCCTGCCTCATTCAATGCTCC (SEQ ID No 24)

Methods:

10 Dextranase activity assay

The Dextranase activity assay measures the release of reducing sugars from dextran with alkalic 3,5-dinitrosalicylic acid (adsorption at 540 nm).

Conditions: 2.5% Dextran 500 (Pharmacia) in 0.1 M sodium acetate, pH 5.4 at 40°C. Enzyme concentrations around 1 DU/ml.

1 DU equals the amount of enzyme that produces an amount of reducing sugars equivalent to 1 mg of maltose in 1 hour.

Dextranase activity can also be measured using AZCL-dextran (MegaZyme). 500 µl 0.4 % AZCL-dextran in 0.1 M sodium acetate, pH 5.5 or 50 mM Britton-Robinson buffer is added 500 µl enzyme sample diluted in MilliQ filtered H₂O and incubated for 10 minutes at 40°C. Then the samples are centrifuged for 2 minutes at 15,000g and 200 µl of supernatant is added to wells in a microtiter plate and the absorption at 595 nm is measured.

25

Molecular characterization of wild-type dextranase from *Paecilomyces lilacinus*

Mass spectrometry

Mass spectrometry of purified wild type dextranase is done using matrix assisted laser desorption ionisation time-of-flight mass spectrometry in a VG Analytical ToFSpec. For mass spectrometry 2 ml of sample is mixed with 2 ml saturated matrix solution (α-cyano-4-hydroxycinnamic acid in 0.1% TFA:acetonitrile (70:30)) and 2 ml of the mixture spotted onto the target plate. Before introduction into the mass spectrometer the solvent is removed by evaporation. Samples are desorbed and ionised by 4 ns laser pulses (337 nm) at threshold laser power and accelerated

into the field-free flight tube by an accelerating voltage of 25 kV. Ions are detected by a microchannel plate set at 1850 V.

Preparation of hydroxyapatite disks (HA)

5 Hydroxyapatite tablets are prepared by compressing 250 mg of hydroxyapatite in a tablet die at about 5,900 kg (13,000 lbs) of pressure for 5 minutes. The tablets are then sintered at 600°C for 4 hours and finally hydrated with sterile deionized water.

10 Plaque coating of Hydroxyapatite disks (HA)

Hydroxyapatite disks (HA) were dry sterilised (121°C, 2 bar, 20 minutes) and coated with filter sterilised saliva for 18 hours at 37°C. The HA disks were placed in a sterile rack in a beaker, Brain Heart Infusion broth (BHI) containing 0.2% sucrose
15 was poured into the beaker covering the disks. Sterile Na₂S (pH 7.0) was added immediately before inoculation given the final concentration of 5 g/litre. A mixture 1:1:1 of *Streptococcus mutans*, *Actinomyces viscosus* and *Fusobacterium nucleatum* grown anaerobically (BHI, 37°C, 24 hour) was used as inoculum in the
20 concentration of approximately 10⁶ cfu/ml. The disks were incubated anaerobic at 37°C for 4 days with slight stirring.

Malthus-method for plaque

The Malthus-method is based on the methods described in
25 Johnston et al., (1995), Journal of Microbiological Methods 21, p. 15-26 and Johanssen et al. (1995), Journal of Applied Bacteriology 78, p. 297-303.

Polymerase Chain Reaction (PCR).

30 PCR reactions contained components from the Advantage cDNA PCR core kit (Clontech, Palo Alto), 0.1 mg of pToC343 and 50 pmol each of the primers o-dexSwaI {GCATTTAAATATG CGT TGG CCT GGT} and o-dexPacI {CGTTAAT TAA TCA TTC AAT GCT CCA GTC} with the following cycles: 1 cycle of 95°C for 4 min.; 25 cycles of
35 (95°C for 1 min, 60°C for 1 min, 72°C for 2 min); 1 cycle of 72°C for 5 min..

EXAMPLES**Example 1****5 Purification of wild-type dextranase****Step 1: Ultra-filtration**

1 litre Dextranase 50 L (Novo Nordisk A/S) was mixed with 8 litre 50 mM Na-acetate/HCl, pH 5.4. The mixture was concentrated to approximately 0.5 litre on a 10 kDa cut-off
10 ultra-filtration cassette (Alpha Minisette from Filtron). Another 8 litre 50 mM Na-acetate/HCl, pH 5.4 was added and the enzyme was again concentrated to approx. 0.5 litre.

Step 2: Chromatography on Phenyl-sepharose FF

15 Saturated ammonium sulphate was added to give a final ammonium sulphate concentration of 1.0M. The pH was adjusted to pH 6.0 with 3% NaOH and the enzyme was filtered on a Seitz EK1 filter plate. The EK1-filtrate was divided in two halves.

A 1 litre Phenyl-sepharose FF (high sub) column was equilibrated in 25mM Na-acetate/HCl, 1.0M (NH₄)₂SO₄, pH 6.0. One
20 half of the EK1-filtrate was applied to the column and the column was washed with 5 column volumes of equilibration buffer to remove non-binding proteins. To elute the dextranase an ammonium sulphate gradient (1.0M --> 0.0M) over 5 column
25 volumes was applied to the column. The Phenyl-sepharose FF column step was repeated with the other half of the EK1-filtrate. Fractions with dextranase activity were pooled.

Step 3: Chromatography on Q-sepharose FF

30 The pooled fractions were concentrated on a 10 kDa cut-off ultrafiltration cassette to approx. 250ml. The ultrafiltrated enzyme was dialyzed against 10mM Na-acetate/HCl, pH 6.0 with several buffer changes.

A 1 litre Q-sepharose FF column was equilibrated in 20mM
35 Na-acetate/HCl, pH 6.0. The enzyme was applied to the column and the column was washed with equilibration buffer until the OD₂₈₀ signal had returned to baseline. The dextranase enzyme

was eluted with a linear NaCl gradient (0 --> 75mM) over five column volumes.

Fractions from the column were analyzed for dextranase activity and fractions with dextranase activity were analyzed by SDS-PAGE. Fractions which were judged to be at least 90% pure were pooled as the purified Dextranase. Finally the enzyme was filtered through a 0.20 μ filter.

Example 2

10 **N-terminal sequencing of wild-type dextranase**

N-terminal amino acid sequencing:

N-terminal amino acid sequencing was carried out in an Applied Biosystems 473A protein sequencer.

To generate peptides reduced and S-carboxymethylated dextranase, wild-type dextranase (> 500 mg), purified as described in the Material and Methods section, was digested with the lysyl-specific protease from *Achromobacter* (20 mg) in 40 mM NH_4HCO_3 containing 1.3 M urea for 16 hours at 37°C. The resulting peptides were separated by reversed phase HPLC using a Vydac C₁₈ column eluted with a linear gradient of 80% 2-propanol containing 0.08% TFA in 0.1% aqueous TFA.

Peptides were re-purified by reversed phase HPLC using a Vydac C₁₈ column eluted with linear gradients of 80% acetonitrile containing 0.08% TFA in 0.1% aqueous TFA before subjected to N-terminal amino acid sequencing.

The amino acid sequences determined are given below. Xaa designates unidentified residues and Asx are residues where it has not been possible to distinguish Asp from Asn.

30 **N-terminal:**

Asp-Gln-Gln-Asn-Gln-Ala-Leu-His-Thr-Trp-Trp-His-Glu-Lys-Ser- (SEQ ID No. 3)

Actually the direct N-terminal amino acid sequencing of the dextranase revealed three sequences staggered with respect to each other. The sequences was found in the ratio 2:1:2 starting at Asp1, Gln3 and Asn4, respectively.

Peptide 1:

Ser-Tyr-Val-Asn-Asp-Gly-Gly-Val-Leu-Val-Ser-Glu-Glu-Pro-Arg-Asn-Ala-Leu-Leu-Ile-Phe-Ala-Ser-Pro-Phe-Ile-Pro-Gln (SEQ ID No. 4).

Peptide 2:

5 Ser-Asp-Arg-Thr-Ser-Leu-Arg-Ile-Phe-Ser-His-Gln-Ala-Val-Ser-Asp-Ser-Gln-Ile-Trp-His-Xaa-Ile (SEQ ID NO. 5)

Peptide 3:

Asn-Asp-Phe-Tyr-Thr-Val-Gly-His-Gly-Val-Val-Ser-Gly-Glu-Asn-Tyr-10 Ala-Tyr-Met-Ala-Asn-Thr-Ala-Lys (SEQ ID No. 6)

Peptide 4:

Ile-Asn-Ala-Ala-Trp-Thr-Gln-Phe-Gln-Tyr-Ala-Lys (SEQ ID No. 7)

15 Peptide 5:

Asp-Gly-Ser-Ala-Leu-Gly-Pro-Thr-Ser-Asn-Val-Val-Ile-Arg-Pro-Ser-Asp-Ile-Arg-Tyr-Asp-Ile-Ser-Ser-Pro-Asp (SEQ ID No. 8)

Peptide 6:

20 Asn-Trp-Gln-Ile-Gly-Gly-Asn-Arg-Val-Asp-Gly-Ser-Asn-Trp-Gln-Val-Asn-Gln (SEQ ID No. 9)

Peptide 7:

Ser-Glu-Thr-Val-Val-Pro-Ser-Ala-Ile-Ile-Gly-Ala-Ser-Pro-Tyr-Tyr-25 Gly-Asp-Pro (SEQ ID No. 10)

Peptide 8:

Leu-Asx-Ala-Asx-Thr-His-Tyr-Val-Tyr-Phe-Glu-Pro-Gly-Thr-Tyr-Ile-Lys (SEQ ID No. 11).

30

Peptide 9:

Val-Ile-His-Thr-Arg-Trp-Phe (SEQ ID No. 12)

Peptide 10:

35 Ser-Ala-Val-Asn-Asp-Ala-Gly-Ala-Val-Ala-Ala-Asp-Glu-Val-Arg-Gln-Ser-Asg-Lys (SEQ ID No. 13)

Peptide 11:

Xaa-His-Asn-Asp-Pro-Val-Ile-Gln-Met-Gly-Xaa-Lys (SEQ ID No. 14).

Example 3

Cloning of the dextranase gene from *Paecilomyces lilacinus*.

5 The cloning of the dextranase gene from *Paecilomyces lilacinus* was based on the knowledge of the partial amino acid sequence described in Example 2. Degenerate PCR primers able to code for peptide 3, 4 and 6 were designed. Primers coding for the same sequences in both orientations were made. All
10 combinations of the six primers were used in PCR reactions with chromosomal DNA from *Paecilomyces lilacinus*. Some of these reactions resulted in DNA fragments which coded for parts of the dextranase genes. These fragment were used as probes on a genomic Southern. A 6kb BamHI fragment hybridized and was
15 subsequently cloned and sequenced. All *in vitro* DNA work was done following standard procedures (Sambrook et al., Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press (1989))

20 PCR fragments

The following primers were synthesized.

8001 GA(A/G)AA(T/C)TA(T/C)GC(T/C/G/A)TA(T/C)ATGGC
8002 GCCAT(G/A)TA(G/A/T/C)GC(A/G)TA(G/A)TT(T/C)TC
8003 TGGAC(A/G/T/C)CA(A/G)TT(T/C)CA(A/G)TA(T/C)GC
25 8004 GC(A/G)TA(T/C)TG(A/G)AA(T/C)TG(A/G/T/C)GTCC
8005 AA(T/C)TGGCA(A/G)AT(T/C/A)GG(A/G/T/C)GG
8006 CC(A/G/T/C)CC(T/A/G)TA(T/C)TGCCA(A/G)TT

The primers 8001 and 8002 encode amino acids no. 14-20 of peptide no. 3 in one or the other direction. Primers 8003 and
30 8004 encode amino acids no. 5-11 of peptide no. 4 and primers 8005 and 8006 encode amino acids no. 1-6 of peptide no. 6.

Chromosomal DNA from *Paecilomyces lilacinus* Li-3 was prepared essential as described by Yelton et al. (Proc. Natl. Acad. Sci., (1984), 81, p. 1470-1474).

35 PCR reactions were run according to standard procedures with all combinations of primers. The primer sets 8001/8006 and 8003/8002 gave fragments that upon cloning in the vector pCRII (Invitrogen TA Cloning Kit) and sequencing on an Applied

Biosystems DNA Sequencer were shown to contain parts of the dextranase gene. The fragment obtained with the primer-set 8001/8006 was approximately 0.9 kb. Sequencing of the ends of this fragment revealed a DNA sequence encoding peptide no. 2.

5 The fragment obtained with 8003/8002 were approximately 0.6 kb. Sequencing revealed that sequences encoding peptide 5, 1 and 8 were contained in this fragment.

Genomic clone

10 A Southern blot of chromosomal DNA from *Paecilomyces lilacinus* cut with *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Sal*I, *Xba*I and *Xho*I was made and hybridized with the ³²P labelled PCR fragments. Both fragments hybridized to an approximately 6 kb *Bam*HI fragment. A library of approximately 6 kb *Bam*HI fragments
15 from *Paecilomyces lilacinus* cloned into pUC19 was made. The library was screened by colony hybridization with one of the PCR fragments and a positive plasmid pToC325 (See Figure 2) was isolated. 2994bp of pToC325 were sequenced, the DNA sequence and the deduced amino acid sequence is shown in SEQ ID No. 1
20 and SEQ ID No. 2, respectively. pToC325 transformed into *E. coli* DH5α was deposited at DSM as strain no. 10706

Example 4

Expression of recombinant dextranase in *Aspergillus oryzae*.

25 Restriction enzyme sites were introduced at the start and stop codon of the dextranase by which the gene was cloned into an *A. oryzae* expression vector, pCaHj483. The resulting dextranase expression plasmid was transformed into an *A. oryzae* strain. Transformants were isolated and analyzed for the
30 expression of dextranase.

Construction of pCaHj483.

pCaHj483 (see Figure 1) was build from the following fragments:

- a) The vector pToC65 (W091/17243) cut with *Eco*RI and *Xba*I.
- 35 b) A 2.7 kb *Xba*I fragment from *A. nidulans* carrying the *amdS* gene (C. M. Corrick et al. (1987), Gene 53, 63-71). The *amdS* gene is used as a selective marker in fungal transformations. The *amdS* gene has been modified so that the *Bam*HI site normally

present in the gene is destroyed. This has been done by introducing a silent point mutation using the primer : 5'-AGAAATCGGGTATCCTTTCAG- 3' (see SEQ ID No. 21)

c) A 0.6 kb *EcoRI*/*BamHI* fragment carrying the *A. niger* NA2 promoter fused to a 60bp DNA fragment of the sequence encoding the 5' un-translated end of the mRNA of the *A. nidulans tpi* gene. The NA2 promoter was isolated from the plasmid pNA2 (EP-B-0 383 779 from Novo Nordisk A/S) and fused to the 60 bp *tpi* sequence by PCR. The primer encoding the 60 bp *tpi* sequence had the following sequence:

5'-GCTCCTCATGGTGGATCCCCAGTTGTGTATATAGAGGATTGAGGAAGGAAGAGAAGTGTGGATAGAGGTAAATTGAGTTGGAACTCCAAGCATGGCATCCTTGC- 3' (See SEQ ID No. 22)

d) A 675 bp *XbaI* fragment carrying the *A. niger* glucoamylase transcription terminator. The fragment was isolated from the plasmid pICAMG/Term (EP 238 023 from Novo Nordisk A/S).

The *BamHI* site of fragment c was connected to the *XbaI* site in front of the transcription terminator on fragment d via the pIC19R linker (*BamHI* to *XbaI*)

Cloning of dextranase into pCaHj483.

BamHI and a *XhoI* sites were introduced in front of the ATG and right after the stop codon of the dextranase gene by PCR with the following primers :

8864 CGCGGATCCACCATGCGTTGGCCTGGTAATTTTC (SEQ ID No. 23)

8867 CCGCTCGAGCCTGCCTCATTCAATGCTCC (SEQ ID No. 24)

The gene was re-sequenced to check for PCR errors and cloned via the *BamHI* and *XhoI* sites into the expression vector pCaHj483. The resulting dextranase expression plasmid was named pToC343 and is depicted in Figure 3.

A. oryzae transformants of pToC343.

A. oryzae JaL 125 was transformed with pToC343 as described in EP 0 238 023. Transformants were selected by their ability to use acetamide as the only nitrogen source. After two re-isolations through conidiospores on minimal acetamid plates the transformants were fermented for four days at 30°C in 10 ml YPD. Samples of the fermentation broth were applied to SDS-

PAGE. The gels were stained by croomasie brilliant blue. A band of approximately 65 kD was visible in the broth from the transformants and not in the broth from JaL125.

Three transformants producing the 65 kD protein were
5 fermented in a 2 liter Kieler fermenter for five days in a maltodextrin containing medium and the content of dextranase was determined enzymatically.

The transformant produced up to 11.000 DU/ml in the fermentation broth, the un-transformed host produced less than
10 100 DU/ml.

Example 5

Purification of recombinant dextranase:

Culture broth was filtered and concentrated in an Amicon
15 cell (membrane cut off: 10 kDa). The sample was diluted with MilliQ filtered H₂O and pH was adjusted to pH 7.5. The sample was then loaded on to a Q-Sepharose column (Pharmacia) equilibrated in 20 mM sodium phosphate, pH 7.5 and the dextranase was eluted in a linear gradient of 0 to 0.5 M NaCl
20 in 20 mM sodium phosphate, pH 7.5. Dextranase-containing fractions were pooled, added (NH₄)₂SO₄ to a concentration of 1 M and loaded onto a Phenyl-Sepharose column (Pharmacia) equilibrated in 1 M (NH₄)₂SO₄. The dextranase was eluted in a linear gradient of 1 to 0 M (NH₄)₂SO₄.

25 The N-terminal amino acid sequence of the purified recombinant dextranase was confirmed by N-terminal protein sequencing. Actually two N-terminal amino acid sequences were found; one beginning at Asp1 (Asp-Gln-Gln-Asn-Gln-) and one beginning at Asn4 (Asn-Gln-) in a ratio of 2:3.

30

Example 6

Molecular weight of dextranase

The purified recombinant dextranase like the wild-type enzyme had a molecular weight of around 65 kDa from SDS-PAGE.
35 This was confirmed by matrix assisted laser desorption ionization time-of-flight mass spectrometry, where a molecular weight around 65 kDa was observed.

Mass spectrometry of the wild-type dextranase revealed an average mass around 65.3 kDa.

Example 7

5 pH-profile of recombinant and wild-type dextranase:

Enzyme samples were incubated with AZCL-dextran in 50 mM Britton-Robinson buffer at various pH. Both recombinant and the wild-type dextranase have a pH optimum around pH 6 (see Figure 4).

10

Example 8

Temperature profile of recombinant and wild-type dextranase:

Enzyme samples were incubated with AZCL-dextran in 0.1 M sodium acetate, pH 5.5 at various temperatures. The recombinant and the wild-type enzyme has similar temperature profiles with an optimum around 60°C (see Figure 5).

Example 9

Temperature stability of recombinant and wild-type dextranase:

Enzyme samples were pre-incubated at various temperatures in 50 mM Britton-Robinson buffer at pH 5.5 or pH 7 for 30 minutes. Then the samples were diluted 10 fold in 0.1 M sodium acetate, pH 5.5 before measuring the residual activity. A comparable temperature stability was obtained for the two enzymes at both pH 5.5 and pH 7. The dextranase is stable at 60°C. After incubation at 70°C little residual activity is observed (see Figure 6).

Example 10

30 Recombinant dextranase against Dental Plaque

A plaque biofilm was grown anaerobic on saliva coated hydroxyapatite disks as described in the Materials and Methods section above. The plaque was a mixed culture of *Streptococcus mutans* (SFAG, CBS 350.71), *Actinomyces viscosus* (DSM no. 43329) and *Fusobacterium nucleatum* subsp. *polymorphum* (DSM no. 20482).

HA disks with plaque were transferred to acetate buffer (pH 5.5) containing 1 KDU/ml recombinant *Paecilomyces lilacinus*

dextranase and whirled for 2 minutes (sterile buffer was used as control).

After enzyme treatment, the HA disks were either DAPI stained or transferred to Malthus cells.

5 Indirect impedance measurements were used when enumerating living adherent cells (Malthus Flexi M2060, Malthus Instrument Limited).

For the impedance measurements 3 ml of BHI were transferred to the outer chamber of the indirect Malthus cells, and 0.5 ml of
10 sterile KOH (0.1 M) was transferred to the inner chamber. The HA disks with plaque were after dextranase treatment slightly rinsed with phosphate buffer and transferred to the outer chamber. The detection times (dt) in Malthus were converted to colony counts by use of a calibration curve relating cfu/ml to dt (See Figure
15 7).

The calibration curve was constructed by a series 10-fold dilution rate prepared from the mixed culture. Conductance dt of each dilution step was determined in BHI and a calibration curve relating cfu/ml of the 10 fold dilutions to dt in BHI was
20 constructed for the mixed culture .

The removal of plaque from the HA disks was also determined by fluorescent microscopy, disks were after enzyme treatment stained with DAPI (3 mM) and incubated in the dark for 5 minutes at 20°C. The DAPI stained cells were examined with the x 100 oil
25 immersion fluorescence objective on an Olympus model BX50 microscope equipped with a 200 W mercury lamp and an UV- filter. The result was compared with the quantitative data obtained by the impedance measurements.

The number of living cells on the saliva treated HA-surface
30 was after the dextranase treatment determined by the Malthus method and shown in Table 1.

However, by the Malthus method it is not possible to distinguish between a bactericidal activity of the enzyme or an enzymatic removal of the plaque. Therefore a decrease in living
35 bacteria on the surface has to be compared with the simultaneously removal of plaque from the surface which is estimated by the DAPI staining.

Dextranase (KDU/ml)	Log ₁₀ reduction (cfu/cm ²)	Removal of plaque (%)	No. of observations
0	0	0	10
1	2.5	99	3

Table 1: Enzymatic plaque removal (pH 5.5, 2 minutes) from saliva treated hydroxyapatite determined by impedance measurements.

A significant removal of plaque was determined by fluorescent microscopy after treatment with dextranase. Thus the
 5 recombinant dextranase reduced the amount of adhering cells. Consequently, the activity was observed as a removal of plaque and not as a bactericidal activity against cells in plaque.

Example 11

10 **Expression of recombinant *Paecilomyces lilacinus* dextranase in *Fusarium venenatum*.**

Construction of dextranase expression plasmid for *F. venenatum*

The dextranase gene was PCR amplified from the expression
 15 plasmid pToC343 using primers o-dexSwa (SEQ ID NO 25) and o-dexPac (SEQ ID NO 26) (see Materials and Methods). The resulting 1860 nt amplicon was digested with *SwaI* and *PacI* and ligated to pDM181 that had been linearized with the same two enzymes. The construct was introduced into *E. coli* and the resulting
 20 colonies were screened by colony hybridization to identify those that contained the dextranase coding region. From this screen, plasmid pJW111 was selected (Figure 8). DNA sequencing of the insert in pJW111 determined that this was the dextranase gene and that the sequence was identical to that of the
 25 dextranase gene in pToC343.

Transformation of *Fusarium venenatum*

The plasmid pJW111 was introduced into a morphological mutant of *Fusarium* A3/5 (ATCC 20334), designated CC1-3, as
 30 follows:

Conidia were generated by growth of strain ATTC 20334 CC1-3 in sporulation medium at 28°C, 150 rpm for 2-4 days. Conidia were filtered through Miracloth, concentrated by centrifugation and re-suspended in sterile water. 50 ml of YPG medium was
5 inoculated with 10^8 conidia, and incubated for 14 hours at 24°C, 150 rpm. The resulting hyphae were resuspended in 20 ml of NOVOZYM 234 solution (2.5 mg/ml in 1.0 M $MgSO_4$) and digested for 15-45 minutes at 28°C at 80 rpm. 30 ml of STC were added and protoplasts were pelleted at 1500 rpm (Sorvall RT 6000
10 centrifuge) for 10 minutes. STC wash steps were repeated twice. Protoplasts were suspended in STC:SPTC:DMSO (8:2:0.1) at a concentration of approximately 5×10^7 protoplasts per ml.

Plasmid DNA (2-20 mg) was added to 200 ml protoplasts and incubated 30 minutes on ice. Two ml SPTC were added slowly,
15 followed by a 20 minute incubation at room temperature. 50 ml melted overlay (1X Vogel's salts, 25mM $NaNO_3$, 0.8M sucrose, 1% low melt agarose) at 40°C were added to the transformation reaction. Samples were mixed by inversion and split between two empty 150 mm petri dishes. After 24 hours 25 ml overlay plus
20 10 mg/ml Basta were added to each plate. Plates were incubated at room temperature.

Expression of dextranase activity

16 transformants were transferred to Vogel's/Basta and
25 grown 7 days at room temperature. 20 ml M400Da medium in a 125 ml flask were inoculated with a 1 cm² piece of mycelia from the Vogel's/Basta plate. Cultures were incubated 7 days at 30°C, 150 rpm. Culture samples were centrifuged and the supernatants were assayed for dextranase (as described above). The best
30 producing transformants were selected by dextranase activity assays and SDS/PAGE analysis. The dextranase band ran at approximately 60kD on a 10-27% gradient tris-glycine gel.

N-terminal sequencing revealed that the dextranase expressed in
35 *F. venenatum* was 100% correctly processed to Asn-Gln-Ala-Leu-, in contrast to the dextranase produced by *A. oryzae*, of which

40% was incorrectly processed to yield the N-terminal sequence Asp-Gln-Gln-Asn-Gln-Ala-Leu-.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: Bagsvaerd
(E) COUNTRY: Denmark
(F) POSTAL CODE (ZIP): DK-2880
(G) TELEPHONE: +45 4444 8888
(H) TELEFAX: +45 4449 3256

(ii) TITLE OF INVENTION: A recombinant enzyme with dextranase activity

(iii) NUMBER OF SEQUENCES: 24

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: *Paecilomyces lilacinus*

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 875..2701

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 875..958

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 959..2701

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GACGACCATG ACAGGTGTCG CACAACAGGA ACAATCAGGA CCCTGATACG GCCATAAGGT	60
GAAACACCCC CTTGATGACA ACGGGAAGAA ACAGCTGGTC CGTATGTTCT AGACAATTCA	120
AAGACACATC TTCCCCTCCC TGTCCATGAC ACTGTGGTAG GACGATGACA CCGATGCATG	180
ATCATGAAAG GACAATGAAC ATGGGCGATC GATTTAGCTG ACATGAAGTG TAGCGAAGAC	240
AAGTGCCTCC GTGTTTCGTA GATCGAGACC AGAGTGGTGG CAATTGCTCC GGCTCCAACC	300
CGATCCAGAG ATGGGCCGAG TATAACTGAT ATGCGCCCGC TTTCGTTAGA CTGCGCATGG	360
AGCTGTGGCA CATCGTCGTC CAGACCAAGG AAGACTAGCA ATGGTTTGGC CGCTGTGTGA	420
GCCACGTTTCG TTTTACATCC AACTGCCGCC GGCCCCCGT GGGGTAACAA GGCGGAGGCC	480
TGGGGTAACC GGGCGGTTCC CGTTCGTAGT AATACGCCTT CTGATTGTGC CAATCTGGAG	540
CGGTGGTCGC TGCAGGGGGA TGGCCCTTCT AACTTCTTTC TTTTAAGCTT ATGAAACTAG	600
GCCAGGTGGT GGCTGTGGCA AGTCTCAACA GCGCCATAGA TTGAATCAGA CATGGACCCC	660
CCGCAACATG TGTCCCGCCC CAGAACTCCT GCGTCTTCGG TCCTCTCCCG GGAAGAGAGT	720
GCGCCGTCAC CAAGGCTATA AATACTGGG GTATGTTAGC ATAGTCTCGA AATGATATCC	780

CATTTCAATC TTTACTGGTC CATCTCTAAA GGCATACACA CAGTGAGGCT GATTTTCGGC	840
CATTGTCCTG TACACTTACC TGTCAGCGG CATC ATG CGT TGG CCT GGT AAT	892
Met Arg Trp Pro Gly Asn	
-28 -25	
TTT CTC ACT CTC GCG ACG GCG CTG CAA GCT GCT GGG AAC CTC GCC GCA	940
Phe Leu Thr Leu Ala Thr Ala Leu Gln Ala Ala Gly Asn Leu Ala Ala	
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AGC GTC CAT CAC AGG TGT GAT CAG CAG AAT CAA GCG CTA CAC ACA TGG	988
Ser Val His His Arg Cys Asp Gln Gln Asn Gln Ala Leu His Thr Trp	
-5 1 5 10	
TGG CAC GAA AAG TCT GCT GTC AAC GAC GCG GGG GCT GTC GCA GCA GAT	1036
Trp His Glu Lys Ser Ala Val Asn Asp Ala Gly Ala Val Ala Ala Asp	
15 20 25	
GAA GTT CGC CAA TCG CGC AAG TAC GAT GTC TCT GTG TCC GTT CGC GAA	1084
Glu Val Arg Gln Ser Arg Lys Tyr Asp Val Ser Val Ser Val Arg Glu	
30 35 40	
GAA TCC AAA TTC CGG GAC TCG TTT GTC TAC GAG ACC ATC CCG CGG AAC	1132
Glu Ser Lys Phe Arg Asp Ser Phe Val Tyr Glu Thr Ile Pro Arg Asn	
45 50 55	
GGC AAC GGC AAG ATG TAC GAC CCG GCC AAT CCT GGT CAG GAA TAC AAC	1180
Gly Asn Gly Lys Met Tyr Asp Pro Ala Asn Pro Gly Gln Glu Tyr Asn	
60 65 70	
CTG GCG GAC GGG GAT GGC ATC ACC GTC GAA GAG GAC GCA AAG ATC AAC	1228
Leu Ala Asp Gly Asp Gly Ile Thr Val Glu Glu Asp Ala Lys Ile Asn	
75 80 85 90	
ATG GCT TGG ACG CAG TTT CAA TAC GCC AAA GAT GTT GAA GTT CGC ATC	1276
Met Ala Trp Thr Gln Phe Gln Tyr Ala Lys Asp Val Glu Val Arg Ile	
95 100 105	
ACC TCC AAG GAT GGT TCT GCA CTG GGG CCA ACT AGC AAC GTT GTC ATC	1324
Thr Ser Lys Asp Gly Ser Ala Leu Gly Pro Thr Ser Asn Val Val Ile	
110 115 120	
CGC CCC TCG GAT ATC AGG TAC GAC ATC AGA AGC CCA GAC AGC AGC ACT	1372
Arg Pro Ser Asp Ile Arg Tyr Asp Ile Arg Ser Pro Asp Ser Ser Thr	
125 130 135	
GTT ATA ATC CAG GTT CCA TAC GAC CTG AGG GGC CGA CGA TTC TCC GTC	1420
Val Ile Ile Gln Val Pro Tyr Asp Leu Arg Gly Arg Arg Phe Ser Val	
140 145 150	
GAG TTC CAA AAC GAC TTA TAC GCC TAC CGC TCG AAC GGC AAA TCA TAT	1468
Glu Phe Gln Asn Asp Leu Tyr Ala Tyr Arg Ser Asn Gly Lys Ser Tyr	
155 160 165 170	
GTC AAT GAC GGC GGC GTT CTC GTG AGC GAG GAA CCG CGC AAT GCG CTG	1516
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175 180 185	
CTT ATC TTC GCC AGT CCA TTC ATT CCC CAG GAA CTC ATC CCG TCC AAG	1564
Leu Ile Phe Ala Ser Pro Phe Ile Pro Gln Glu Leu Ile Pro Ser Lys	
190 195 200	
ACA TCA GGC GAT ACG CAA GTC CTC AAG CCG GGC AAG ATC ACC GAC AGC	1612
Thr Ser Gly Asp Thr Gln Val Leu Lys Pro Gly Lys Ile Thr Asp Ser	
205 210 215	
ACC ATT GGC GCG AAG CCG ACA CTC TAC TTT GAG GCA GGC ACC TAC TGG	1660
Thr Ile Gly Ala Lys Pro Thr Leu Tyr Phe Glu Ala Gly Thr Tyr Trp	
220 225 230	

GTA	GAG	AAA	GAC	GGC	CGC	CTC	GGT	AAA	AGT	CAC	ATC	AAG	CTG	AAC	GCC	1708
Val	Glu	Lys	Asp	Gly	Arg	Leu	Gly	Lys	Ser	His	Ile	Lys	Leu	Asn	Ala	
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AAC	ACG	CAC	TAC	GTC	TAC	TTC	GAG	CCA	GGA	ACT	TAT	ATC	AAA	GGC	GCC	1756
Asn	Thr	His	Tyr	Val	Tyr	Phe	Glu	Pro	Gly	Thr	Tyr	Ile	Lys	Gly	Ala	
				255					260					265		
TTT	GAG	TAC	ACC	ACT	TCG	AAG	AAT	GAC	TTT	TAT	ACC	GTC	GGA	CAT	GGA	1804
Phe	Glu	Tyr	Thr	Thr	Ser	Lys	Asn	Asp	Phe	Tyr	Thr	Val	Gly	His	Gly	
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GTA	GTC	TCG	GGC	GAA	AAT	TAC	GCA	TAC	ATG	GCA	AAC	ACT	GCC	AAG	AAC	1852
Val	Val	Ser	Gly	Glu	Asn	Tyr	Ala	Tyr	Met	Ala	Asn	Thr	Ala	Lys	Asn	
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TAT	GTT	GCG	GAA	AAG	AGT	GAC	CGG	ACC	AGT	CTT	AGG	ATC	TTT	TCG	CAC	1900
Tyr	Val	Ala	Glu	Lys	Ser	Asp	Arg	Thr	Ser	Leu	Arg	Ile	Phe	Ser	His	
	300					305					310					
CAG	GCA	GTT	TCG	GAC	AGC	CAG	ATA	TGG	CAT	TGC	ATT	GGA	CCT	ACC	CTT	1948
Gln	Ala	Val	Ser	Asp	Ser	Gln	Ile	Trp	His	Cys	Ile	Gly	Pro	Thr	Leu	
315					320					325					330	
AAT	GCA	CCG	CCC	TTT	AAT	ACC	GTG	GAC	CTG	TTT	CCA	AAG	AAC	CAG	ACG	1996
Asn	Ala	Pro	Pro	Phe	Asn	Thr	Val	Asp	Leu	Phe	Pro	Lys	Asn	Gln	Thr	
				335					340					345		
CCA	AAC	GAG	GAA	GAC	AAC	AAG	GTG	CGG	AAC	GAC	ATC	TCT	GAC	TAC	AAA	2044
Pro	Asn	Glu	Glu	Asp	Asn	Lys	Val	Arg	Asn	Asp	Ile	Ser	Asp	Tyr	Lys	
			350					355					360			
CAG	GTC	GGC	GCG	TTC	TAC	TTC	CAG	ACT	GAT	GGG	CCG	CAA	ATA	TAC	TCT	2092
Gln	Val	Gly	Ala	Phe	Tyr	Phe	Gln	Thr	Asp	Gly	Pro	Gln	Ile	Tyr	Ser	
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GGA	ACC	GTC	AAG	GAC	TGC	TTC	TGG	CAT	GTT	AAT	GAC	GAC	GCT	ATC	AAG	2140
Gly	Thr	Val	Lys	Asp	Cys	Phe	Trp	His	Val	Asn	Asp	Asp	Ala	Ile	Lys	
	380					385					390					
TTG	TAC	CAC	TCG	GAC	GCG	AAG	GTC	GAA	CGG	GTG	ACC	ATC	TGG	AAG	TGT	2188
Leu	Tyr	His	Ser	Asp	Ala	Lys	Val	Glu	Arg	Val	Thr	Ile	Trp	Lys	Cys	
395					400					405					410	
CAC	AAC	GAC	CCC	GTT	ATC	CAA	ATG	GGC	TGG	AAA	CCA	CGT	GGA	GTC	TCT	2236
His	Asn	Asp	Pro	Val	Ile	Gln	Met	Gly	Trp	Lys	Pro	Arg	Gly	Val	Ser	
				415					420					425		
GGA	ACT	ACC	ATT	TCT	GAA	CTC	AAG	GTC	ATC	CAC	ACT	CGA	TGG	TTT	AAG	2284
Gly	Thr	Thr	Ile	Ser	Glu	Leu	Lys	Val	Ile	His	Thr	Arg	Trp	Phe	Lys	
			430					435					440			
AGC	GAG	ACG	GTT	GTT	CCT	TCC	GCC	ATT	ATT	GGA	GCC	TCA	CCC	TAC	TAC	2332
Ser	Glu	Thr	Val	Val	Pro	Ser	Ala	Ile	Ile	Gly	Ala	Ser	Pro	Tyr	Tyr	
		445					450					455				
GGC	GAC	CCA	AAG	ATT	GTG	GAT	GCG	TCT	AGG	ACA	ATG	AGC	GTT	CGA	ATT	2380
Gly	Asp	Pro	Lys	Ile	Val	Asp	Ala	Ser	Arg	Thr	Met	Ser	Val	Arg	Ile	
	460					465					470					
TCT	GAC	GTG	ACC	TGC	GAA	GGT	CGT	TGC	CCT	GCG	CTC	CTT	CGG	ATT	GGT	2428
Ser	Asp	Val	Thr	Cys	Glu	Gly	Arg	Cys	Pro	Ala	Leu	Leu	Arg	Ile	Gly	
	475				480					485					490	
CCG	CTC	CAG	AAT	TAT	GAC	ATG	ACC	ATT	GAG	AAC	GTG	AAA	TTC	GAT	GAA	2476
Pro	Leu	Gln	Asn	Tyr	Asp	Met	Thr	Ile	Glu	Asn	Val	Lys	Phe	Asp	Glu	
				495					500					505		

CTT TTG AGG GAT GAC AAC GTC AAG CTA GGA CAG AGT CTG GTT GGT ATG Leu Leu Arg Asp Asn Val Lys Leu Gly Gln Ser Leu Val Gly Met 510 515 520	2524
AGG ATC AGC GAC CAA GAG GAC GCC TAC ATA CCC GGC CAA GAA AAG CTC Arg Ile Ser Asp Gln Glu Asp Ala Tyr Ile Pro Gly Gln Glu Lys Leu 525 530 535	2572
AAG CTA GGG ATA CAT ATC AAG AAT TGG CAG ATT GGG GGC AAC AGA GTG Lys Leu Gly Ile His Ile Lys Asn Trp Gln Ile Gly Gly Asn Arg Val 540 545 550	2620
GAT GGA TCA AAC TGG CAA GTC AAC CAA CTT GGG CAG TTG AAC ATC CAC Asp Gly Ser Asn Trp Gln Val Asn Gln Leu Gly Gln Leu Asn Ile His 555 560 565 570	2668
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GTGTGTTTCC GTGTTGGCGC ACTTCCAAAC CCATCACGCC GACTGTTTCA ATTCTTCGCA	2781
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TTATCGAAAA ACCAGACCTC ACCAAAGAAA GTGCACGTGG TTAAC TAGGG ACATGAGATG	2901
CCCGACACTG TAGACTCTGC TCATCAAGAT AATCCTTCTT GCACAGCGCT GATAACGTGA	2961
TGGCGCCCGAG TACGTGTAGG GGCATCCGAG TC	2993

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 609 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Arg Trp Pro Gly Asn Phe Leu Thr Leu Ala Thr Ala Leu Gln Ala
-28 -25 -20 -15

Ala Gly Asn Leu Ala Ala Ser Val His His Arg Cys Asp Gln Gln Asn
-10 -5 1

Gln Ala Leu His Thr Trp Trp His Glu Lys Ser Ala Val Asn Asp Ala
5 10 15 20

Gly Ala Val Ala Ala Asp Glu Val Arg Gln Ser Arg Lys Tyr Asp Val
25 30 35

Ser Val Ser Val Arg Glu Glu Ser Lys Phe Arg Asp Ser Phe Val Tyr
40 45 50

Glu Thr Ile Pro Arg Asn Gly Asn Gly Lys Met Tyr Asp Pro Ala Asn
55 60 65

Pro Gly Gln Glu Tyr Asn Leu Ala Asp Gly Asp Gly Ile Thr Val Glu
70 75 80

Glu Asp Ala Lys Ile Asn Met Ala Trp Thr Gln Phe Gln Tyr Ala Lys
85 90 95 100

Asp Val Glu Val Arg Ile Thr Ser Lys Asp Gly Ser Ala Leu Gly Pro
105 110 115

Thr Ser Asn Val Val Ile Arg Pro Ser Asp Ile Arg Tyr Asp Ile Arg
120 125 130

Ser Pro Asp Ser Ser Thr Val Ile Ile Gln Val Pro Tyr Asp Leu Arg
 135 140 145
 Gly Arg Arg Phe Ser Val Glu Phe Gln Asn Asp Leu Tyr Ala Tyr Arg
 150 155 160
 Ser Asn Gly Lys Ser Tyr Val Asn Asp Gly Gly Val Leu Val Ser Glu
 165 170 175 180
 Glu Pro Arg Asn Ala Leu Leu Ile Phe Ala Ser Pro Phe Ile Pro Gln
 185 190 195
 Glu Leu Ile Pro Ser Lys Thr Ser Gly Asp Thr Gln Val Leu Lys Pro
 200 205 210
 Gly Lys Ile Thr Asp Ser Thr Ile Gly Ala Lys Pro Thr Leu Tyr Phe
 215 220 225
 Glu Ala Gly Thr Tyr Trp Val Glu Lys Asp Gly Arg Leu Gly Lys Ser
 230 235 240
 His Ile Lys Leu Asn Ala Asn Thr His Tyr Val Tyr Phe Glu Pro Gly
 245 250 255 260
 Thr Tyr Ile Lys Gly Ala Phe Glu Tyr Thr Thr Ser Lys Asn Asp Phe
 265 270 275
 Tyr Thr Val Gly His Gly Val Val Ser Gly Glu Asn Tyr Ala Tyr Met
 280 285 290
 Ala Asn Thr Ala Lys Asn Tyr Val Ala Glu Lys Ser Asp Arg Thr Ser
 295 300 305
 Leu Arg Ile Phe Ser His Gln Ala Val Ser Asp Ser Gln Ile Trp His
 310 315 320
 Cys Ile Gly Pro Thr Leu Asn Ala Pro Pro Phe Asn Thr Val Asp Leu
 325 330 335 340
 Phe Pro Lys Asn Gln Thr Pro Asn Glu Glu Asp Asn Lys Val Arg Asn
 345 350 355
 Asp Ile Ser Asp Tyr Lys Gln Val Gly Ala Phe Tyr Phe Gln Thr Asp
 360 365 370
 Gly Pro Gln Ile Tyr Ser Gly Thr Val Lys Asp Cys Phe Trp His Val
 375 380 385
 Asn Asp Asp Ala Ile Lys Leu Tyr His Ser Asp Ala Lys Val Glu Arg
 390 395 400
 Val Thr Ile Trp Lys Cys His Asn Asp Pro Val Ile Gln Met Gly Trp
 405 410 415 420
 Lys Pro Arg Gly Val Ser Gly Thr Thr Ile Ser Glu Leu Lys Val Ile
 425 430 435
 His Thr Arg Trp Phe Lys Ser Glu Thr Val Val Pro Ser Ala Ile Ile
 440 445 450
 Gly Ala Ser Pro Tyr Tyr Gly Asp Pro Lys Ile Val Asp Ala Ser Arg
 455 460 465
 Thr Met Ser Val Arg Ile Ser Asp Val Thr Cys Glu Gly Arg Cys Pro
 470 475 480
 Ala Leu Leu Arg Ile Gly Pro Leu Gln Asn Tyr Asp Met Thr Ile Glu
 485 490 495 500

Asn Val Lys Phe Asp Glu Leu Leu Arg Asp Asp Asn Val Lys Leu Gly
 505 510 515
 Gln Ser Leu Val Gly Met Arg Ile Ser Asp Gln Glu Asp Ala Tyr Ile
 520 525 530
 Pro Gly Gln Glu Lys Leu Lys Leu Gly Ile His Ile Lys Asn Trp Gln
 535 540 545
 Ile Gly Gly Asn Arg Val Asp Gly Ser Asn Trp Gln Val Asn Gln Leu
 550 555 560
 Gly Gln Leu Asn Ile His Pro Asp Tyr Trp Gly Asp Trp Ser Ile Glu
 565 570 575 580

*

- (2) INFORMATION FOR SEQ ID NO: 3:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (v) FRAGMENT TYPE: N-terminal
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Gln Gln Asn Gln Ala Leu His Thr Trp Trp His Glu Lys Ser
 1 5 10 15

- (2) INFORMATION FOR SEQ ID NO: 4:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide 1
 (v) FRAGMENT TYPE: N-terminal
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Tyr Val Asn Asp Gly Gly Val Leu Val Ser Glu Glu Pro Arg Asn
 1 5 10 15
 Ala Leu Leu Ile Phe Ala Ser Pro Phe Ile Pro Gln
 20 25

- (2) INFORMATION FOR SEQ ID NO: 5:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide 2
 (v) FRAGMENT TYPE: N-terminal
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Asp Arg Thr Ser Leu Arg Ile Phe Ser His Gln Ala Val Ser Asp Ser Gln
 1 5 10 15

Ile Trp His Xaa Ile
 20

- (2) INFORMATION FOR SEQ ID NO: 6:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide 3
 (v) FRAGMENT TYPE: N-terminal

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide 8
(v) FRAGMENT TYPE: N-terminal
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Leu Asx Ala Asx Thr His Tyr Val Tyr Phe Glu Pro Gly Thr Tyr Ile Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 12:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide 9
 (v) FRAGMENT TYPE: N-terminal
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Val Ile His Thr Arg Trp Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 13:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide 10
 (v) FRAGMENT TYPE: N-terminal
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ser Ala Val Asn Asp Ala Gly Ala Val Ala Ala Asp Glu Val Arg Gln
1 5 10 15

Ser Asg Lys

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide 11
- (v) FRAGMENT TYPE: N-terminal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Xaa His Asn Asp Pro Val Ile Gln Met Gly Xaa Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 15:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "Primer 8001"
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GA(A/G)AA(T/C)TA(T/C)G C(T/C/G/A)TA(T/C)ATGGC

20

(2) INFORMATION FOR SEQ ID NO: 16:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8002"

GCCAT(G/A)TA(G/A/T/C)GC (A/G)TA(G/A)TT(T/C)TC 19

- (2) INFORMATION FOR SEQ ID NO: 17:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8003"

TGGAC(A/G/T/C)CA(A/G)T T(T/C)CA(A/G)TA(T/C)GC 20

- (2) INFORMATION FOR SEQ ID NO: 18:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8004"

GC(A/G)TA(T/C)TG(A/G)A A(T/C)TG(A/G/T/C)GTCC 19

- (2) INFORMATION FOR SEQ ID NO: 19:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8005"

AA(T/C)TGGCA(A/G)A T(T/C/A)GG(A/G/T/C)GG 17

- (2) INFORMATION FOR SEQ ID NO: 20:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8006"

CC(A/G/T/C)CC(T/A/G)TA(T/C)T GCCA(A/G)TT 17

- (2) INFORMATION FOR SEQ ID NO: 21:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer"

AGAAATCGGG TATCCTTTCA G 21

- (2) INFORMATION FOR SEQ ID NO: 22:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 105 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (A) DESCRIPTION: /desc = "Primer"

GCTCCTCATG GTGGATCCCC AGTTGTGTAT ATAGAGGATT GAGGAAGGAA GAGAAGTGTG 60
GATAGAGGTA AATTGAGTTG GAAACTCCAA GCATGGCATC CTTGC 105

- (2) INFORMATION FOR SEQ ID NO: 23:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8864"

CGCGGATCCA CCATGCGTTG GCCTGGTAAT TTTC 34

- (2) INFORMATION FOR SEQ ID NO: 24:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Primer 8867"

CCGCTCGAGC CTGCCTCATT CAATGCTCC 29

- (2) INFORMATION FOR SEQ ID NO: 25:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer o-dexSwaI"

GCATTTAAAT ATG CGT TGG CCT GGT 25

- (2) INFORMATION FOR SEQ ID NO: 26:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer o-dexPacI"

CGTTAAT TAA TCA TTC AAT GCT CCA GTC 28

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 9 _____, line 1-10 _____.	
B. IDENTIFICATION OF DEPOSIT <div style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></div>	
Name of depositary institution DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	
Address of depositary institution (including postal code and country) Mascheroder Weg 1b, D-38124 Braunschweig, GERMANY	
Date of deposit 5 June 1996	Accession Number DSM 10706
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional <input type="checkbox"/>	
Until the publication of the mention of grant of a European patent or, where applicable, for twenty years from the date of filing if the application has been refused, withdrawn or deemed withdrawn, a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. Rule 28(4) EPC). And as far as Australia is concerned, the expert option is likewise requested, reference being had to Regulation 3.25 of Australia Statutory Rules 1991 No 71. Also, for Canada we request that only an independent expert nominated by the Commissioner is authorized to have access to a sample of the microorganism deposited.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	
<input type="checkbox"/>	This sheet was received with the international application
Authorized officer	

For International Bureau use only	
<input type="checkbox"/>	This sheet was received by the International Bureau on:
Authorized officer	

PATENT CLAIMS

1. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting dextranase activity, which DNA sequence comprises
- 5 a) the dextranase encoding part of the DNA sequence shown in SEQ ID no. 1, and/or the DNA sequence obtainable from *E. coli* DSM 10706, or
- b) an analogue of the DNA sequence shown defined in a), which
- 10 i) is 80% homologous with the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from *E. coli* DSM 10706, or
- ii) hybridizes with the same oligonucleotide probe as the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from *E. coli* DSM 10706, or
- 15 iii) encodes a polypeptide which is at least 80% homologous with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID no. 1 and/or the DNA sequence obtainable from *E. coli* DSM , or
- iv) encodes a polypeptide which is immunologically reactive with an antibody raised against a purified dextranase encoded by the DNA sequence shown in SEQ ID no. 1 derived from *Paecilomyces lilacinus* and/or obtainable from *E. coli*, DSM 10706.
- 20
- 25 2. The DNA construct according to claim 1, in which the DNA sequence is obtainable from a fungal microorganism, such as a filamentous fungus or a yeast.
3. The DNA construct according to claim 2, in which the DNA
- 30 sequence is obtainable from a strain of *Paecilomyces*, such as *Paecilomyces lilacinus*, obtainable from a strain of *Penicillium*,, such as *Penicillium lilacinum* or *Penicillium minioluteum*.
- 35 4. The DNA construct according to claim 3, in which the DNA sequence is isolated from or produced on the basis of a nucleic acid library of *Paecilomyces lilacinus*.

5. A recombinant expression vector comprising the DNA construct according to any of claims 1 to 4.
6. A cell comprising a DNA construct according to claims 1 to 4 or a recombinant expression vector according to claim 5.
7. The cell according to claim 6, which is a filamentous fungus.
- 10 8. The cell according to claim 7, wherein the cell belongs to a strain within the genus *Aspergillus*, in particular a strain of *Aspergillus niger* or *Aspergillus oryzae*, or a strain within the genus *Fusarium*, such as a strain of *Fusarium oxysporium*, *Fusarium graminearum*, *Fusarium sulphureum*, *Fusarium cerealis* or
15 *Fusarium venenatum*, or a strain within the genus *Penicillium*, such as *Penicillium lilacinum* or *Penicillium minioluteum*, or a strain within the genus *Paecilomyces*, such as *Paecilomyces lilacinus*.
- 20 9. A method for producing a recombinant enzyme exhibiting dextranase activity, which method comprises cultivating a host cell according to any of claims 6 to 8 in suitable culture medium under conditions permitting the expression of the DNA construct according to any of claims 1 to 4 or expression
25 vector according to claim 5, and recovering the enzyme from the culture.
10. A recombinant enzyme with dextranase activity encoded by a DNA construct of claims 1 to 4.
- 30 11. The recombinant enzyme produced according to the method according to claim 9.
12. A composition comprising an recombinant dextranase
35 according to any of claims 10 and 11.
13. An oral care product comprising an enzyme according to claims 10 and 11 or a composition according to claim 12 further

comprising ingredients conventionally used in oral care products.

14. The oral care product according to claim 13, being a dentifrice, such as a toothpaste, tooth powder or a mouth wash.

15. The oral care product according to any of claims 13 and 14 further comprising enzyme activities selected from the group of mutanases, oxidases, peroxidases, haloperoxidases, laccases, proteases, endoglucosidases, lipases, amylases, anti-microbial enzymes, and mixtures thereof.

16. Use of the recombinant dextranase according to claim 10 and 11 or a composition of claim 12 or oral care product according to claims 13 and 14 for preventing the formation of dental plaque or removing dental plaque.

17. Use of the recombinant dextranase according to claims 10 and 11 or a composition for claims 12 for hydrolysis of sugar juice or syrup.

18. Use of the recombinant dextranase according to claims 10 and 11 or composition according to claim 12 or oral care composition according to claims 13 and 14 for oral care products for animals.

19. Use of the recombinant dextranase according to claims 10 and 11 or a composition of claims 12 in food, feed and/or pet food products.

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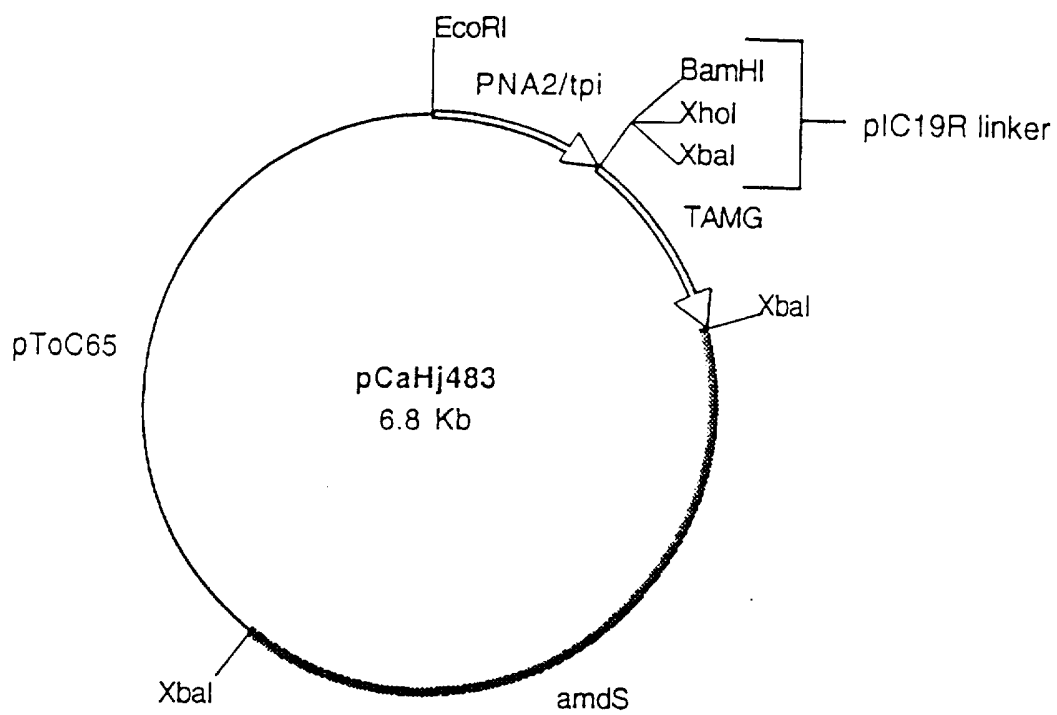


Fig. 1

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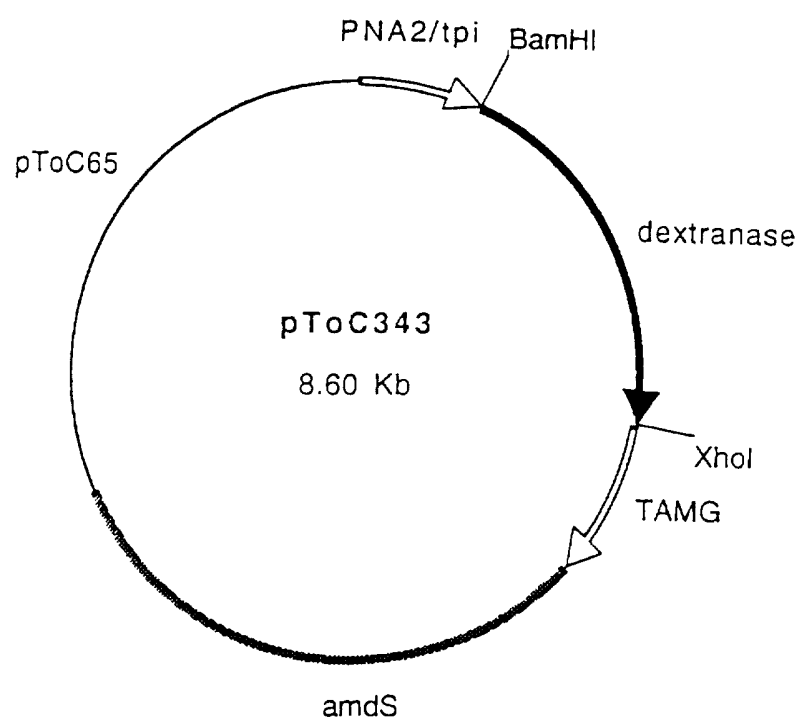


Fig. 2

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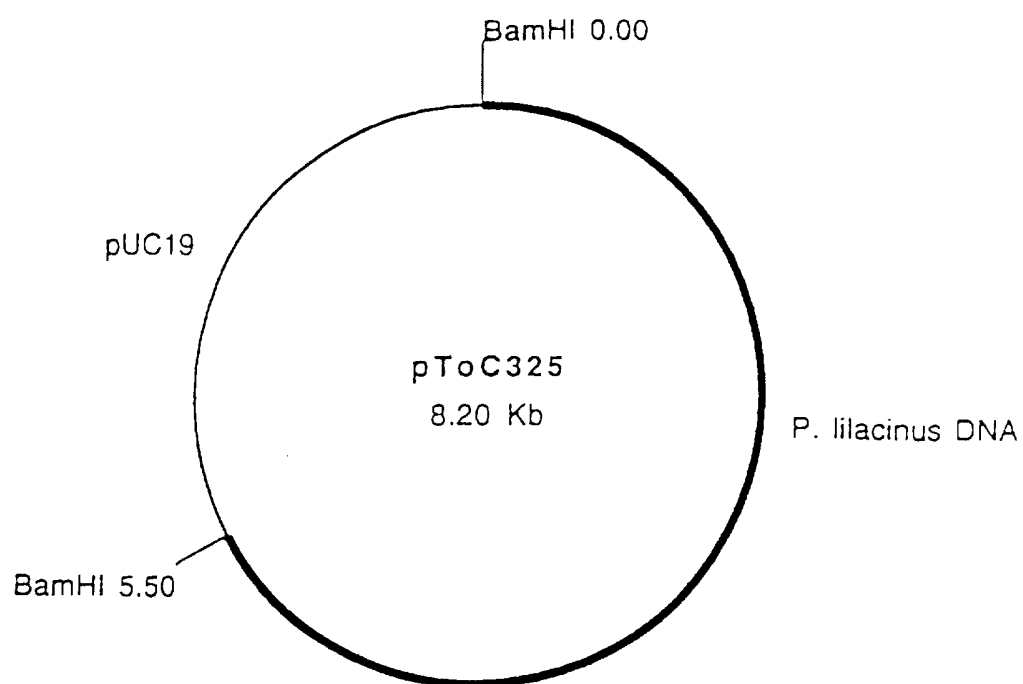


Fig. 3

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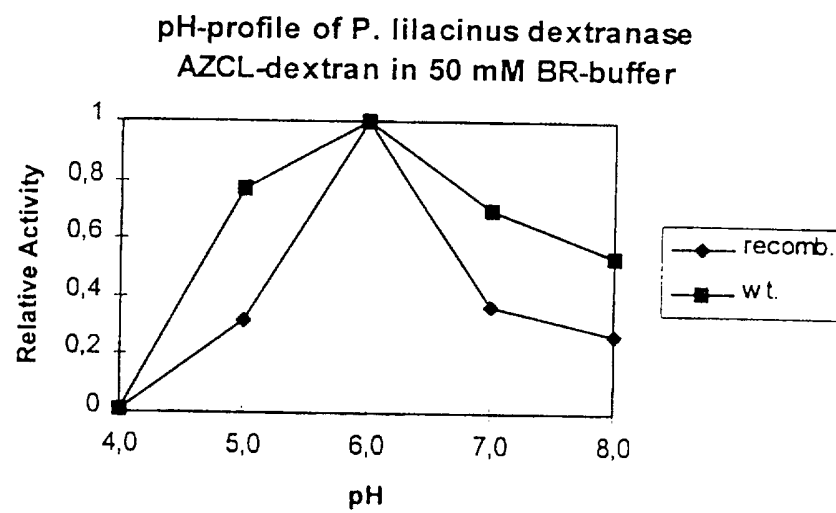


Fig. 4

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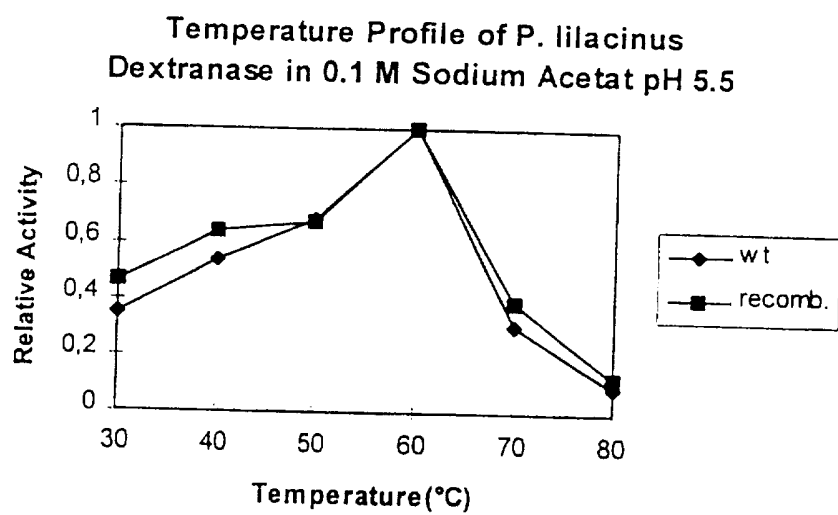


Fig. 5

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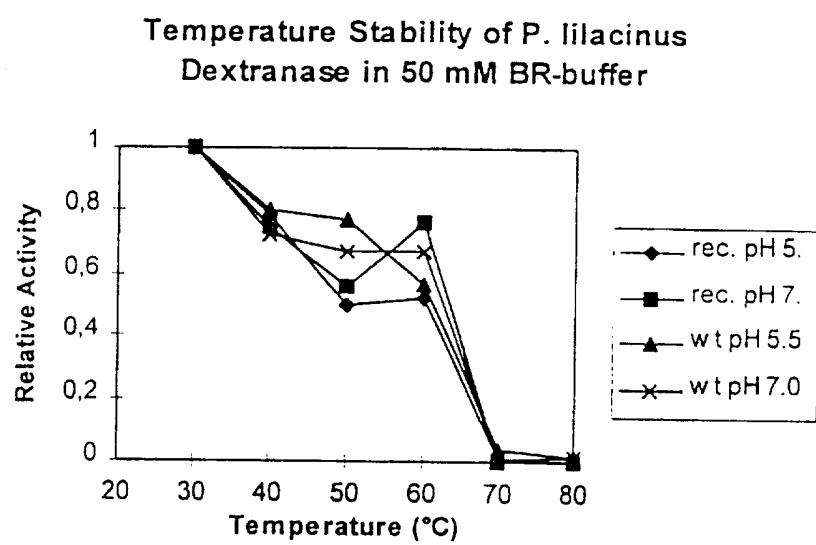


Fig. 6

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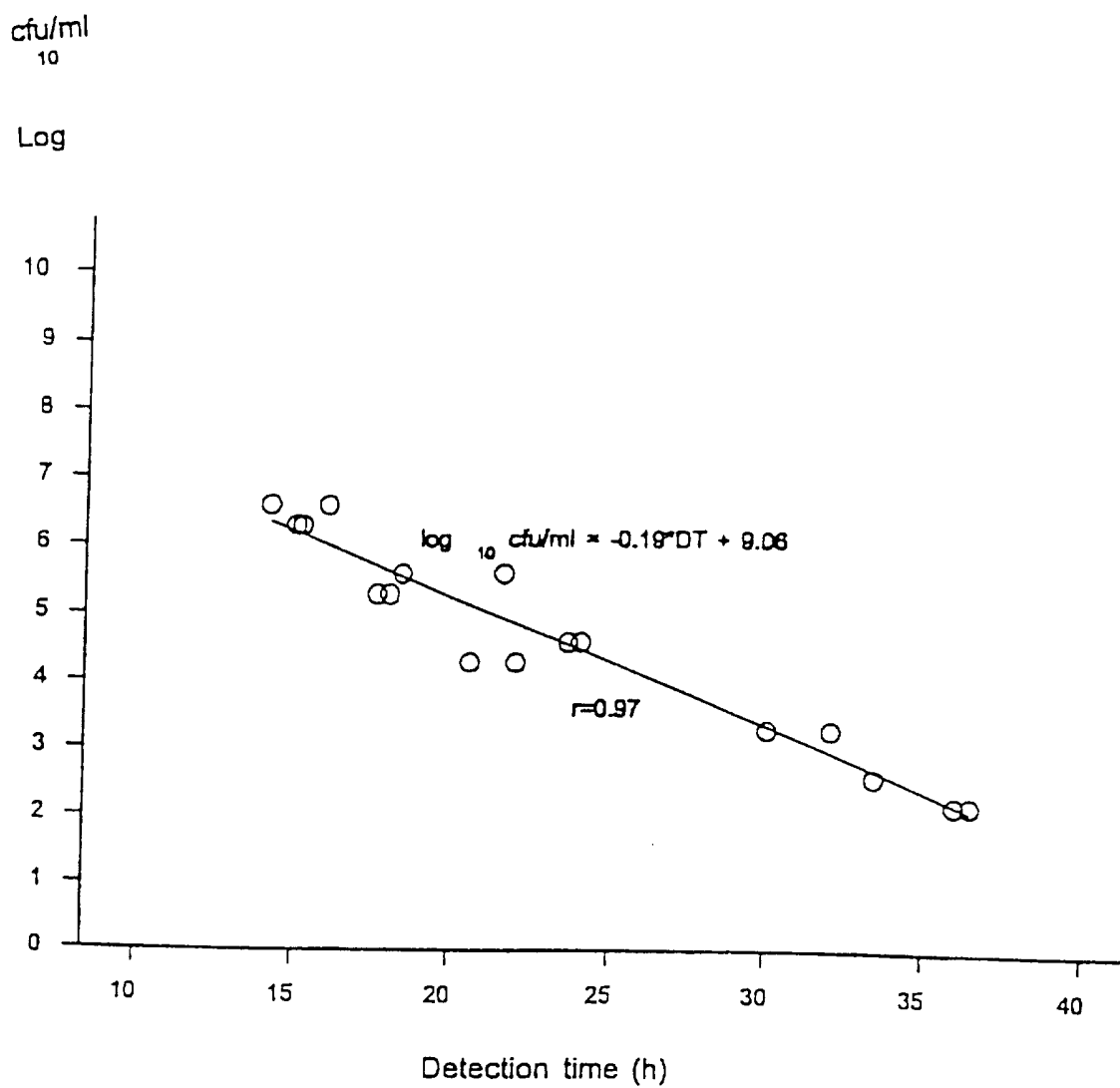


Fig. 7

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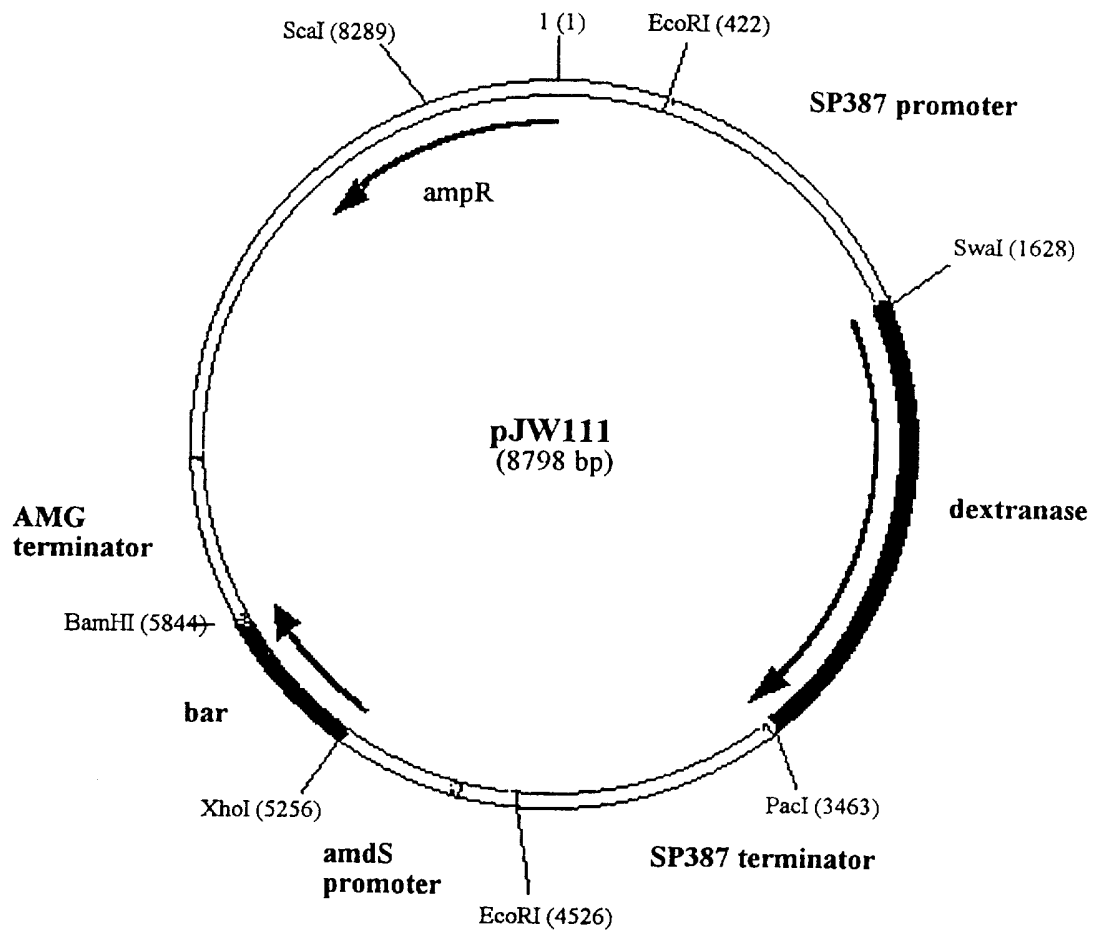


Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00284

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/46, C12N 15/56

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, EMBL/GENBANK/DBJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0663443 A1 (CENTRO DE INGENIERIA GENETICA Y BIOTECNOLOGIA), 19 July 1995 (19.07.95), see page 8, lines 18-37 --	1-19
X	Chemical Abstracts, Volume 104, No 3, 20 January 1986 (20.01.86), (Columbus, Ohio, USA), Lee, J. M. et al, "Purification and characterization of Paecilomyces lilacinus dextranase", page 240, THE ABSTRACT No 16885u, Enzyme Microb. Technol. 1985, 7 (11), 573-577 --	1-19

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

26 Sept. 1997

Date of mailing of the international search report

29 -09- 1997

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00284

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Volume 110, No 25, 19 June 1989 (19.06.89), (Columbus, Ohio, USA), Sun, Jinwu et al, "A strain of Paecilomyces lilacinus producing high quality dextranase", page 5, THE ABSTRACT No 227559c, Ann. N. Y. Acad. Sci. 1988, 542, 192-194 --	1-19
A	Chemical Abstracts, Volume 119, No 17, 25 October 1993 (25.10.93), (Columbus, Ohio, USA), Cheng, Xiulan et al, "Conditions for dextranase formation by Paecilomyces lilacinus", page 654, THE ABSTRACT No 179275u, Weishengwu Xuebao 1992, 32 (5), 334-339 --	1-19
A	Chemical Abstracts, Volume 116, No 7, 17 February 1992 (17.02.92), (Columbus, Ohio, USA), Galvez-Mariscal, Amanda et al, "Production and characterization of a dextranase from an isolated Paecilomyces lilacinus strain", page 367, THE ABSTRACT No 54253s, Appl. Microbiol. Biotechnol. 1991, 36 (3), 327-331 --	1-19
A	Chemical Abstracts, Volume 108, No 301, 20 June 1988 (20.06.88), (Columbus, Ohio, USA), Sun, Jinwu et al, "Screening of dextranase producing strains and comparison of their enzymic properties", page 301, THE ABSTRACT No 218770w, Weishengwu Xuebao 1988, 28 (1), 45-55 -----	1-19

