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(54) Titre : VARIANTES DU XYLANASE AYANT UNE SENSIBILITE MODIFIEE AUX INHIBITEURS DE XYLANASE
(54) Title: XYLANASE VARIANTS HAVING ALTERED SENSITIVITY TO XYLANASE INHIBITORS

(57) **Abrégé/Abstract:**

The present invention relates to a variant xylanase polypeptide, or fragment thereof having xylanase activity, comprising one or more amino acid modifications such that the polypeptide or fragment thereof has an altered sensitivity to a xylanase inhibitor as compared with the parent enzyme.



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(54) Title: XYLANASE VARIANTS HAVING ALTERED SENSITIVITY TO XYLANASE INHIBITORS

(57) Abstract: The present invention relates to a variant xylanase polypeptide, or fragment thereof having xylanase activity, comprising one or more amino acid modifications such that the polypeptide or fragment thereof has an altered sensitivity to a xylanase inhibitor as compared with the parent enzyme.

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XYLANASE VARIANTS HAVING ALTERED SENSITIVITY TO XYLANASE INHIBITORS

Field of the invention

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The present invention relates to mutant xylanase enzymes having an altered sensitivity to xylanase inhibitors. The present invention also relates to the use of these mutant enzymes in processing plant materials.

10 Background to the invention

For many years, endo- β -1,4-xylanases (EC 3.2.1.8) (referred to herein as xylanases) have been used for the modification of complex carbohydrates derived from plant cell wall material. It is well known in the art that the functionality of different xylanases (derived
15 from different micro organisms or plants) differs enormously.

Comprehensive studies characterising the functionality of xylanases have been done on well characterised and pure substrates (Kormelink *et al.*, 1992). These studies show that different xylanases have different specific requirements with respect to substitution of the
20 xylose backbone of the arabinoxylan (AX). Some xylanases require three un-substituted xylose residues to hydrolyse the xylose backbone; others require only one or two. The reasons for these differences in specificity is thought to be due to the three dimensional structure within the catalytic domains, which in turn is dependent on the primary structure of the xylanase, i.e. the amino acid sequence. However, the translation of these
25 differences in the amino acid sequences into differences in the functionality of the xylanases, has up until now not been documented when the xylanase acts in a complex environment, such as plant material.

The xylanase substrates found in wheat (wheat flour), have traditionally been divided into
30 two fractions: The water un-extractable AX (WU-AX) and the water extractable AX (WE-AX). The WU-AX:WE-AX ratio is approx. 70:30 in wheat flour. There have been numerous explanations as to why there are two different fractions of AX. The older literature (D'Appolonia and MacArthur (1976) and Montgomery and Smith (1955))

describes quite high differences in the substitution degree between WE-AX and WU-AX. The highest degree of substitution was found in WE-AX. This was used to explain why some of the AX was extractable. The high degree of substitution made the polymer soluble, compared to a lower substitution degree, which would cause hydrogen bonding
5 between polymers and consequently precipitation.

The difference between the functionality of different xylanases has been thought to be due to differences in xylanase specificity and thereby their preference for the WU-AX or the WE-AX substrates.

10

In some applications (e.g. bakery) it is desirable to produce high molecular weight (HMW) soluble polymers from the WU-AX fraction. Such polymers have been correlated to a volume increase in bread making (Rouau, 1993; Rouau *et al.*, 1994 and Courtin *et al.*, 1999).

15

In other applications it is desirable to modify the HMW WU-AX, making the molecular weight lower, reducing their hydrocolloid effect and hence water-binding in the product (crackers, flour separation, etc.)

20 These different applications require different functionalities of the xylanases used to do the job. As mentioned above, the difference in functionality has been explained by the different substrate specificities of the xylanases.

Summary of the invention

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By contrast to earlier studies, we have now shown that other factors are more important in determining xylanase functionality than the substrate specificity of the xylanases determined on pure well-characterised substrates. The data presented herein show that endogenous xylanase inhibitors dictate the functionality of the xylanases currently used in,
30 for example, wheat flour systems. This means that a xylanase that normally modifies the WU-AX, giving increased dough liquid viscosity in a wheat flour system, has a different functionality if the endogenous xylanase inhibitor is absent in the wheat flour. Thus, our

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findings indicate that the design and application of uninhibited xylanases, for example, using site-directed mutagenesis could be a way to mimic the absence of xylanase inhibitors in various plant materials, giving new xylanases with completely new functionality. Such xylanases would be very effective in applications where a reduction in viscosity is required. The uninhibited xylanase would act rapidly on the AX, and be primarily influenced by its specific activity, rather than by endogenous inhibitors. From our studies, we consider that the inhibitory effects are likely to be far more important than the specific activity. Indeed our results show for the first time that there are 10 to 50 fold differences in inhibition levels between the family 11 xylanases.

10

Furthermore, we have gone on to design and test a series of xylanases modified by site-directed mutagenesis to demonstrate that xylanases can be produced that have reduced sensitivity to xylanase inhibitors present in plant materials. In particular, we have identified a number of residues in family 11 xylanases which influence the degree of inhibition of the xylanase.

15

Thus, it will be possible to produce variant xylanases having reduced sensitivity to xylanase inhibitors and hence altered functionality. This will, for example, allow a reduction in the amount of xylanase required in a number of applications such as animal feed, starch production, bakery, flour separation (wetmilling) and, paper and pulp production.

20

Accordingly, the present invention provides a variant xylanase polypeptide, or fragment thereof having xylanase activity, comprising one or more amino acid modifications such that the polypeptide or fragment thereof has an altered sensitivity to a xylanase inhibitor as compared with the parent enzyme.

25

Here, the "parent enzyme" is the xylanase enzyme from which the variant xylanase enzyme is derived or derivable. With respect to the term "derivable", the variant need not necessarily be derived from the parent enzyme. Instead, the variant could be prepared, for example, by use of recombinant DNA techniques that utilise nucleotide sequence(s) encoding said variant xylanase sequence - i.e. here the nucleotide sequence(s) are similar

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to mutated nucleotide sequence(s) but they are not prepared by mutation of the parent nucleotide sequence(s). The variant may even be prepared by chemically modifying a parent enzyme.

- 5 For some embodiments the parent enzyme is the wild type enzyme. The term "wild type" is a term of art understood by skilled persons and includes a phenotype that is characteristic of most of the members of a species occurring naturally and contrasting with the phenotype of a mutant. Thus, in the present context, the wild type enzyme may be a form of the enzyme naturally found in most members of the relevant species.
- 10 Generally, the relevant wild type enzyme in relation to the variant polypeptides of the invention is the most closely related corresponding wild type enzyme in terms of sequence homology. For example, for the particular mutant xylanases described in the examples, the corresponding wild type enzyme is the wild type *B. subtilis* xylanase A, more specifically the wild type *B. subtilis* xylanase A published by Paice *et al.*, 1986 and shown
- 15 as SEQ I.D. 1. However, where a particular wild type sequence has been used as the basis for producing a variant polypeptide of the invention, this will be the corresponding wild type sequence regardless of the existence of another wild type sequence that is more closely related in terms of amino acid sequence homology.
- 20 For some embodiments, preferably the variant polypeptide is derived from a family 11 xylanase.

One of our surprising findings is that in our studies so far a mutation in the xylanase active site has no measurable effect on inhibition against the xylanase inhibitor. This is in

25 direct contrast to the mutation(s) that are made outside of the active site – which mutations are discussed in more detail below.

In a preferred aspect the amino acid modification is of one or more surface amino acid residues.

In a more preferred aspect the amino acid modification is of one or more solvent accessible residues. Here, the solvent is water.

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In a more preferred aspect the amino acid modification is of one or more surface residues outside of the active site.

5 In a highly preferred aspect the amino acid modification is of one or more surface residues outside of the active site and which is/are at least 8 % solvent accessible. Here, the solvent is water.

In a highly preferred aspect the amino acid modification is of one or more surface residues outside of the active site and which is/are at least 10 % solvent accessible. Here, the solvent is water.

10

Solvent accessibility can be determined using Swiss-PdbViewer (version 3.5b1), which can be located on the ExPASy website. The Swiss-PdbViewer is presented by Glaxo Wellcome Experimental Research.

15 The surface amino acids of xylanase enzymes are determinable by a person skilled in the art.

By way of example, the *B. subtilis* amino acid sequence for xylanase A is shown as SEQ I.D. No. 1. With respect to this sequence, the surface amino acid residues are:

20

Ala1-Trp6, Asn8, Thr10-Gly23, Asn25, Ser27, Asn29, Ser31-Asn32, Gly34, Thr43-Thr44, Ser46-Thr50, Asn52, Asn54, Gly56-Asn61, Asn63, Arg73-Leu76, Thr87-Arg89, Thr91-Lys95, Thr97, Lys99, Asp101-Gly102, Thr104, Thr109-Thr111, Tyr113-Asn114, Asp119-Thr124, Thr126, Gln133-Asn141, Thr143, 25 Thr145, Thr147-Asn148, Asn151, Lys154-Gly157, Asn159-Leu160, Ser162-Trp164, Gln175, Ser177, Ser179, Asn181, Thr183, Trp185.

As indicated, the surface amino acids of other xylanase enzymes (such as *Thermomyces lanuginosus* xylanase A, whose coding nucleotide sequence is presented as SEQ ID No. 30 9) are determinable by a person skilled in the art.

Hence, for some aspects the present invention encompasses a variant xylanase polypeptide, or fragment thereof having xylanase activity, which variant xylanase polypeptide or fragment comprises one or more amino acid modifications at any one of
5 amino acid residues:

Ala1-Trp6, Asn8, Thr10-Gly23, Asn25, Ser27, Asn29, Ser31-Asn32, Gly34, Thr43-Thr44, Ser46-Thr50, Asn52, Asn54, Gly56-Asn61, Asn63, Arg73-Leu76, Thr87-Arg89, Thr91-Lys95, Thr97, Lys99, Asp101-Gly102, Thr104, Thr109-
10 Thr111, Tyr113-Asn114, Asp119-Thr124, Thr126, Gln133-Asn141, Thr143, Thr145, Thr147-Asn148, Asn151, Lys154-Gly157, Asn159-Leu160, Ser162-Trp164, Gln175, Ser177, Ser179, Asn181, Thr183, Trp185

of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or its/their equivalent
15 positions in other homologous xylanase polypeptides.

Thus, in one embodiment, the present invention provides a variant xylanase polypeptide, or fragment thereof having xylanase activity, comprising one or more amino acid modifications at any one of amino acid residues numbers:

20

11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175

of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent
positions in other homologous xylanase polypeptides.

25

In one embodiment, the present invention provides a variant xylanase polypeptide or fragment thereof having xylanase activity, comprising one or more amino acid modifications at any one of amino acid residues numbers 11, 12 and 13 of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent positions in other
30 homologous xylanase polypeptides.

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Specific preferred examples of modifications made are presented in the Examples section herein.

For some embodiments, preferably the variant xylanase polypeptide, or fragment thereof
5 having xylanase activity, comprises one or more amino acid modifications at any one of amino acid residues numbers: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 30, 31, 32, 33, 34, 35, 36, 37, 61, 62, 63, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 173, 174, 175, 176, 177, 178 of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent positions in other homologous xylanase
10 polypeptides.

For convenience, we sometimes refer to the amino acid residues numbers: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 30, 31, 32, 33, 34, 35, 36, 37, 61, 62, 63, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 173, 174, 175, 176, 177, 178 as
15 BAND 1.

Figure 1 shows the 3-D structure of *B. subtilis* xylanase having the amino acid sequence shown as SEQ I.D. No. 1. BAND 1 is depicted in Figure 1 as the upper layer of the molecule and extends approximately 13 Ångstroms from the top of the molecule when the
20 molecule is orientated as shown in Figure 1. BAND 1 ends with the residue Phe 125 on the left hand side when viewing Figure 1 and with the residue Asn 61 on the right hand side when viewing Figure 1.

In addition, or in the alternative, for some embodiments, preferably the variant xylanase
25 polypeptide, or fragment thereof having xylanase activity, comprises one or more amino acid modifications at any one of the other amino acid residues.

Preferably said other modifications may occur at any one or more of amino acid residues numbers: 3, 4, 5, 6, 7, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 38, 39, 40, 41, 42, 43, 44, 45,
30 55, 56, 57, 58, 59, 60, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 108, 109, 110, 126, 127, 128, 129, 130, 131, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 179, 180,

181, 182, 183 of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent positions in other homologous xylanase polypeptides.

For convenience, we sometimes refer to the amino acid residues numbers: 3, 4, 5, 6, 7, 19,
20, 21, 22, 23, 24, 25, 26, 27, 28, 38, 39, 40, 41, 42, 43, 44, 45, 55, 56, 57, 58, 59, 60, 64,
65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88,
89, 90, 91, 92, 93, 94, 108, 109, 110, 126, 127, 128, 129, 130, 131, 158, 159, 160, 161,
162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 179, 180, 181, 182, 183 of the *B. subtilis* amino acid sequence shown as BAND 2.

Preferably said other modifications may occur at any one or more of the surface amino acid residues numbers: 3, 4, 5, 6, 19, 20, 21, 22, 23, 25, 27, 43, 44, 56, 57, 58, 59, 60, 73, 74, 75, 76, 87, 89, 91, 92, 93, 94, 109, 110, 126, 159, 160, 162, 163, 164, 179, 181, 183 of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent positions in other homologous xylanase polypeptides.

Preferably, the present invention encompasses a variant xylanase polypeptide, or fragment thereof having xylanase activity, which comprises one or more amino acid modifications in BAND 1 and optionally/or BAND 2 of the *B. subtilis* amino acid sequence or their equivalent positions (bands) in other homologous xylanase polypeptides. Hence, the modification is in at least BAND 1; but could be in just BAND 2 alone.

The variant xylanase polypeptide may comprise other modifications in other amino acid residues, such as modification at any one of amino acid residues: 1, 2, 46, 47, 48, 49, 50, 51, 52, 53, 54, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 184, 185 of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent positions in other homologous xylanase polypeptides.

The variant xylanase polypeptide may comprise other modifications in other surface amino acid residues, such as modification at any one of the surface amino acid residues: 1, 2, 46, 47, 48, 49, 50, 52, 54, 95, 97, 99, 101, 102, 104, 133, 134, 135, 136, 137, 138,

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139, 140, 141, 143, 145, 147, 148, 151, 154, 155, 156, 157, 185 of the *B. subtilis* amino acid sequence shown as SEQ I.D. No. 1 or their equivalent positions in other homologous xylanase polypeptides.

- 5 Preferably, the inhibitor is an inhibitor found naturally in plant tissues. Preferably the sensitivity of the variant xylanase enzyme to the inhibitor is reduced as compared to the parent xylanase enzyme.

10 The present invention also provides a nucleic acid molecule (a nucleotide sequence) encoding a polypeptide of the invention. Also provided is a vector comprising a nucleic acid of the invention, optionally operably linked to a regulatory sequence capable of directing expression of said nucleic acid in a suitable host cell. A host cell comprising a nucleic acid or a vector of the invention is also provided.

- 15 In another aspect the present invention provides a method of making a polypeptide of the invention comprising transforming a host cell with a nucleic acid encoding said polypeptide, culturing the transformed cell and expressing said polypeptide.

20 Our results show that these variant polypeptides have improved properties that make them suitable for a variety of applications, such as bakery, animal feed, starch production, flour separation (wetmilling) and, paper and pulp production.

Accordingly, the present invention also provides the use of a variant polypeptide of the invention in a method of modifying plant materials.

25

Also provided is the use of a variant polypeptide of the invention in baking. The invention further provides the use of a variant polypeptide of the invention in processing cereals, starch production and animal feed and the use of a variant polypeptide of the invention in processing wood, for example in enhancing the bleaching of wood pulp.

30

In a further aspect, the present invention provides a method of altering the sensitivity of a xylanase polypeptide to an inhibitor which method comprises modifying one or more

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amino acid residues of said enzyme selected from amino acid numbers 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 based on the amino acid numbering of *B. subtilis* xylanase shown as SEQ ID No. 1, or the equivalent residues in
5 other homologous xylanase polypeptides. Preferably the sensitivity is reduced.

In accordance with another aspect of the present invention there is provided a method of altering the sensitivity of a xylanase polypeptide to an inhibitor, which method comprises modifying one or more amino acid residues of said polypeptide; and measuring the
10 sensitivity of said xylanase polypeptide to a xylanase inhibitor; such that the polypeptide or fragment thereof has altered sensitivity to a xylanase inhibitor as compared with the parent xylanase enzyme, wherein said xylanase has at least one of said amino acids modifications at any one of position 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 or 175 of the *B. subtilis* amino acid sequences shown as SEQ. ID. No.1, or at equivalent
15 position(s) in other homologous xylanases, wherein at least one of said amino acids modifications is at least at position 11, 12 or 13 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1 or at equivalent position(s) in other homologous xylanases.

In accordance with another aspect of the invention, there is provided a method of altering
20 the sensitivity of a xylanase polypeptide to an inhibitor, which method comprises modifying two or more amino acid residues of said polypeptide; and optionally, measuring the sensitivity of said xylanase polypeptide to a xylanase inhibitor; such that the polypeptide or fragment thereof has altered sensitivity to a xylanase inhibitor as compared with the parent xylanase enzyme, wherein said modifications are to two or more of positions 11, 12, 13, 15,
25 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other homologous xylanases, wherein one of said amino acid modifications is at position 11.

In accordance with another aspect of the present invention, there is provided a method a
30 method of altering the sensitivity of a xylanase polypeptide to an inhibitor, which method comprises modifying two or more amino acid residues of said polypeptide; and optionally, measuring the sensitivity of said xylanase polypeptide to a xylanase inhibitor; such that the polypeptide or fragment thereof has altered sensitivity to a xylanase inhibitor as compared

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with the parent xylanase enzyme, wherein said modifications are to two or more of positions 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other homologous xylanases, wherein said amino acid modifications are carried out by site-directed mutagenesis, and wherein one of said amino acid modifications is at position 11, 12 or 13.

In accordance with another aspect of the present invention, there is provided a variant polypeptide or fragment thereof having xylanase activity, comprising two or more amino acid modifications, wherein said amino acid modifications are at two or more of positions 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other homologous xylanases, such that the polypeptide or fragment thereof has altered sensitivity as compared with the parent xylanase, wherein one of said amino acid modifications is at position 11 and wherein said amino acids modifications are any of: D11Y, D11N, D11F, D11K, D11S, D11W, G12F, G13F, I15K, N17K, N17Y, N17D, N29K, N29Y, N29D, S31K, S31Y, S31D, N32K, G34D, G34F, G34T, Y113A, Y113D, Y113K, N114A, N114D, N114F, N114K, D119K, D119Y, D119N, G120K, G120D, G120F, G120Y, G120N, D121N, D121K, D121F, D121A, R122D, R122F, R122A, T123K, T123Y, T123D, T124K, T124Y, T124D, Q175E, Q175S and Q175L.

In accordance with another aspect of the present invention there is provided, a variant polypeptide or fragment thereof having xylanase activity, comprising two or more amino acid modifications, wherein said amino acid modifications are at two or more of positions 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other homologous xylanases, such that the polypeptide or fragment thereof has altered sensitivity as compared with the parent xylanase, wherein said amino acid modifications are introduced by site-directed mutagenesis and wherein at least one of said amino acid modifications is at position 11, 12 or 13 and wherein said amino acids modifications are any of: D11Y, D11N, D11F, D11K, D11S, D11W, G12F, G13F, I15K, N17K, N17Y, N17D, N29K, N29Y, N29D, S31K, S31Y, S31D, N32K, G34D, G34F, G34T, Y113A, Y113D, Y113K, N114A,

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N114D, N114F, N114K, D119, D119Y, D119N, G120K, G120D, G120F, G120Y, G120N, D121N, D121K, D121F, D121A, R122D, R122F, R122A, T123K, T123Y, T123D, T124K, T124Y, T124D, Q175E, Q175S and Q175L.

- 5 In accordance with another aspect of the present invention, there is provided a composition comprising a variant polypeptide described herein.

In accordance with another aspect of the present invention, there is provided a method of degrading or modifying a plant cell wall which method comprises contacting said plant wall
10 with a polypeptide described herein or a composition described herein.

In accordance with another aspect of the present invention, there is provided a method of processing a plant material which method comprises contacting said plant wall with a polypeptide described herein or a composition described here.

15

In accordance with another aspect of the present invention there is provided a nucleotide sequence encoding a variant polypeptide described herein.

In accordance with another aspect of the present invention there is provided a construct
20 comprising a nucleotide sequence described herein.

In accordance with another aspect of the present invention there is provided use of a variant polypeptide described herein in a method of modifying plant materials.

- 25 In accordance with another aspect of the present invention there is provided use of a variant polypeptide described herein in one or more of: baking, processing cereals, starch production, in processing wood, enhancing the bleaching process of wood pulp.

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Importantly, our results also show for the first time that xylanase inhibitors play an important role in determining the functionality of xylanase enzymes in a complex system, such as a plant material. By the term "functionality", we mean the biochemical properties of the xylanase in a given system. These properties include substrate specificity, K_m and V_{max} kinetic parameters (where appropriate) and the nature of the reaction products obtained by the action of the xylanase in that system. Functionality may also consequently be described in terms of the effect on the physical and/or chemical properties of the plant materials on which the xylanase acts, for example the extent to which the viscosity of the material is altered.

In the same way that variant xylanases may be used in a variety of processing applications, xylanase inhibitors may be used in a variety of processing applications such as bakery, wood pulp processing and cereal processing.

Detailed description of the invention

Although in general any molecular techniques mentioned herein are well known in the art, reference may be made in particular to Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (1989) and Ausubel *et al.*, Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc.

A. Variant xylanase polypeptides

Xylanase enzymes have been reported from nearly 100 different organisms, including plants, fungi and bacteria. The xylanase enzymes are classified into several of the more than 40 families of glycosyl hydrolase enzymes. The glycosyl hydrolase enzymes, which include xylanases, mannanases, amylases, β -glucanases, cellulases, and other

carbohydrases, are classified based on such properties as the sequence of amino acids, the three dimensional structure and the geometry of the catalytic site (Gilkes, et al., 1991, Microbiol. Reviews 55: 303-315).

- 5 Of particular interest for baking applications are the enzymes classified in Family 11. All of these are xylanases and are known as the "Family 11 xylanases". Some publications refer to these synonymously as the Family G xylanases, but the term "Family 11 xylanases" will be used herein to refer to both Family G and Family 11 xylanases.
- 10 Table A lists a number of known Family 11 xylanases. Most of them have a molecular mass of about 21,000 Da. Three of the Family 11 xylanases (*Clostridium stercorarium* XynA, *Streptomyces lividans* XynB, and *Thermomonospora fusca* XynA) have a higher molecular mass of 31,000 to 50,000 Da. However, these xylanases have a catalytic core sequence of about 21,000 Da similar to the other Family 11 xylanases. The amino acid
- 15 sequences of the Family 11 xylanases (or, for the larger enzymes, the catalytic core) show a high degree of similarity, usually with more than 40 % identical amino acids in a proper amino acid alignment. The Family 11 xylanases, which are of bacterial, yeast, or fungal origin, share the same general molecular structure.
- 20 Figure 2 shows amino acid sequence alignment data in respect of 51 Family 11 xylanases.

TABLE A - Family 11 xylanases

<i>Aspergillus niger</i> Xyn A	<i>Aspergillus kawachii</i> Xyn C
<i>Aspergillus tubigenensis</i> Xyn A	<i>Bacillus circulans</i> Xyn A
<i>Bacillus pumilus</i> Xyn A	<i>Bacillus subtilis</i> Xyn A
<i>Cellulomonas fimi</i> Xyn D	<i>Chainia</i> spp. Xyn
<i>Clostridium acetobutylicum</i> Xyn B	<i>Clostridium stercorarium</i> Xyn A
<i>Fibrobacter succinogenes</i> Xyn C	<i>Neocallimastix patriciarum</i> Xyn A
<i>Nocardiopsis dassonvillei</i> Xyn II	<i>Ruminococcus flavefaciens</i> Xyn A

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<i>Schizophyllum commune</i> Xyn	<i>Streptomyces lividans</i> Xyn B
<i>Streptomyces lividans</i> Xyn C	<i>Streptomyces</i> sp. No. 36a Xyn
<i>Streptomyces thermoviolaceus</i> Xyn II	<i>Thermomonospora fusca</i> Xyn A
<i>Trichoderma harzianum</i> Xyn	<i>Trichoderma reesei</i> Xyn I
<i>Trichoderma reesei</i> Xyn II	<i>Trichoderma viride</i> Xyn

Variant xylanases of the invention

A variant xylanase polypeptide of the invention is typically obtained by modifying a xylanase polypeptide by substituting, deleting or adding one or more amino acid residues within the amino acid sequence of the xylanase polypeptide. Preferably the modification comprises one or more amino acid substitutions. Modification of polypeptide sequences can be carried out using standard techniques such as site directed mutagenesis. The modification may also occur by chemical techniques – such as chemical modification of one or more amino acid residues.

The starting sequence may be a wild type sequence or a non-naturally occurring sequence, for example a derivative that has already been subjected to protein engineering. The xylanase sequence to be modified may be from any source, for example a bacterial, fungal or plant source. Preferably the xylanase sequence to be modified is that of a Family 11 xylanase, more preferably a Family 11 xylanase selected from *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma harzianum* xylanase, *Trichoderma viride* xylanase, *Bacillus circulans* xylanase A, *Bacillus subtilis* xylanase A, *Aspergillus niger* xylanase A, *Aspergillus kawachii* xylanase C, *Aspergillus tubigenensis* xylanase A, *Streptomyces lividans* xylanase B, and *Streptomyces lividans* xylanase C.

In a particularly preferred embodiment, the xylanase sequence to be modified is the *B subtilis* xylanase sequence shown as SEQ ID No. 1 or a homologue thereof. Preferably said homologue has at least 40, 50, 60 or 80% homology over at least 50 or 100 amino acid residues as determined using the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.*, 1984, Nucleic Acids Research 12:387).

Specific modifications that are preferred according to the present invention include one or more amino acid substitutions at positions 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 based on the amino acid numbering of *B. subtilis* xylanase shown as SEQ ID No. 1, or the equivalent residues in other homologous xylanase polypeptides.

Particularly preferred substitutions include one or more of D11 $\rightarrow$ Y, D11 $\rightarrow$ N, D11 $\rightarrow$ F, D11 $\rightarrow$ K, D11 $\rightarrow$ S, D11 $\rightarrow$ W, G12 $\rightarrow$ F, G13 $\rightarrow$ F, I15 $\rightarrow$ K, N17 $\rightarrow$ K, N17 $\rightarrow$ Y, N17 $\rightarrow$ D, N29 $\rightarrow$ K, N29 $\rightarrow$ Y, N29 $\rightarrow$ D, S31 $\rightarrow$ K, S31 $\rightarrow$ Y, S31 $\rightarrow$ D, N32 $\rightarrow$ K, G34 $\rightarrow$ D, G34 $\rightarrow$ F, G34 $\rightarrow$ T, Y113 $\rightarrow$ A, Y113 $\rightarrow$ D, Y113 $\rightarrow$ K, N114 $\rightarrow$ A, N114 $\rightarrow$ D, N114 $\rightarrow$ F, N114 $\rightarrow$ K, D119 $\rightarrow$ K, D119 $\rightarrow$ Y, D119 $\rightarrow$ N, G120 $\rightarrow$ K, G120 $\rightarrow$ D, G120 $\rightarrow$ F, G120 $\rightarrow$ Y, G120 $\rightarrow$ N, D121 $\rightarrow$ N, D121 $\rightarrow$ K, D121 $\rightarrow$ F, D121 $\rightarrow$ A, R122 $\rightarrow$ D, R122 $\rightarrow$ F, R122 $\rightarrow$ A, T123 $\rightarrow$ K, T123 $\rightarrow$ Y, T123 $\rightarrow$ D, T124 $\rightarrow$ K, T124 $\rightarrow$ Y, T124 $\rightarrow$ D, Q175 $\rightarrow$ E, Q175 $\rightarrow$ S and Q175 $\rightarrow$ L (with reference to the amino acid sequence of *B. subtilis* xylanase) or their equivalents in other homologous xylanase polypeptides. Further references to specific residues of the *B. subtilis* xylanase shown as SEQ ID No. 1 will also include their equivalents in other homologous xylanase polypeptides.

A combination of mutations may be carried out, for example mutations at two or more of the above-mentioned residues. Examples of such combinations are presented in the Examples section herein.

In a further embodiment, the variant polypeptides of the invention may be purified and isolated naturally occurring mutant xylanases. Alternatively, mutant xylanases may be generated by subjecting organisms to mutagens and then screening for individuals comprising mutations in their xylanase genes. Naturally occurring mutants and mutants generated by random mutagenesis may be identified/screened using a variety of techniques such as PCR screening using suitable nucleic acid primers to amplify regions of xylanase genes and sequencing the resulting fragments.

30

Thus variant polypeptides of the invention include naturally occurring mutant xylanases (purified and isolated from the organisms in which they occur or obtained recombinantly),

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mutant xylanases obtained by random mutagenesis and mutant xylanases obtained by site-directed mutagenesis.

Variant polypeptides of the invention may also be subjected to further modifications that do not necessarily affect sensitivity to inhibitors, including any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acids from or to the sequence providing the resultant amino acid sequence retains xylanase activity, preferably having at least substantially the same xylanase activity as the unmodified sequence.

10

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

15

Polypeptides of the invention also include fragments of the full length sequences mentioned above having xylanase activity.

Polypeptides of the invention may further comprise heterologous amino acid sequences, typically at the N-terminus or C-terminus, preferably the N-terminus. Heterologous sequence may include sequences that affect intra or extracellular protein targeting (such as leader sequences).

Polypeptides of the invention are typically made by recombinant means, for example as described below. However they may also be made by synthetic means using techniques

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well known to skilled persons such as solid phase synthesis. Polypeptides of the invention may also be produced as fusion proteins, for example to aid in extraction and purification. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion
5 protein sequences, such as a thrombin cleavage site. Preferably the fusion protein will not hinder the function of the protein sequence of interest.

The use of appropriate host cells is expected to provide for such post-translational modifications as may be needed to confer optimal biological activity on recombinant
10 expression products of the invention.

Polypeptides of the invention may be in a substantially isolated form. It will be understood that the protein may be mixed with carriers or diluents which will not interfere with the intended purpose of the protein and still be regarded as substantially isolated. A
15 polypeptide of the invention may also be in a substantially purified form, in which case it will generally comprise the protein in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the protein in the preparation is a polypeptide of the invention.

Variant polypeptides of the invention have altered sensitivity to xylanase inhibitors
20 compared to the parent xylanase sequence – which may be a corresponding wild type xylanase. Preferably, variant polypeptides have reduced sensitivity to xylanase inhibitors. The term “altered sensitivity to xylanase inhibitors” means that extent to which the endo- β -1,4-xylanase activity of a variant polypeptide of the invention is inhibited by the xylanase inhibitor is different to that of the parent xylanase enzyme – which may be the
25 corresponding wild type xylanase. Preferably the extent to which the variant polypeptide is inhibited by the inhibitor is less than that of the parent xylanase enzyme – which may be the wild type protein. This may, for example, be due to a change in the three-dimensional structure of the variant polypeptide such that the inhibitor no longer binds with the same affinity as it does to the parent xylanase enzyme – which may be the wild
30 type enzyme.

The sensitivity of the variant polypeptides of the invention to xylanase inhibitors can be assayed using, for example, the assay described in example 4 and below. A suitable

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inhibitor for use in the assay is the inhibitor purified from wheat flour in example 1. Other inhibitors are described below.

Xylanase assay (Endo- β -1,4-Xylanase activity)

5

Xylanase samples are diluted in citric acid (0.1 M) - *di*-sodium-hydrogen phosphate (0.2 M) buffer, pH 5.0, to obtain approx. OD = 0.7 in the final assay. Three dilutions of the sample and an internal standard with a defined activity are thermostated for 5 minutes at 40°C. At time = 5 minutes, 1 Xylazyme tab (crosslinked, dyed xylan substrate) is added to the enzyme solution. At time = 15 minutes (or in some cases longer, depending on the xylanase activity present in the sample) the reaction is terminated, by adding 10 ml of 2% TRIS. The reaction mixture is centrifuged and the OD of the supernatant is measured at 590 nm. Taking into account the dilutions and the amount of xylanase, the activity (TXU, Total-Xylanase-Units) of the sample can be calculated relative to the standard.

15

Xylanase inhibitors

As used herein, the term "xylanase inhibitor" refers to a compound, typically a protein, whose role is to control the depolymerisation of complex carbohydrates, such as arabinoxylan, found in plant cell walls. These xylanase inhibitors are capable of reducing the activity of naturally occurring xylanase enzymes as well as those of fungal or bacterial origin. Although the presence of xylanase inhibitors have been reported in cereal seeds (see for example McLauchlan et al 1999a; Rouau and Suget 1998) their impact on the efficacy of xylanase enzymes has not been extensively examined.

25

McLauchlan et al (1999a) disclose the isolation and characterisation of a protein from wheat that binds to and inhibits two family-11 xylanases. Likewise, WO 98/49278 demonstrates the effect of a wheat flour extract on the activity of a group of microbial xylanases all of which are classified as family 11 xylanases. Debyser et al. (1999) also disclose that endoxylanases from *Aspergillus niger* and *Bacillus subtilis*, which are both members of the family 11 xylanases were inhibited by a wheat xylanase inhibitor called

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TAXI. McLauchlan *et al* (1999b) teach that extracts from commercial flours such as wheat, barley, rye and maize are capable of inhibiting both family 10 and 11 xylanases.

The xylanase inhibitor may be any suitable xylanase inhibitor. By way of example, the xylanase inhibitor may be the inhibitor described in WO-A-98/49278 and/or the xylanase inhibitor described by Rouau, X. and Surget, A. (1998), McLauchlan, R., et al. (1999) and/or the xylanase inhibitor described in UK patent application number 9828599.2 (filed 23 December 1998), UK patent application number 9907805.7 (filed 6 April 1999) and UK patent application number 9908645.6 (filed 15 April 1999).

Xylanase inhibitor assay

100 µl of an candidate inhibitor fraction, 250 µl xylanase solution (containing 12 TXU microbial xylanase/ml) and 650 µl buffer (0.1 M citric acid - 0.2M *di*-sodium hydrogen phosphate buffer, pH 5.0) are mixed. The mixture is thermostated for 5 minutes at 40.0°C. At time = 5 minutes one Xylazyme tab is added. At time = 15 minutes the reaction is terminated by adding 10 ml 2% TRIS. The reaction mixture is centrifuged (3500 g, 10 minutes, room temperature) and the supernatant is measured at 590 nm. The inhibition is calculated as residual activity compared to the blank. The blank is prepared the same way, except that the 100 µl inhibitor is substituted with 100 µl buffer (0.1 M citric acid - 0.2 M *di*-sodium hydrogen phosphate buffer, pH 5.0).

Specific xylanase inhibitor

As indicated, a xylanase inhibitor that may be used in accordance with the present invention is the xylanase inhibitor described in UK patent application number 9828599.2 (filed 23 December 1998), UK patent application number 9907805.7 (filed 6 April 1999) and UK patent application number 9908645.6 (filed 15 April 1999).

This endogenous endo-β-1,4-xylanase inhibitor is obtainable from wheat flour. The inhibitor is a di-peptide, having a MW of about 40 kDa (as measured by SDS-PAGE or mass spectrometry) and a pI of about 8 to about 9.5.

Sequence analysis to date has revealed the that the inhibitor has at least one or more of the sequences presented as SEQ ID No. 2, SEQ ID No. 3, SEQ ID No 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7 and/or SEQ ID No. 8.

5

These inhibitors described in the prior art may also be used in assays to determine the sensitivity of a variant polypeptide of the invention to xylanase inhibitors. They may also be used as described below to modulate the functionality of a xylanase.

10 Polynucleotides

Polynucleotides of the invention comprise nucleic acid sequences encoding the variant polypeptide sequences of the invention. It will be understood by a skilled person that numerous different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides of the invention to reflect the codon usage of any particular host organism in which the polypeptides of the invention are to be expressed.

Polynucleotides of the invention may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides of the invention.

30 Nucleotide vectors and host cells

Polynucleotides of the invention can be incorporated into a recombinant replicable vector.

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The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, the invention provides a method of making polynucleotides of the invention by introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under
5 conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells include bacteria such as *E. coli*, yeast and fungi.

Preferably, a polynucleotide of the invention in a vector is operably linked to a regulatory sequence which is capable of providing for the expression of the coding sequence by the
10 host cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. The term "regulatory sequences"
15 includes promoters and enhancers and other expression regulation signals.

Enhanced expression of the polynucleotide encoding the polypeptide of the invention may also be achieved by the selection of heterologous regulatory regions, e.g. promoter, secretion leader and terminator regions, which serve to increase expression and, if desired,
20 secretion levels of the protein of interest from the chosen expression host and/or to provide for the inducible control of the expression of the polypeptide of the invention.

Aside from the promoter native to the gene encoding the polypeptide of the invention, other promoters may be used to direct expression of the polypeptide of the invention. The
25 promoter may be selected for its efficiency in directing the expression of the polypeptide of the invention in the desired expression host.

In another embodiment, a constitutive promoter may be selected to direct the expression of the desired polypeptide of the invention. Examples of strong constitutive and/or
30 inducible promoters which are preferred for use in fungal expression hosts are those which are obtainable from the fungal genes for xylanase (*xlnA*), phytase, ATP-synthetase, subunit 9 (*oliC*), triose phosphate isomerase (*tpi*), alcohol dehydrogenase (*AdhA*),

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α -amylase (*amy*), amyloglucosidase (AG - from the *glaA* gene), acetamidase (*amdS*) and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) promoters.

5 Examples of strong yeast promoters are those obtainable from the genes for alcohol dehydrogenase, lactase, 3-phosphoglycerate kinase and triosephosphate isomerase.

Examples of strong bacterial promoters are the α -amylase and *SP02* promoters as well as promoters from extracellular protease genes.

10 Hybrid promoters may also be used to improve inducible regulation of the expression construct.

Often, it is desirable for the polypeptide of the invention to be secreted from the expression host into the culture medium from where the polypeptide of the invention may
15 be more easily recovered. According to the present invention, the polypeptide of the invention's native secretion leader sequence may be used to effect the secretion of the expressed polypeptide of the invention. However, an increase in the expression of the polypeptide of the invention sometimes results in the production of the protein in levels beyond that which the expression host is capable of processing and secreting, creating a
20 bottleneck such that the protein product accumulates within the cell. Accordingly, the present invention also provides heterologous leader sequences to provide for the most efficient secretion of the polypeptide of the invention from the chosen expression host.

According to the present invention, the secretion leader may be selected on the basis of
25 the desired expression host. A heterologous secretion leader may be chosen which is homologous to the other regulatory regions of the expression construct. For example, the leader of the highly secreted amyloglucosidase (AG) protein may be used in combination with the amyloglucosidase (AG) promoter itself, as well as in combination with other promoters. Hybrid signal sequences may also be used with the context of the present
30 invention.

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Examples of preferred heterologous secretion leader sequences are those originating from the fungal amyloglucosidase (AG) gene (*glaA* - both 18 and 24 amino acid versions e.g. from *Aspergillus*), the α -factor gene (yeasts e.g. *Saccharomyces* and *Kluyveromyces*) or the α -amylase gene (*Bacillus*).

5

Such vectors may be transformed into a suitable host cell as described above to provide for expression of a polypeptide of the invention. Thus, in a further aspect the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell transformed or transfected with an expression vector as described
10 above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides. Suitable host cells include, for example, fungal cells, such as *Aspergillus* and yeast cells, such as yeast cells of the genus *Kluyveromyces* or *Saccharomyces*. Other suitable host cells are discussed below.

15

The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes. The most suitable selection systems for industrial micro-organisms are
20 those formed by the group of selection markers which do not require a mutation in the host organism. Examples of fungal selection markers are the genes for acetamidase (*amdS*), ATP synthetase, subunit 9 (*oliC*), orotidine-5'-phosphate-decarboxylase (*pvrA*), phleomycin and benomyl resistance (*benA*). Examples of non-fungal selection markers are the bacterial G418 resistance gene (this may also be used in yeast, but not in fungi),
25 the ampicillin resistance gene (*E. coli*), the neomycin resistance gene (*Bacillus*) and the *E. coli uidA* gene, coding for β -glucuronidase (GUS). Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell.

A further embodiment of the invention provides host cells transformed or transfected with
30 a polynucleotide of the invention. Preferably said polynucleotide is carried in a vector for the replication and expression of said polynucleotides. The cells will be chosen to be

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compatible with the said vector and may for example be prokaryotic (for example bacterial), fungal, yeast or plant cells.

Bacteria from the genus *Bacillus* are very suitable as heterologous hosts because of their capability to secrete proteins into the culture medium. Other bacteria suitable as hosts are those from the genera *Streptomyces* and *Pseudomonas*.

Depending on the nature of the polynucleotide encoding the polypeptide of the invention, and/or the desirability for further processing of the expressed protein, eukaryotic hosts such as yeasts or fungi may be preferred. In general, yeast cells are preferred over fungal cells because they are easier to manipulate. However, some proteins are either poorly secreted from the yeast cell, or in some cases are not processed properly (e.g. hyperglycosylation in yeast). In these instances, a fungal host organism should be selected.

15

A heterologous host may also be chosen wherein the polypeptide of the invention is produced in a form which is substantially free from other xylanases. This may be achieved by choosing a host which does not normally produce such enzymes.

Examples of preferred expression hosts within the scope of the present invention are fungi such as *Aspergillus* species and *Trichoderma* species; bacteria such as *Bacillus* species, *Streptomyces* species and *Pseudomonas* species; and yeasts such as *Kluyveromyces* species and *Saccharomyces* species.

Particularly preferred expression hosts may be selected from *Aspergillus niger*, *Aspergillus niger* var. *tubigenis*, *Aspergillus niger* var. *awamori*, *Aspergillus aculeatis*, *Aspergillus nidulans*, *Aspergillus oryzae*, *Trichoderma reesei*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Kluyveromyces lactis* and *Saccharomyces cerevisiae*.

30

According to the present invention, the production of the polypeptide of the invention can be effected by the culturing of microbial expression hosts, which have been transformed

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with one or more polynucleotides of the present invention, in a conventional nutrient fermentation medium.

The fermentation medium can comprise a known culture medium containing a carbon
5 source (e.g. glucose, maltose, molasses, etc.), a nitrogen source (e.g. ammonium sulphate, ammonium nitrate, ammonium chloride, etc.), an organic nitrogen source (e.g. yeast extract, malt extract, peptone, etc.) and inorganic nutrient sources (e.g. phosphate, magnesium, potassium, zinc, iron, etc.). Optionally, an inducer may be added.

10 The selection of the appropriate medium may be based on the choice of expression hosts and/or based on the regulatory requirements of the expression construct. Such media are well-known to those skilled in the art. The medium may, if desired, contain additional components favouring the transformed expression hosts over other potentially contaminating microorganisms.

15

After fermentation, the cells can be removed from the fermentation broth by means of centrifugation or filtration. After removal of the cells, the variant polypeptide of the invention may then be recovered and, if desired, purified and isolated by conventional means.

20

Organisms

The term "organism" in relation to the present invention includes any organism that could comprise the nucleotide sequence coding for the variant xylanase protein according to the
25 present invention and/or products obtained therefrom, wherein a transcriptional regulatory sequence can allow expression of the nucleotide sequence according to the present invention when present in the organism. Suitable organisms may include a prokaryote, fungus, yeast or a plant. For the xylanase aspect of the present invention, a preferable organism may be a bacterium, preferably of the genus *Bacillus*, more preferably *Bacillus subtilis*.

30

The term "transgenic organism" in relation to the present invention includes any organism that comprises the nucleotide sequence coding for the protein according to the present

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invention and/or products obtained therefrom, wherein the transcriptional regulatory sequence can allow expression of the nucleotide sequence according to the present invention within the organism. Preferably the nucleotide sequence is incorporated in the genome of the organism.

5

The term "transgenic organism" does not cover native nucleotide coding sequences in their natural environment when they are under the control of their native promoter which is also in its natural environment.

10 Therefore, the transgenic organism of the present invention includes an organism comprising any one of, or combinations of, the nucleotide sequence coding for the amino acid sequence according to the present invention, constructs according to the present invention (including combinations thereof), vectors according to the present invention, plasmids according to the present invention, cells according to the present invention, tissues
15 according to the present invention or the products thereof. The transformed cell or organism could prepare acceptable quantities of the desired compound which would be easily retrievable from, the cell or organism.

Transformation of Host Cells/Host Organisms

20

As indicated earlier, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook et al (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring
25 Harbor Laboratory Press) and Ausubel *et al.*, Short Protocols in Molecular Biology (1999), 4th Ed., John Wiley & Sons, Inc.

If a prokaryotic host is used then the nucleotide sequence may need to be suitably modified before transformation - such as by removal of introns.

30

As mentioned above, a preferred host organism is of the genus *Bacillus*, such as *Bacillus subtilis*.

In another embodiment the transgenic organism can be a yeast. In this regard, yeasts have also been widely used as a vehicle for heterologous gene expression. The species *Saccharomyces cerevisiae* has a long history of industrial use, including its use for
5 heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey et al (1987, Yeast Biotechnology, D R Berry et al, eds, pp 401-429, Allen and Unwin, London) and by King et al (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

10 For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of
15 knowledge about the genetics and physiology as well as large-scale fermentation characteristics of *Saccharomyces cerevisiae*.

A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for
20 the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating
25 plasmid vectors.

In order to prepare the transgenic *Saccharomyces*, expression constructs are prepared by inserting the nucleotide sequence of the present invention into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been
30 developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence of the present invention, usually a promoter of yeast origin, such as the GAL1

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promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic *Saccharomyces* according to the present invention can be prepared by following the teachings of Hinnen et al (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D (1978, Nature, London, 275, 104); and Ito, H et al (1983, J Bacteriology 153, 163-168).

10 The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, e.g. G418.

15 Another host organism is a plant. The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

A transgenic plant of the invention may be produced from any plant such as the seed-bearing plants (angiosperms), and conifers. Angiosperms include dicotyledons and monocotyledons. Examples of dicotyledonous plants include tobacco, (*Nicotiana plumbaginifolia* and *Nicotiana tabacum*), arabidopsis (*Arabidopsis thaliana*), *Brassica napus*, *Brassica nigra*, *Datura innoxia*, *Vicia narbonensis*, *Vicia faba*, pea (*Pisum sativum*), cauliflower, carnation and lentil (*Lens culinaris*). Examples of monocotyledonous plants include cereals such as wheat, barley, oats and maize.

Techniques for producing transgenic plants are well known in the art. Typically, either whole plants, cells or protoplasts may be transformed with a suitable nucleic acid construct encoding a zinc finger molecule or target DNA (see above for examples of nucleic acid constructs). There are many methods for introducing transforming DNA constructs into cells, but not all are suitable for delivering DNA to plant cells. Suitable methods include *Agrobacterium* infection (see, among others, Turpen et al., 1993, J.

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Virol. Methods, 42: 227-239) or direct delivery of DNA such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles. Acceleration methods are generally preferred and include, for example, microprojectile bombardment. A typical protocol for producing transgenic plants (in particular
5 monocotyledons), taken from U.S. Patent No. 5, 874, 265, is described below.

An example of a method for delivering transforming DNA segments to plant cells is microprojectile bombardment. In this method, non-biological particles may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles
10 include those comprised of tungsten, gold, platinum, and the like.

A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly stably transforming both dicotyledons and monocotyledons, is that neither the isolation of protoplasts nor the susceptibility to
15 *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into plant cells by acceleration is a Biolistics Particle Delivery System, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or NytexTM screen, onto a filter surface covered with plant cells cultured in suspension. The screen disperses the tungsten-DNA particles so that they are not
20 delivered to the recipient cells in large aggregates. It is believed that without a screen intervening between the projectile apparatus and the cells to be bombarded, the projectiles aggregate and may be too large for attaining a high frequency of transformation. This may be due to damage inflicted on the recipient cells by projectiles that are too large.

25 For the bombardment, cells in suspension are preferably concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the macroprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more clusters of cells transiently expressing a marker
30 gene ("foci") on the bombarded filter. The number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from 1 to 10 and average 2 to 3.

After effecting delivery of exogenous DNA to recipient cells by any of the methods discussed above, a preferred step is to identify the transformed cells for further culturing and plant regeneration. This step may include assaying cultures directly for a screenable
5 trait or by exposing the bombarded cultures to a selective agent or agents.

An example of a screenable marker trait is the red pigment produced under the control of the R-locus in maize. This pigment may be detected by culturing cells on a solid support containing nutrient media capable of supporting growth at this stage, incubating the cells
10 at, e.g., 18°C and greater than $180 \mu\text{E m}^{-2} \text{ s}^{-1}$, and selecting cells from colonies (visible aggregates of cells) that are pigmented. These cells may be cultured further, either in suspension or on solid media.

An exemplary embodiment of methods for identifying transformed cells involves
15 exposing the bombarded cultures to a selective agent, such as a metabolic inhibitor, an antibiotic, herbicide or the like. Cells which have been transformed and have stably integrated a marker gene conferring resistance to the selective agent used, will grow and divide in culture. Sensitive cells will not be amenable to further culturing.

20 To use the bar-bialaphos selective system, bombarded cells on filters are resuspended in nonselective liquid medium, cultured (e.g. for one to two weeks) and transferred to filters overlaying solid medium containing from 1-3 mg/l bialaphos. While ranges of 1-3 mg/l will typically be preferred, it is proposed that ranges of 0.1-50 mg/l will find utility in the practice of the invention. The type of filter for use in bombardment is not believed to be
25 particularly crucial, and can comprise any solid, porous, inert support.

Cells that survive the exposure to the selective agent may be cultured in media that supports regeneration of plants. Tissue is maintained on a basic media with hormones for about 2-4 weeks, then transferred to media with no hormones. After 2-4 weeks, shoot
30 development will signal the time to transfer to another media.

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Regeneration typically requires a progression of media whose composition has been modified to provide the appropriate nutrients and hormonal signals during sequential developmental stages from the transformed callus to the more mature plant. Developing plantlets are transferred to soil, and hardened, e.g., in an environmentally controlled chamber at about 85% relative humidity, 600 ppm CO₂, and 250 $\mu\text{E m}^{-2} \text{ s}^{-1}$ of light. Plants are preferably matured either in a growth chamber or greenhouse. Regeneration will typically take about 3-12 weeks. During regeneration, cells are grown on solid media in tissue culture vessels. An illustrative embodiment of such a vessel is a petri dish. Regenerating plants are preferably grown at about 19°C to 28°C. After the regenerating plants have reached the stage of shoot and root development, they may be transferred to a greenhouse for further growth and testing.

Genomic DNA may be isolated from callus cell lines and plants to determine the presence of the exogenous gene through the use of techniques well known to those skilled in the art such as PCR and/or Southern blotting.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a construct encoding a variant xylanase polypeptide according to the present invention and which is capable of introducing the construct into the genome of a plant.

The vector system may comprise one vector, but it can comprise at least two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An et al. (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

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One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* (An et al. (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. et al. (1980), *Tissue Culture*
5 *Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208).

Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above.

10 B. Uses

In a general sense, a variant xylanase of the invention may be used to alter, for example reduce, the viscosity derived from the presence of hemicellulose or arabinoxylan in a solution or system comprising plant cell wall material. Typically said plant cell wall
15 materials will comprise one or more xylanase inhibitors.

Specifically, a variant xylanase of the invention may be used in processing plant materials for use as foodstuffs, such as animal feed, in starch production, in baking and in the processing of wood pulp to make paper.

20

Preparation of foodstuffs

A variant xylanase of the invention may be used to process plant materials such as cereals that are used in foodstuffs including animal feed. As used herein, the term "cereal" means
25 any kind of grain used for food and/or any grass producing this grain such as but not limited to any one of wheat, milled wheat, barley, maize, sorghum, rye, oats, triticale and rice or combinations thereof. In one preferred embodiment, the cereal is a wheat cereal.

The xylan in the food and/or feed supplement is modified by contacting the xylan with the
30 variant xylanase of the present invention.

As used herein, the term "contacting" includes but is not limited to spraying, coating, impregnating or layering the food and/or feed supplement with the variant xylanase enzyme of the present invention.

- 5 In one embodiment, the food and/or feed supplement of the present invention may be prepared by mixing the variant xylanase enzyme directly with a food and/or feed supplement. By way of example, the variant xylanase enzyme may be contacted (for example, by spraying) onto a cereal-based food and/or feed supplement such as milled wheat, maize or soya flour.

10

- It is also possible to incorporating the variant xylanase enzyme it into a second (and different) food and/or feed or drinking water which is then added to the food and/or feed supplement of the present invention. Accordingly, it is not essential that the variant xylanase enzyme provided by the present invention is incorporated into the cereal-based food and/or feed supplement itself, although such incorporation forms a particularly preferred aspect of the present invention.

15

- In one embodiment of the present invention, the food and/or feed supplement may be combined with other food and/or feed components to produce a cereal-based food and/or feed. Such other food and/or feed components may include one or more other (preferably thermostable) enzyme supplements, vitamin food and/or feed supplements, mineral food and/or feed supplements and amino acid food and/or feed supplements. The resulting (combined) food and/or feed supplement comprising possibly several different types of compounds can then be mixed in an appropriate amount with the other food and/or feed components such as cereal and protein supplements to form a human food and/or an animal feed.

20

25

- In one preferred embodiment, the food and/or feed supplement of the present invention can be prepared by mixing different enzymes having the appropriate activities to produce an enzyme mix. By way of example, a cereal-based food and/or feed supplement formed from e.g. milled wheat or maize may be contacted (e.g. by spraying) either simultaneously or sequentially with the xylanase enzyme and other enzymes having

30

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appropriate activities. These enzymes may include but are not limited to any one or more of an amylase, a glucoamylase, a mannanase, an a galactosidase, a phytase, a lipase, a glucanase, an-arabinofuranosidase, a pectinase, a protease, a glucose oxidase, a hexose oxidase and a xylanase. Enzymes having the desired activities may for instance be mixed
5 with the xylanase of the present invention either before contacting these enzymes with a cereal-based food and/or feed supplement or alternatively such enzymes may be contacted simultaneously or sequentially on such a cereal based supplement. The food and/or feed supplement is then in turn mixed with a cereal-based food and/or feed to prepare the final food and/or feed. It is also possible to formulate the food and/or feed supplement as a
10 solution of the individual enzyme activities and then mix this solution with a food and/or feed material prior to processing the food and/or feed supplement into pellets or as a mash.

Bakery products

15

The present invention provides the use of a variant xylanase polypeptide of the invention in a process for preparing a foodstuff. Typical bakery (baked) products in accordance with the present invention include bread - such as loaves, rolls, buns, pizza bases etc. - pretzels, tortillas, cakes, cookies, biscuits, crackers etc. The preparation of foodstuffs
20 such as bakery products is well know in the art. Dough production, for example, is described in example 2. The use of variant xylanases of the invention to alter the viscosity of a flour slurry in described in the example 5.

Starch production

25

A variant xylanase of the invention may also be used in starch production from plant materials derived from cereals and tubers, such as potatoes.

Processing of wood pulp

30

A variant xylanase of the invention may also be used in processing wood pulp, for example in the preparation of paper.

As discussed above, we have shown that a major determinant of xylanase functionality is the presence of endogenous inhibitors in plant material. Consequently, although one method for altering xylanase functionality is to modify a xylanase to change its sensitivity to endogenous inhibitors, another method would be to vary the amount and/or type of inhibitor present in the plant material. Thus, the present invention also provides the use of a xylanase inhibitor to alter the functionality of a xylanase and consequently the use of a xylanase inhibitor in the methods of processing plant materials described above.

The present invention will now be further described with reference to the following examples which are intended to be illustrative only and non-limiting.

EXAMPLES

Example 1 - Purification and characterisation of wheat endogenous xylanase inhibitor.

2 kg wheat flour (Danish reform, batch 99056) was extracted with water, using a flour:water ratio of 1:2, during 10 minutes of stirring. The soluble endogenous xylanase inhibitor was separated from the flour-water slurry by centrifugation. The extraction and centrifugation was performed at 4°C. The inhibitor was purified from the water extract by the following chromatographic techniques and concentration techniques: HPLC-SEC, HPLC-CIEC, rotary evaporation, HPLC-HIC, HPLC-SEC and rotary evaporation. The xylanase inhibitor could be monitored and quantified during purification, using the following quantification method.

Inhibitor quantification method

1 XIU (Xylanase Inhibitor Unit) is defined as the amount of inhibitor that decreases 1 TXU to 0.5 TXU under the conditions described below.

The xylanase used in this assay is *Bacillus subtilis* wild type xylanase.

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250 μ l xylanase solution containing 12 TXU/ml, approx. 100 μ l xylanase inhibitor solution and citric acid (0.1 M) - *di*-sodium-hydrogen phosphate (0.2 M) buffer, pH 5, to react a reaction volume of 1000 μ l is pre-incubated for 5 minutes at 40°C. At t = 5 minutes, 1 Xylazyme (Megazyme, Ireland) tablet is added to the reaction mixture. At t = 15 minutes the reaction is terminated, by addition of 10 ml 2 % TRIS/NaOH, pH 12. The solution is filtered and the absorbency of the supernatant is measured at 590 nm. By choosing several different concentrations of inhibitor in the above assay, it is possible to create a plot of OD versus inhibitor concentration. Using the slope (a) and intercept (b) from this plot and the concentration of the xylanase it is possible to calculate the amount of XIU in a given inhibitor solution (equation 1).

Equation 1 amount of XIU in solution = ((b/2)/-a)/ TXU

From the endogenous xylanase inhibitor purification, the following inhibitor yield was recovered (table 1).

Table 1. Wheat endogenous xylanase inhibitor recovery after purification.

Sample	Amount	XIU	XIU, total	Recovery, %
Flour	2000 g	590/g	1.180.000	100
Purified inhibitor	90 ml	4658/ml	419.220	35.5

The inhibitor sample was pure and free from wheat endogenous xylanolytic activities.

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Example 2 - Fractionation and reconstruction of wheat flour free of xylanase inhibitor and xylanases functionality in this flour as a function of added xylanase inhibitor.

5 Flour fractionation and reconstitution

The flour used was: Danish Reform flour, batch No 99056. The fractionation, inhibitor inactivation and reconstitution were as follows:

10 A simple dough was made by mixing 1600 gram flour, with optimal water addition, according to a baker's absorption at 500 BU and mixing time according to Farinograph results. This resulted in 2512 gram dough. The gluten was manually washed out from the dough, using a water dough ratio of approx. 5:1. The water used was pre-chilled to 4°C to prevent further enzyme activity in the dough. The resulting wash-water contained the
15 soluble proteins (including the xylanase inhibitor), lipids, non-starch polysaccharides and starch. The starch and other non-soluble components were separated from the wash-water by centrifugation (5000 g, 10 minutes, 10°C). To inactivate the endogenous xylanase inhibitor in the wash-water, the supernatant from the centrifugation was boiled for three minutes using a heat-evaporator.

20

All three fractions (gluten, starch and solubles) were frozen in flasks and placed in a freeze dryer. After drying, the fractions were weighed, grounded using a mortar and pestle, coffee mill and sieved through a 250 µm sieve. All fractions were weighed again and flour was reconstituted, based on the ratios obtained after fractionation.

25

Enzymes

The xylanases listed in table 2 have been used in the study. The xylanases are purified, meaning no other xylonolytic activity is present in the sample.

30

Table 2. Xylanases used in the study and activity, TXU.

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ID	Origin	TXU
<i>B. sub</i>	<i>B. subtilis.</i>	5100
<i>A. nig</i>	<i>A. niger</i>	8800

Xylanase assay (Endo- β -1,4-Xylanase activity)

Xylanase samples are diluted in citric acid (0.1 M) - *di*-sodium-hydrogen phosphate
5 (0.2 M) buffer, pH 5.0, to obtain approx. OD = 0.7 in the final assay. Three dilutions of
the sample and an internal standard with a defined activity are thermostated for 5 minutes
at 40°C. At time = 5 minutes, 1 Xylazyme tab (crosslinked, dyed xylan substrate) is
added to the enzyme solution. At time = 15 minutes (or in some cases longer, depending
on the xylanase activity present in the sample) the reaction is terminated, by adding 10 ml
10 of 2% TRIS. The reaction mixture is centrifuged and the OD of the supernatant is
measured at 590 nm. Taking into account the dilutions and the amount of xylanase, the
activity (TXU, Total-Xylanase-Units) of the sample can be calculated relative to the
standard.

15 Baking trials

Baking trials were done with (1.44 x initial inhibitor level in Danish Reform flour, batch
No 99056) and without addition of purified endogenous xylanase inhibitor to the
reconstituted flour, respectively. The baking trials were done using the xylanases listed in
20 table 2 and the compositions listed in table 3..

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Table 3. Composition of dough made within the baking trials.

Dough No	ID	TXU	Inh. add, XIU/50g
1	Control	0	0
2	<i>B. sub</i>	7500	0
3	<i>A. nig</i>	7500	0
4	<i>B. sub</i>	7500	850
5	<i>A. nig</i>	7500	850
6	Control	0	850

Dough analysis

5

The dough were analysed with respect to:

Stickiness

Dough stickiness was measured on a TX-XT2 system (Stable Micro Systems) using a
 10 SMS Dough Stickiness Cell according to the method described by Chen And Hosney
 (Lebensmittel Wiss u.- Technol., 28, 467-473. 1995).

Viscosity analysis of dough liquid

The viscosity of extracted dough liquid was measured using a Brookfield viscosimeter
 15 after extraction.

Pentosan analysis of dough liquid

Solubilised pentosan was measured in the dough liquid using the method of Rouau and
 Surget (Carbohydrate polymers, 24, 123-132, 1994).

20

RESULTS

Flour fractionation and reconstitution

The fractionation and reconstitution of the dough resulted in 168.15 grams of freeze dried
5 gluten, 111.13 grams of freeze dried soluble fraction and 1143.56 grams of freeze dried
starch.

Inhibitor quantification in flour

Using the inhibitor quantification method, the inhibitor level in the 99056 flour and the
10 reconstituted flour could be detected. The results from these analyses are listed in table 4.

Table 4. Results from inhibitor quantification in native flour (99056) and reconstituted
flour.

Flour	Inhibitor concentration, XIU/g flour
99056	590
Reconstituted flour	42

15

Comparing the inhibitor level in the two portions of flour a 93% ($100 - (42\text{XIU}/590\text{XIU})$
x 100 %)) decrease of inhibitor level in the reconstituted flour is shown.

Baking trials

20 The results from the baking trial are listed in tables 5 and 6.

Table 5. Data from baking trials with reconstituted flour, xylanase and +/- xylanase
inhibitor addition. Std. dev., % represents the standard deviation over two days of
baking.

25

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ID	TXU	Inh., XIU/50g	Avg. spec. vol, ml/gram	Std. dev., %
Control	0	42	3.04	4.06
<i>B. sub</i>	7500	42	3.23	12.51
<i>A. nig.</i>	7500	42	3.44	5.24
<i>B. sub</i>	7500	850	3.22	4.26
<i>A. nig.</i>	7500	850	3.38	0.70
Control	0	850	2.94	0.05

The standard deviation shown in table 5 reflects the dough handling properties of the tested dough. The dough made without the endogenous xylanase inhibitor (42 XIU), were very difficult to handle. The standard deviation for these doughs are in the area of 3 to 12.5 %. Compared to the dough with the inhibitor added, this is quite high. If these standard deviations are compared with the actual changes in the bread volume, it can be seen that the figures are approximately the same value. This means that we can not conclude anything about the absence of the inhibitor's influence on the bread volume. If we look at the dough made with addition of the endogenous xylanase inhibitor (850 XIU) in table 5, we can see that we were able to produce bread from the reconstituted flour in a reproducible way over a two day period. The standard deviation was within the area of 0.05 to 4.2 %, which is acceptable. From table 6 it can be seen, that the xylanases all increased the volume of the baked bread.

Table 6. Volume increase in bread baked from reconstituted flour as a function of xylanase and xylanase inhibitor addition.

ID	TXU	Inh., XIU/50g	Avg. spec. vol, ml/gram	Volume increase as function of xylanase, %
Control	0	42	3.04	0.0
<i>B. sub</i>	7500	42	3.23	6.2
<i>A. nig.</i>	7500	42	3.44	13.3
<i>B. sub</i>	7500	850	3.22	9.7
<i>A. nig.</i>	7500	850	3.38	15.0
Control	0	850	2.94	0.0

What can be deduced from table 5 and table 6, is that the absence of the xylanase inhibitor in the flour made the handling of the dough very difficult. Therefore, what may seem as a positive response in volume by addition of inhibitor in table 6, probably can be explained
 5 by the high standard deviation in the dough lacking the inhibitor, due to difficult handling properties. Furthermore, it can be concluded that all the xylanases tested increased the bread volume significantly compared to the blank control.

Stickiness

10 The same dough, that was used for the baking trials, was used for stickiness measurements. The results are listed in table 7.

Table 7. Data representing stickiness as a function of time, xylanase and xylanase inhibitor addition to reconstituted flour.

15

ID	TXU	<u>Inh.,</u> <u>XIU/50g</u>	Avg. stickiness after 10 min, g x s	Avg. stickiness after 60 min, g x s
Control	0	42	4.71	4.79
<i>B. sub.</i>	7500	42	12.20	13.39
<i>A. nig.</i>	7500	42	9.22	12.58
<i>B. sub.</i>	7500	850	2.51	3.66
<i>A. nig.</i>	7500	850	5.24	6.45
Control	0	850	4.10	4.15

The results in table 7 clearly indicate the influence of the inhibitor that was observed in the experiment. The dough with a low level of xylanase inhibitor in combination with xylanase, was very difficult to handle and mould. However, when the inhibitor was
 20 added, the dough became dry and very easy to handle. As can be seen from table 7, addition of the 990202 xylanase in combination with the inhibitor decreased the stickiness. The dough became drier.

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- Table 7 also shows that there is only a small effect of time on the stickiness. It seems that the xylanases act very rapidly. Within the first 10 minutes most of the arabinoxylan is modified when the first xylanase (*B. sub*) is added. The second xylanase tested (*A. nig*), seems to act less rapidly. A function of time can easily be observed using this xylanase.
- 5 This is also the xylanase that shows the least effect as a function of inhibitor level when analysed on stickiness.

Dough Viscosity

- The dough viscosity and the pentosan analysis results were obtained from the same
- 10 extraction of dough prepared from reconstituted flour added xylanase and xylanase inhibitor. This dough was analysed after two proofing times, 30 and 120 minutes.

The results of the viscosity analysis are presented in table 8.

- 15 Table 8. Data representing dough liquid viscosity as a function of time, xylanase and xylanase inhibitor addition to reconstituted flour.

ID	TXU	Inh., XIU/50g	Avg. dough viscosity, cP, 30 min proofing	Avg. dough viscosity, cP, 120 min proofing
Control	0	42	5.21	5.56
<i>B. sub.</i>	7500	42	5.07	4.55
<i>A. nig.</i>	7500	42	5.78	4.14
<i>B. sub.</i>	7500	850	9.03	11.09
<i>A. nig.</i>	7500	850	8.44	8.55
Control	0	850	5.96	6.95

- As can be seen from table 8 the inhibitor has a significant effect on the functionality of the
- 20 xylanases. Without addition of the inhibitor, the arabinoxylan is being de-polymerised to Low Molecular Weight (LMW) arabinoxylan with a low viscosity. Addition of inhibitor prevents this very extensive de-polymerisation of the arabinoxylan.

Pentosan analysis of dough liquid

- 25 The results from the pentosan (arabinoxylan) analysis of the dough liquid are presented in table 9.

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Table 9. Data representing pentosan solubilisation as a function of time, xylanase and xylanase inhibitor addition to reconstituted flour.

ID	TXU	Inh., XIU/50g	Avg. Pentosan, %, 30 min proofing	Avg. Pentosan, %, 120 min proofing
Control	0	42	0.387	0.458
<i>B. sub.</i>	7500	42	0.766	0.819
<i>A. nig.</i>	7500	42	0.719	0.798
<i>B. sub.</i>	7500	850	0.410	0.544
<i>A. nig.</i>	7500	850	0.560	0.673
Control	0	850	0.400	0.528

- 5 As can be seen from the results in table 9, the addition of endogenous xylanase inhibitor decreased the solubilisation of the arabinoxylan. When evaluated after 30 minutes proofing time, the amount of arabinoxylan solubilised in absence of the inhibitor is almost twice the amount as in presence of the inhibitor. Calculated on the basis of the relating control samples, the solubilisation is much higher in absence of the inhibitor, as illustrated
10 in the following example:

$$(0.766 - 0.387) / (0.410 - 0.400) = 37.9 \text{ times higher solubilisation}$$

- The above example was calculated on basis of solubilisation of arabinoxylan using the
15 *Bacillus* xylanase, 30 minutes proofing and +/- inhibitor.

Example 3 - Site-directed mutagenesis on xylanases.

- Specific mutants of *Bacillus subtilis* xylanase may be obtained by site directed
20 mutagenesis of the wild type enzyme, by the use of any of a number of commercially available mutagenesis kits. An example of how to obtain the D11F mutant using the Quick Exchange kit, available from Stratagene Cloning Systems, 11011 North Torrey Pines Road, La Jolla, CA 92037, USA is given below :

- 25 The DNA sequence encoding *Bacillus subtilis* xylanase A has been published by Paice et al., 1986.

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The sequence of the coding region is as follows, with the sequence encoding the mature part of the protein shown in capitals:

```

catatgtttaagtttaaaaagaattttcttagttggattatcggcagctttaatgagtatt
5 agcttggttttcggcaaccgcctctgcaGCTAGCACAGACTACTGGCAAAATTGGACTGAT
GGGGGCGGTATAGTAAACGCTGTCAATGGGTCTGGCGGGAATTACAGTGTTAATTGGTCT
AATACCGGAAATTTTGTGTGTGGTAAAGGTTGGACTACAGGTTCCGCCATTTAGGACGATA
AACTATAATGCCGGAGTTTGGGCGCCGAATGGCAATGGATATTTAACTTTATATGGTTGG
ACGAGATCACCTCTCATAGAATATTATGTAGTGGATTCATGGGGTACTTATAGACCTACT
10 GGAACGTATAAAGGTACTGTAAAAAGTGATGGGGGTACATATGACATATATACTACTACA
CGTTATAACGCACCTTCCATTGATGGCGATCGCACTACTTTTACGCAGTACTGGAGTGTT
CGCCAGTCGAAGAGACCAACCGGAAGCAACGCTACAATCACTTTCAGCAATCATGTGAAC
GCATGGAAGAGCCATGGAATGAATCTGGGCAGTAATTGGGCTTACCAAGTCATGGCGACA
GAAGGATATCAAAGTAGTGGAAGTTCTAACGTAACAGTGTGGTAA

```

15

The part of the gene encoding the mature part of the wild type enzyme may be expressed intracellularly in *E.coli* by methods well known to people skilled in the art of molecular biology. For example :

- 20 1. Generating a copy of the capitalised part of the above described gene by use of the Polymerase Chain Reaction (PCR) with an added NdeI restriction enzyme site (CATATG) before the GCTAGCACA and an added HindIII restriction site (AAGCTT) after the GTGTGGTAA.
2. Inserting the resultant modified copy of the gene by use of the above mentioned
25 enzymes into the expression vector pET24a(+), which can be obtained from Novagen, Inc. 601 Science Drive, Madison, WI 53711, USA.
3. Transforming into a suitable *E.coli* strain and expression by fermentation as described by the vendor of pET24a(+).
- 30 4. Our D11F mutant enzyme may be obtained by using the "Quick Exchange" mutagenesis kit according to the manufacturer, and using the above described *Bacillus subtilis* wild type xylanase-pET24a(+) construct and the following PCR mutagenesis primers :

Sense primer:

```

35 CTACTGGCAAAATTGGACTTTTGGAGGAGGTATAGTAAACGCTG

```

Antisense primer :

CAGCGTTTACTATACCTCCTCCAAAAGTCCAATTTTGCCAGTAG

- 5 The mutant enzyme is expressed and purified using the same protocols as for the wild type enzyme.

Example 4 - Inhibition studies of xylanase mutants.

- 10 Xylanase mutants expressed in *E. coli* (see Example 3) were fermented and purified (meaning no other xylanolytic activity was present in the purified preparation) using a de-salting step and a cation exchange chromatography step.

- These pure xylanase mutant preparations were diluted to 12 TXU/ml using 0.1 M citric
15 acid - 0.2 M *di*-sodium-hydrogen phosphate, pH 5.0 and used in the following assay.

- A stable inhibitor preparation was made according to the protocol described in Example 1. This stable inhibitor preparation is used as stock for all xylanase-xylanase inhibitor studies. Using the inhibitor quantification method described in example 1, the inhibitor
20 preparation was analysed to contain 126 XIU/ml.

Assay

- To 250 µl diluted xylanase mutant preparations, are added 0, 10, 25, 50 or 100 µl inhibitor preparation, respectively. To these inhibitor-xylanase mixtures were added 0.1 M citric
25 acid - 0.2 M *di*-sodium-hydrogen phosphate, pH 5.0 making the end-volume 1000 µl. These reaction mixtures were pre-incubated for 5 minutes at 40°C. Hereafter 1 xylazyme tablet (Megazyme, Ireland) were added to all inhibitor-xylanase mixtures. After 10 minutes of incubation at 40°C, the reactions were terminated, by adding 10 ml 2% Tris/NaOH, pH 12.0. The mixtures were centrifuged and the liberated blue colour from
30 the substrate was measured at 590 nm.

The results are presented in table 10.

Table 10. Relative inhibition of xylanase mutants and parent xylanase (here wildtype enzyme) as a function of xylanase inhibitor.

Mutant ID	0	1,26	3,15	6,3	12,6
			Relative inhibition, %		
Wildtype	100	77	48	29	23
D11Y	100	120	114	126	124
D11N	100	93	72	53	32
D11F	100	114	119	116	115
D11K	100	109	112	113	116
D11S	100	98	81	60	38
D11W	100	101	88	70	50
G34D	100	94	83	70	53
G34F	100	76	53	34	29
G34T	100	99	99	93	86
Y113A	100	96	80	62	43
Y113D	100	96	81	63	45
Y113K	100	103	85	63	47
N114A	100	80	49	28	22
N114D	100	84	57	39	29
N114F	100	84	54	39	34
N114K	100	87	56	33	24
D121N	100	80	36	16	14
D121K	100	104	95	85	75
D121F	100	101	89	72	60
D121A	100	81	50	27	21
R122D	100	85	59	41	28
R122F	100	93	74	58	58
R122A	100	78	46	33	26
Q175E	100	87	59	40	31
Q175S	100	88	59	30	19
Q175L	100	78	42	25	23
G12F	100	110	106	100	92
G13F	100	104	95	87	84
I15K	100	84	47	28	23
N32K	100	82	42	19	14
G120K	100	85	52	29	22
G120D	100	84	47	24	18
G120F	100	71	35	18	15
G120Y	100	81	40	18	16
G120N	100	84	49	29	23
D119K	100	94	67	40	26
D119Y	100	87	50	28	22
D119N	100	91	74	44	22
T123K	100	80	46	30	25
T123Y	100	80	47	28	27
T123D	100	83	36	20	17
T124K	100	110	92	73	57
T124Y	100	101	76	49	33
T124D	100	87	52	32	25

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N17K	100	88	48	31	26
N17Y	100	79	42	23	19
N17D	100	90	81	50	22
N29K	100	83	50	30	23
N29Y	100	85	49	30	24
N29D	100	74	44	26	20
S31K	100	77	42	23	23
S31Y	100	83	50	27	22
S31D	100	79	52	30	24
D11F/R122D	100	109	111	110	109
D11F/G34D	100	104	106	103	104

From the results in table 10, it can be seen the xylanase mutants D11Y, D11F, D11K, D11F/R122D and D11F/G34D are uninhibited by the wheat endogenous xylanase inhibitor. These xylanase mutants would be expected to act more aggressively/specifically on the soluble arabinoxylan, compared to the other xylanase mutants or other xylanases. They would therefore be superior in applications where a decrease in viscosity (as a function of HMW arabinoxylan) is wanted.

Example 5 - Functionality studies of xylanase mutants.

10

Xylanase mutants expressed in *E. coli* (see Example 3) were fermented and purified (meaning no other xylanolytic activity were present in the purified preparation).

These pure xylanase mutant preparations were diluted to 400 TXU/ml using water and used in the following assay.

Assay

200 ml 30 % (w/w) flour slurry was made using water (thermostated to 25°C), by stirring for 5 minutes. 60.0 ml of this flour slurry is poured into a Ford-cup, and the time for drainage of 50.0 ml is measured. This measurement is the blank measurement. The 60.0 ml flour slurry is poured back and 1000 µl diluted xylanase mutant preparation is added to the flour slurry under stirring. After 2, 5, 10 and 20 minutes, 60.0 ml is poured into the Ford-cup, and the drainage time for 50.0 ml is recorded. Each measurement were done in triplicate.

25

The results are presented in table 11.

Table 11. Relative viscosity of flour slurry as a function of xylanase mutant and parent xylanase (here wild type xylanase)

		Incubation time,			
Mutant ID	0	2	5	10	20
		Relative viscosity change, %			
Wildtype	100	112	120	131	141
D11Y	100	97	93	83	75
D11N	100	112	125	130	136
D11F	100	93	87	78	69
D11K	100	105	95	88	78
D11S	100	102	110	113	117
D11W	100	106	115	121	122
G34D	100	110	120	128	124
G34F	100	111	126	128	146
G34T	100	100	108	111	106
Y113A	100	118	129	130	124
Y113D	100	116	127	124	114
Y113K	100	118	123	121	115
N114A	100	117	128	127	131
N114D	100	125	144	162	170
N114F	100	113	119	131	150
N114K	100	119	129	141	147
D121N	100	104	103	106	104
D121K	100	122	132	141	162
D121F	100	107	117	128	147
D121A	100	101	102	103	107
R122D	100	120	119	124	115
R122F	100	127	144	150	160
R122A	100	123	138	144	153
Q175E	100	116	134	142	149
Q175S	100	110	113	121	129
Q175L	100	111	111	119	126
G12F	100	127	132	122	101
G13F	100	106	119	124	113
I15K	100	109	108	113	118
N32K	100	97	98	101	101
G120K	100	103	111	115	121
G120D	100	112	122	120	126
G120F	100	103	111	117	130
G120Y	100	106	106	108	126
G120N	100	119	123	130	141
D119K	100	118	119	127	125
D119Y	100	102	102	111	110
D119N	100	126	137	145	146
T123K	100	106	109	121	120
T123Y	100	101	106	108	116
T123D	100	113	123	125	126
T124K	100	117	131	128	127
T124Y	100	112	123	132	135

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T124D	100	103	110	111	118
N17K	100	114	119	119	132
N17Y	100	102	102	108	108
N17D	100	120	131	135	143
N29K	100	98	100	100	104
N29Y	100	115	117	132	143
N29D	100	104	104	113	111
S31K	100	119	115	124	134
S31Y	100	110	118	122	137
S31D	100	99	103	109	110
D11F/R122D	100	91	89	82	77
D11F/G34D	100	96	93	84	80

Example 6. Site-directed mutation in the active site of *Bacillus subtilis* xylanase A, does not influence the xylanase : xylanase inhibitor interaction.

5

A residue in the active site of the *Bacillus subtilis* wildtype xylanase A enzyme was altered by a site-directed mutation (see ex. 3) In the mutated residue (Y166F) a potential hydrogen bond is lost. The mutant xylanase, was expressed in *E. coli*, fermented and purified. Hereafter, the mutant was investigated for its interaction with the xylanase inhibitor (see example 4).

10

As can be seen below (table 12), the exchange of an amino acid in the active site, did surprisingly not have any effect on interactions with the xylanase inhibitor as compared to the *Bacillus subtilis* wildtype xylanase enzyme.

15

Table 12. Relative inhibition of *Bacillus subtilis* wildtype xylanase and the xylanase mutant Y166F.

			XIU/ml		
Xylanase ID	0	1,26	3,15	6,3	12,6
			Relative inhibition, %		
Wildtype	100	75	40	24	20
Y166F	100	74	39	22	20

Hence, in summary the experiment described above shows a site-directed mutation in the active site of the *Bacillus subtilis* xylanase A, which mutation does not influence the xylanase's interactions with the xylanase inhibitor.

5 Example 7. Site-directed mutation in family 11 xylanases other than the *Bacillus subtilis* xylanase A, influencing the xylanase - xylanase inhibitor interactions.

D19 residue of the *Thermomyces lanuginosus* xylanase A enzyme was mutated to F19 by
 10 site-directed mutagenesis. D19 corresponds to D11 residue in the *Bacillus subtilis* xylanase (SEQ ID NO. 1). *Thermomyces lanuginosus* xylanase A gene is described as SEQ ID NO. 9.

The primers for PCR construction of the D19F mutant may be the following :

15

Sense primer :

GGTTATTACTATTCCTGGTGGAGTTTTGGAGGAGCGCAGGCCACG

Antisense primer :

CGTGGCCTGCGCTCCTCCAAAACCTCCACCAGGAATAGTAATAACC

20

The obtained mutant xylanase (D19F), was expressed in *E. coli*, fermented and purified. Hereafter, the mutant and the *Thermomyces lanuginosus* wildtype xylanase A was investigated for to its interaction with the xylanase inhibitor (see example 4). As can be seen from the results in table 13, the D19F mutant of the *Thermomyces lanuginosus*
 25 xylanase A is significantly less inhibited by the xylanase inhibitor as compared to the *Thermomyces lanuginosus* wildtype xylanase A.

Table 13. Relative inhibition of *Thermomyces lanoginosus* wildtype xylanase A (TLX) and the *Thermomyces lanoginosus* mutant xylanase, D19F (D19F).

30

			XIU/ml		
Xylanase ID	0	1,26	3,15	6,3	12,6
			Relative inhibition, %		
TLX	100	45	24	17	14
D19F	100	73	38	24	20

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Hence, in summary the experiment described above shows a site-directed mutation in the *Thermomyces lanuginosus* xylanase A. The results show that a mutation introducing a substitution of an amino acid on the surface of the xylanase molecule (analogue to the D11F in *B. subtilis*) changes the xylanase:xylanase inhibitor interactions. Thus, our invention (i.e. that surface residues control the level of inhibition of xylanase) holds true for xylanases that are homologous to the *B. subtilis* xylanase.

SUMMARY

10

Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

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 Gln Ser Lys Arg Pro Thr Gly Ser Asn Ala Thr Ile Thr Phe Thr Asn
 165 170 175
 His Val Asn Ala Trp Lys Ser His Gly Met Asn Leu Gly Ser Asn Trp
 180 185 190
 Ala Tyr Gln Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser
 195 200 205
 Asn Val Thr Val Trp
 210

<210> 18

<211> 211

<212> PRT

<213> Bacillus stearothermophilus

<400> 18

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

- 52H -

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

<210> 19
 <211> 211
 <212> PRT
 <213> A. caviae

<400> 19

Met Phe Lys Phe Gly Lys Lys Leu Met Thr Val Val Leu Ala Ala Ser
 1 5 10 15
 Met Ser Phe Gly Val Phe Ala Ala Thr Ser Ser Ala Ala Thr Asp Tyr
 20 25 30
 Trp Gln Asn Trp Thr Asp Gly Gly Gly Thr Val Asn Ala Val Asn Gly
 35 40 45
 Ser Gly Gly Asn Tyr Ser Val Ser Trp Gln Asn Thr Gly Asn Phe Val
 50 55 60
 Val Gly Lys Gly Trp Thr Tyr Gly Thr Pro Asn Arg Val Val Asn Tyr
 65 70 75 80
 Asn Ala Gly Val Phe Ala Pro Ser Gly Asn Gly Tyr Leu Thr Phe Tyr
 85 90 95

- 52I -

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

Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Asn Ser Asp
 115 120 125

Gly Gly Thr Tyr Asp Ile Tyr Thr Thr Met Arg Tyr Asn Ala Pro Ser
 130 135 140

Ile Asp Gly Thr Gln Thr Phe Pro Gln Tyr Trp Ser Val Arg Gln Ser
 145 150 155 160

Lys Arg Pro Thr Gly Val Asn Ser Thr Ile Thr Phe Ser Asn His Val
 165 170 175

Asn Ala Trp Pro Ser Lys Gly Met Tyr Leu Gly Asn Ser Trp Ser Tyr
 180 185 190

Gln Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Asn Ala Asn Val
 195 200 205

Thr Val Trp
 210

<210> 20
 <211> 221
 <212> PRT
 <213> C. carbonum

<400> 20

Met Val Ser Phe Thr Ser Ile Ile Thr Ala Ala Val Ala Ala Thr Gly
 1 5 10 15

Ala Leu Ala Ala Pro Ala Thr Asp Val Ser Leu Val Ala Arg Gln Asn
 20 25 30

Thr Pro Asn Gly Glu Gly Thr His Asn Gly Cys Phe Trp Ser Trp Trp
 35 40 45

Ser Asp Gly Gly Ala Arg Ala Thr Tyr Thr Asn Gly Ala Gly Gly Ser
 50 55 60

Tyr Ser Val Ser Trp Gly Ser Gly Gly Asn Leu Val Gly Gly Lys Gly
 65 70 75 80

Trp Asn Pro Gly Thr Ala Arg Thr Ile Thr Tyr Ser Gly Thr Tyr Asn
 85 90 95

Tyr Asn Gly Asn Ser Tyr Leu Ala Val Tyr Gly Trp Thr Arg Asn Pro
 100 105 110

Leu Val Glu Tyr Tyr Val Val Glu Asn Phe Gly Thr Tyr Asp Pro Ser
 115 120 125

Ser Gln Ser Gln Asn Lys Gly Thr Val Thr Ser Asp Gly Ser Ser Tyr
 130 135 140

- 52J -

Lys Ile Ala Gln Ser Thr Arg Thr Asn Gln Pro Ser Ile Asp Gly Thr
145 150 155 160

Arg Thr Phe Gln Gln Tyr Trp Ser Val Arg Gln Asn Lys Arg Ser Ser
165 170 175

Gly Ser Val Asn Met Lys Thr His Phe Asp Ala Trp Ala Ser Lys Gly
180 185 190

Met Asn Leu Gly Gln His Tyr Tyr Gln Ile Val Ala Thr Glu Gly Tyr
195 200 205

Phe Ser Thr Gly Asn Ala Gln Ile Thr Val Asn Cys Pro
210 215 220

<210> 21

<211> 227

<212> PRT

<213> H. turcicum

<400> 21

Met Val Ser Phe Thr Ser Ile Ile Thr Ala Ala Val Ala Ala Thr Gly
1 5 10 15

Ala Leu Ala Ala Pro Ala Thr Asp Ile Ala Ala Arg Ala Pro Ser Asp
20 25 30

Leu Val Ala Arg Gln Ser Thr Pro Asn Gly Glu Gly Thr His Asn Gly
35 40 45

Cys Phe Tyr Ser Trp Trp Ser Asp Gly Gly Ala Arg Ala Thr Tyr Thr
50 55 60

Asn Gly Ala Gly Gly Ser Tyr Ser Val Ser Trp Gly Thr Gly Gly Asn
65 70 75 80

Leu Val Gly Gly Lys Gly Trp Asn Pro Gly Thr Ala Arg Thr Ile Thr
85 90 95

Tyr Ser Gly Gln Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ala Ile Tyr
100 105 110

Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Val Val Glu Asn Phe
115 120 125

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

Ser Asp Gly Ser Ser Tyr Lys Ile Ala Gln Ser Thr Arg Thr Asn Gln
145 150 155 160

Pro Ser Ile Asp Gly Thr Arg Thr Phe Gln Gln Tyr Trp Ser Val Arg
165 170 175

Gln Asn Lys Arg Ser Ser Gly Ser Val Asn Met Lys Thr His Phe Asp
180 185 190

- 52K -

Ala Trp Ala Ser Lys Gly Met Asn Leu Gly Ser His Tyr Tyr Gln Ile
 195 200 205

Val Ala Thr Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val
 210 215 220

Asn Cys Pro
 225

<210> 22
 <211> 227
 <212> PRT
 <213> A. pisi

<400> 22

Met Val Ser Phe Thr Ser Ile Phe Thr Ala Ala Val Ala Ala Thr Gly
 1 5 10 15

Ala Leu Ala Val Pro Val Thr Asp Leu Ala Thr Arg Ser Leu Gly Ala
 20 25 30

Leu Thr Ala Arg Ala Gly Thr Pro Ser Ser Gln Gly Thr His Asn Gly
 35 40 45

Cys Phe Tyr Ser Trp Trp Thr Asp Gly Gly Ala Gln Ala Thr Tyr Thr
 50 55 60

Asn Gly Ala Gly Gly Ser Tyr Ser Val Asn Trp Lys Thr Gly Gly Asn
 65 70 75 80

Leu Val Gly Gly Lys Gly Trp Asn Pro Gly Ala Ala Arg Thr Ile Thr
 85 90 95

Tyr Ser Gly Thr Tyr Ser Pro Ser Gly Asn Ser Tyr Leu Ala Val Tyr
 100 105 110

Gly Trp Thr Arg Asn Pro Leu Ile Glu Tyr Tyr Val Val Glu Asn Phe
 115 120 125

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

Ala Asp Gly Ser Ser Tyr Lys Ile Ala Gln Thr Gln Arg Thr Asn Gln
 145 150 155 160

Pro Ser Ile Asp Gly Thr Gln Thr Phe Gln Gln Tyr Trp Ser Val Arg
 165 170 175

Gln Asn Lys Arg Ser Ser Gly Ser Val Asn Met Lys Thr His Phe Asp
 180 185 190

Ala Trp Ala Ala Lys Gly Met Lys Leu Gly Thr His Asn Tyr Gln Ile
 195 200 205

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

- 52L -

Asn Cys Ala
225

<210> 23
<211> 201
<212> PRT
<213> S. commune

<400> 23

Ala Ala Ser Gly Thr Pro Ser Ser Thr Gly Thr Asp Gly Gly Tyr Tyr
1 5 10 15

Tyr Ser Trp Trp Thr Asp Gly Ala Gly Asp Ala Thr Tyr Gln Asn Asn
20 25 30

Gly Gly Gly Ser Tyr Thr Leu Thr Trp Ser Gly Asn Asn Gly Asn Leu
35 40 45

Val Gly Gly Lys Gly Trp Asn Pro Gly Ala Ala Ser Arg Ser Ile Ser
50 55 60

Tyr Ser Gly Thr Tyr Gln Pro Asn Gly Asn Ser Tyr Leu Ser Val Tyr
65 70 75 80

Gly Trp Thr Arg Ser Ser Leu Ile Glu Tyr Tyr Ile Val Glu Ser Tyr
85 90 95

Gly Ser Tyr Asp Pro Ser Ser Ala Ala Ser His Lys Gly Ser Val Thr
100 105 110

Cys Asn Gly Ala Thr Tyr Asp Ile Leu Ser Thr Trp Arg Tyr Asn Ala
115 120 125

Pro Ser Ile Asp Gly Thr Gln Thr Phe Glu Gln Phe Trp Ser Val Arg
130 135 140

Asn Pro Lys Lys Ala Pro Gly Gly Ser Ile Ser Gly Thr Val Asp Val
145 150 155 160

Gln Cys His Phe Asp Ala Trp Lys Gly Leu Gly Met Asn Leu Gly Ser
165 170 175

Glu His Asn Tyr Gln Ile Val Ala Thr Glu Gly Tyr Gln Ser Ser Gly
180 185 190

Thr Ala Thr Ile Thr Val Thr Ala Ser
195 200

<210> 24
<211> 225
<212> PRT
<213> T. lanuginosus

<400> 24

Met Val Gly Phe Thr Pro Val Ala Leu Ala Ala Leu Ala Ala Thr Gly
1 5 10 15

- 52M -

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

Gly
225

<210> 25
 <211> 231
 <212> PRT
 <213> C. carbonum

<400> 25

Met Val Ser Phe Lys Ser Leu Leu Leu Ala Ala Val Ala Thr Thr Ser
 1 5 10 15
 Val Leu Ala Ala Pro Phe Asp Phe Leu Arg Glu Arg Asp Asp Val Asn
 20 25 30
 Ala Thr Ala Leu Leu Glu Lys Arg Gln Ser Thr Pro Ser Ala Glu Gly
 35 40 45

- 52N -

Tyr His Asn Gly Tyr Phe Tyr Ser Trp Trp Thr Asp Gly Gly Gly Ser
50 55 60

Ala Gln Tyr Thr Met Gly Glu Gly Ser Arg Tyr Ser Val Thr Trp Arg
65 70 75 80

Asn Thr Gly Asn Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Ser Gly
85 90 95

Arg Val Ile Asn Tyr Gly Gly Ala Phe Asn Pro Gln Gly Asn Gly Tyr
100 105 110

Leu Ala Val Tyr Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Val
115 120 125

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

Gly Ser Phe Gln Thr Asp Gly Gly Thr Tyr Asn Val Ala Val Ser Thr
145 150 155 160

Arg Tyr Asn Gln Pro Ser Ile Asp Gly Thr Arg Thr Phe Gln Gln Tyr
165 170 175

Trp Ser Val Arg Thr Gln Lys Arg Val Gly Gly Ser Val Asn Met Gln
180 185 190

Asn His Phe Asn Ala Trp Ser Arg Tyr Gly Leu Asn Leu Gly Gln His
195 200 205

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

Asp Ile Tyr Val Gln Thr Gln
225 230

<210> 26
<211> 231
<212> PRT
<213> C. sativus

<400> 26

Met Val Ser Phe Lys Ser Leu Leu Leu Ala Ala Val Ala Thr Thr Ser
1 5 10 15

Val Leu Ala Ala Pro Phe Asp Phe Leu Arg Glu Arg Asp Asp Gly Asn
20 25 30

Ala Thr Ala Leu Leu Glu Lys Arg Gln Ser Thr Pro Ser Ser Glu Gly
35 40 45

Tyr His Asn Gly Tyr Phe Tyr Ser Trp Trp Thr Asp Gly Gly Gly Ser
50 55 60

Ala Gln Tyr Thr Met Gly Glu Gly Ser Arg Tyr Ser Val Thr Trp Arg
65 70 75 80

- 520 -

Asn Thr Gly Asn Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Thr Gly
85 90 95

Arg Val Ile Asn Tyr Gly Gly Ala Phe Asn Pro Gln Gly Asn Gly Tyr
100 105 110

Leu Ala Val Tyr Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Val
115 120 125

Ile Glu Ser Tyr Gly Thr Tyr Asn Pro Ser Ser Gly Ala Gln Val Lys
130 135 140

Gly Ser Phe Gln Thr Asp Gly Gly Thr Tyr Asn Val Ala Val Ser Thr
145 150 155 160

Arg Tyr Asn Gln Pro Ser Ile Asp Gly Thr Arg Thr Phe Gln Gln Tyr
165 170 175

Trp Ser Val Arg Gln Gln Lys Arg Val Gly Gly Ser Val Asn Met Gln
180 185 190

Asn His Phe Asn Ala Trp Ser Arg Tyr Gly Leu Asn Leu Gly Gln His
195 200 205

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

Asp Ile Tyr Val Gln Thr Gln
225 230

<210> 27

<211> 227

<212> PRT

<213> H. insolens

<400> 27

Met Val Ser Leu Lys Ser Val Leu Ala Ala Ala Thr Ala Val Ser Ser
1 5 10 15

Ala Ile Ala Ala Pro Phe Asp Phe Val Pro Arg Asp Asn Ser Thr Ala
20 25 30

Leu Gln Ala Arg Gln Val Thr Pro Asn Ala Glu Gly Trp His Asn Gly
35 40 45

Tyr Phe Tyr Ser Trp Trp Ser Asp Gly Gly Gly Gln Val Gln Tyr Thr
50 55 60

Asn Leu Glu Gly Ser Arg Tyr Gln Val Arg Trp Arg Asn Thr Gly Asn
65 70 75 80

Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Thr Gly Arg Thr Ile Asn
85 90 95

Tyr Gly Gly Tyr Phe Asn Pro Gln Gly Asn Gly Tyr Leu Ala Val Tyr
100 105 110

- 52P -

Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Val Ile Glu Ser Tyr
 115 120 125

Gly Thr Tyr Asn Pro Gly Ser Gln Ala Gln Tyr Lys Gly Thr Phe Tyr
 130 135 140

Thr Asp Gly Asp Gln Tyr Asp Ile Phe Val Ser Thr Arg Tyr Asn Gln
 145 150 155 160

Pro Ser Ile Asp Gly Thr Arg Thr Phe Gln Gln Tyr Trp Ser Ile Arg
 165 170 175

Lys Asn Lys Arg Val Gly Gly Ser Val Asn Met Gln Asn His Phe Asn
 180 185 190

Ala Trp Gln Gln His Gly Met Pro Leu Gly Gln His Tyr Tyr Gln Val
 195 200 205

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

Gln Thr His
 225

<210> 28
 <211> 233
 <212> PRT
 <213> M. grisea

<400> 28

Met Val Ser Phe Thr Ser Ile Val Thr Ala Val Val Ala Leu Ala Gly
 1 5 10 15

Ser Ala Leu Ala Ile Pro Ala Pro Asp Gly Asn Met Thr Gly Phe Pro
 20 25 30

Phe Glu Gln Leu Met Arg Arg Gln Ser Thr Pro Ser Ser Thr Gly Arg
 35 40 45

His Asn Gly Tyr Tyr Tyr Ser Trp Trp Thr Asp Gly Ala Ser Pro Val
 50 55 60

Gln Tyr Gln Asn Gly Asn Gly Gly Ser Tyr Ser Val Gln Trp Gln Ser
 65 70 75 80

Gly Gly Asn Phe Val Gly Gly Lys Gly Trp Met Pro Gly Gly Ser Lys
 85 90 95

Ser Ile Thr Tyr Ser Gly Thr Phe Asn Pro Val Asn Asn Gly Asn Ala
 100 105 110

Tyr Leu Cys Ile Tyr Gly Trp Thr Gln Asn Pro Leu Val Glu Tyr Tyr
 115 120 125

Ile Leu Glu Asn Tyr Gly Glu Tyr Asn Pro Gly Asn Ser Ala Gln Ser
 130 135 140

- 52Q -

Arg Gly Thr Leu Gln Ala Ala Gly Gly Thr Tyr Thr Leu His Glu Ser
145 150 155 160

Thr Arg Val Asn Gln Pro Ser Ile Glu Gly Thr Arg Thr Phe Gln Gln
165 170 175

Tyr Trp Ala Ile Arg Gln Gln Lys Arg Asn Ser Gly Thr Val Asn Thr
180 185 190

Gly Glu Phe Phe Gln Ala Trp Glu Arg Ala Gly Met Arg Met Gly Asn
195 200 205

His Asn Tyr Met Ile Val Ala Thr Glu Gly Tyr Arg Ser Ala Gly Asn
210 215 220

Ser Asn Ile Asn Val Gln Thr Pro Ala
225 230

<210> 29

<211> 219

<212> PRT

<213> C. gracile

<400> 29

Met Val Ser Phe Lys Ala Leu Leu Leu Gly Ala Ala Gly Ala Leu Ala
1 5 10 15

Phe Pro Phe Asn Val Thr Gln Met Asn Glu Leu Val Ala Arg Ala Gly
20 25 30

Thr Pro Ser Gly Thr Gly Thr Asn Asn Gly Tyr Phe Tyr Ser Phe Trp
35 40 45

Thr Asp Gly Gly Gly Thr Val Asn Tyr Gln Asn Gly Ala Gly Gly Ser
50 55 60

Tyr Ser Val Gln Trp Gln Asn Cys Gly Asn Phe Val Gly Gly Lys Gly
65 70 75 80

Trp Asn Pro Gly Ala Ala Arg Thr Ile Asn Phe Ser Gly Thr Phe Ser
85 90 95

Pro Gln Gly Asn Gly Tyr Leu Ala Ile Tyr Gly Trp Thr Gln Asn Pro
100 105 110

Leu Val Glu Tyr Tyr Ile Val Glu Ser Phe Gly Thr Tyr Asp Pro Ser
115 120 125

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

Thr Ile Ala Lys Thr Thr Arg Val Asn Gln Pro Ser Ile Glu Gly Thr
145 150 155 160

Ser Thr Phe Asp Gln Phe Trp Ser Val Arg Gln Asn His Arg Ser Ser
165 170 175

- 52R -

Gly Ser Val Asn Val Ala Ala His Phe Asn Ala Trp Ala Gln Ala Gly
 180 185 190

Leu Lys Leu Gly Ser His Asn Tyr Gln Ile Val Ala Thr Glu Gly Tyr
 195 200 205

Gln Ser Ser Gly Ser Ser Ser Ile Thr Val Ser
 210 215

<210> 30

<211> 223

<212> PRT

<213> T. reesei

<400> 30

Met Val Ser Phe Thr Ser Leu Leu Ala Gly Val Ala Ala Ile Ser Gly
 1 5 10 15

Val Leu Ala Ala Pro Ala Ala Glu Val Glu Pro Val Ala Val Glu Lys
 20 25 30

Arg Gln Thr Ile Gln Pro Gly Thr Gly Tyr Asn Asn Gly Tyr Phe His
 35 40 45

Ser Tyr Trp Asn Asp Gly His Gly Gly Val Thr Tyr Thr Asn Gly Pro
 50 55 60

Gly Gly Gln Phe Ser Val Asn Trp Ser Asn Ser Gly Asn Phe Val Gly
 65 70 75 80

Gly Lys Gly Trp Gln Pro Gly Thr Lys Asn Lys Val Ile Asn Phe Ser
 85 90 95

Gly Ser Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ser Val Tyr Gly Trp
 100 105 110

Ser Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Val Gly Asn Phe Gly Thr
 115 120 125

Tyr Asn Pro Ser Thr Gly Ala Thr Lys Leu Gly Glu Val Thr Ser Asp
 130 135 140

Gly Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser
 145 150 155 160

Ile Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn
 165 170 175

His Arg Ser Ser Gly Ser Val Asn Thr Ala Asn His Phe Asn Ala Trp
 180 185 190

Ala Gln Gln Gly Leu Thr Leu Gly Thr Met Asp Tyr Gln Ile Val Ala
 195 200 205

Val Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val Ser
 210 215 220

<210> 31

Met Val Ser Phe Thr Ser Leu Leu Ala Ala Ser Pro Pro Ser Arg Ala
1 5 10 15

- 52T -

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

<210> 33
 <211> 190
 <212> PRT
 <213> T. harzianum

<400> 33

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

- 52U -

Ser Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ser Ile Tyr Gly Trp Ser
 65 70 75 80
 Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Val Glu Asn Phe Gly Thr Tyr
 85 90 95
 Asn Pro Ser Thr Gly Ala Thr Lys Leu Gly Glu Val Thr Ser Asp Gly
 100 105 110
 Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile
 115 120 125
 Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His
 130 135 140
 Arg Ser Ser Gly Ser Val Asn Thr Ala Asn His Phe Asn Ala Trp Ala
 145 150 155 160
 Ser His Gly Leu Thr Leu Gly Thr Met Asp Tyr Gln Ile Val Ala Val
 165 170 175
 Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val Ser
 180 185 190

<210> 34
 <211> 223
 <212> PRT
 <213> T. viride

<400> 34

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

- 52V -

Gly Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser
145 150 155 160

Ile Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn
165 170 175

His Ala Pro Ala Ala Arg Ser Arg Leu Arg Thr Thr Ser Asn Ala Trp
180 185 190

Arg Asn Leu Gly Leu Thr Leu Gly Thr Leu Asp Tyr Gln Ile Ile Ala
195 200 205

Val Glu Gly Tyr Phe Ser Ser Gly Asn Ala Asn Ile Asn Val Ser
210 215 220

<210> 35

<211> 241

<212> PRT

<213> C. gracile

<400> 35

Met Val Asn Phe Ser Ser Leu Phe Leu Ala Ala Ser Ala Ala Val Val
1 5 10 15

Ala Val Ala Ala Pro Gly Glu Leu Pro Gly Met His Lys Arg Gln Thr
20 25 30

Leu Thr Ser Ser Gln Thr Gly Thr Asn Asn Gly Tyr Tyr Tyr Ser Phe
35 40 45

Trp Thr Asp Gly Gln Gly Asn Val Gln Tyr Thr Asn Glu Ala Gly Gly
50 55 60

Gln Tyr Ser Val Thr Trp Ser Gly Asn Gly Asn Trp Val Gly Gly Lys
65 70 75 80

Gly Trp Asn Pro Gly Ser Ala Arg Thr Ile Asn Tyr Thr Ala Asn Tyr
85 90 95

Asn Pro Asn Gly Asn Ser Tyr Leu Ala Val Tyr Gly Trp Thr Arg Asn
100 105 110

Pro Leu Ile Glu Tyr Tyr Val Val Glu Asn Phe Gly Thr Tyr Asn Pro
115 120 125

Ser Thr Gly Ala Thr Arg Leu Gly Ser Val Thr Thr Asp Gly Ser Cys
130 135 140

Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Glu Gly
145 150 155 160

Thr Ser Thr Phe Tyr Gln Phe Trp Ser Val Arg Gln Asn Lys Arg Ser
165 170 175

Gly Gly Ser Val Asn Met Ala Ala His Phe Asn Ala Trp Ala Ala Ala
180 185 190

- 52W -

Gly Leu Gln Leu Gly Thr His Asp Tyr Gln Ile Val Ala Thr Glu Gly
 195 200 205

Tyr Tyr Ser Ser Gly Ser Ala Thr Val Asn Val Gly Ala Ser Ser Asp
 210 215 220

Gly Ser Thr Gly Gly Gly Ser Thr Gly Gly Gly Ser Thr Asn Val Ser
 225 230 235 240

Phe

<210> 36

<211> 225

<212> PRT

<213> A. niger

<400> 36

Met Leu Thr Lys Asn Leu Leu Leu Cys Phe Ala Ala Ala Lys Ala Ala
 1 5 10 15

Leu Ala Val Pro His Asp Ser Val Ala Gln Arg Ser Asp Ala Leu His
 20 25 30

Met Leu Ser Glu Arg Ser Thr Pro Ser Ser Thr Gly Glu Asn Asn Gly
 35 40 45

Phe Tyr Tyr Ser Phe Trp Thr Asp Gly Gly Gly Asp Val Thr Tyr Thr
 50 55 60

Asn Gly Asp Ala Gly Ala Tyr Thr Val Glu Trp Ser Asn Val Gly Asn
 65 70 75 80

Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Ser Ala Gln Asp Ile Thr
 85 90 95

Tyr Ser Gly Thr Phe Thr Pro Ser Gly Asn Gly Tyr Leu Ser Val Tyr
 100 105 110

Gly Trp Thr Thr Asp Pro Leu Ile Glu Tyr Tyr Ile Val Glu Ser Tyr
 115 120 125

Gly Asp Tyr Asn Pro Gly Ser Gly Gly Thr Tyr Lys Gly Thr Val Thr
 130 135 140

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

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

Gln Asn Lys Arg Val Gly Gly Thr Val Thr Thr Ser Asn His Phe Asn
 180 185 190

Ala Trp Ala Lys Leu Gly Met Asn Leu Gly Thr His Asn Tyr Gln Ile
 195 200 205

- 52X -

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

Gln
 225

<210> 37
 <211> 221
 <212> PRT
 <213> Penicillium sp 40

<400> 37

Met Lys Ser Phe Ile Ala Tyr Leu Leu Ala Ser Val Ala Val Thr Gly
 1 5 10 15

Val Met Ala Val Pro Gly Glu Tyr His Lys Arg His Asp Lys Arg Gln
 20 25 30

Thr Ile Thr Ser Ser Gln Thr Gly Thr Asn Asn Gly Tyr Tyr Tyr Ser
 35 40 45

Phe Trp Thr Asn Gly Gly Gly Thr Val Gln Tyr Thr Asn Gly Ala Ala
 50 55 60

Gly Glu Tyr Ser Val Thr Trp Glu Asn Cys Gly Asp Phe Thr Ser Gly
 65 70 75 80

Lys Gly Trp Ser Thr Gly Ser Ala Arg Asp Ile Thr Phe Glu Gly Thr
 85 90 95

Phe Asn Pro Ser Gly Asn Ala Tyr Leu Ala Val Tyr Gly Trp Thr Thr
 100 105 110

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

Pro Gly Asn Ser Met Thr Tyr Lys Gly Thr Val Thr Ser Asp Gly Ser
 130 135 140

Val Tyr Asp Ile Tyr Glu His Gln Gln Val Asn Gln Pro Ser Ile Ser
 145 150 155 160

Gly Thr Ala Thr Phe Asn Gln Tyr Trp Ser Ile Arg Gln Asn Thr Arg
 165 170 175

Ser Ser Gly Thr Val Thr Thr Ala Asn His Phe Asn Ala Trp Ala Lys
 180 185 190

Leu Gly Met Asn Leu Gly Ser Phe Asn Tyr Gln Ile Val Ser Thr Glu
 195 200 205

Gly Tyr Glu Ser Ser Gly Ser Ser Thr Ile Thr Val Ser
 210 215 220

<210> 38
 <211> 240
 <212> PRT
 <213> Streptomyces sp

- 52Y -

<400> 38

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

<210> 39

<211> 228

<212> PRT

<213> Streptomyces sp

<400> 39

Met	Thr	Lys	Asp	Asn	Thr	Pro	Ile	Arg	Pro	Val	Ser	Arg	Arg	Gly	Phe
1				5					10					15	

- 52Z -

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

<210> 40
 <211> 239
 <212> PRT
 <213> S. thermocyaneoviolaceus

<400> 40

Met Asn Thr Leu Val His Pro Gln Gly Arg Ala Gly Gly Leu Arg Leu
 1 5 10 15
 Leu Val Arg Ala Ala Trp Ala Leu Ala Leu Ala Ala Leu Ala Ala Met
 20 25 30
 Met Phe Gly Gly Thr Ala Arg Ala Asp Thr Ile Thr Ser Asn Gln Thr
 35 40 45

- 52AA -

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

<210> 41
 <211> 242
 <212> PRT
 <213> S. viridosporus

<400> 41

Met Asn Ala Phe Ala His Pro Arg Gly Arg Arg His Gly Arg Ser Ala
 1 5 10 15
 Pro Met Ser Pro Arg Ser Thr Trp Ala Val Leu Leu Ala Ala Leu Ala
 20 25 30
 Val Met Leu Leu Pro Gly Thr Ala Thr Ala Ala Pro Val Ile Thr Thr
 35 40 45
 Asn Gln Thr Gly Thr Asn Asn Gly Trp Trp Tyr Ser Phe Trp Thr Asp
 50 55 60
 Ala Gln Gly Thr Val Ser Met Asp Leu Gly Ser Gly Gly Thr Tyr Ser
 65 70 75 80

- 52BB -

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

Gly Gly

<210> 42
 <211> 240
 <212> PRT
 <213> T. fusca

<400> 42

Met Asn His Ala Pro Ala Ser Leu Lys Ser Arg Arg Arg Phe Arg Pro
 1 5 10 15
 Arg Leu Leu Ile Gly Lys Ala Phe Ala Ala Ala Leu Val Ala Val Val
 20 25 30
 Thr Met Ile Pro Ser Thr Ala Ala His Ala Ala Val Thr Ser Asn Glu
 35 40 45
 Thr Gly Tyr His Asp Gly Tyr Phe Tyr Ser Phe Trp Thr Asp Ala Pro
 50 55 60
 Gly Thr Val Ser Met Glu Leu Gly Pro Gly Gly Asn Tyr Ser Thr Ser
 65 70 75 80
 Trp Arg Asn Thr Gly Asn Phe Val Ala Gly Lys Gly Trp Ala Thr Gly
 85 90 95

- 52CC -

Gly Arg Arg Thr Val Thr Tyr Ser Ala Ser Phe Asn Pro Ser Gly Asn
 100 105 110
 Ala Tyr Leu Thr Leu Tyr Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr
 115 120 125
 Tyr Ile Val Glu Ser Trp Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Met
 130 135 140
 Gly Thr Val Thr Thr Asp Gly Gly Thr Tyr Asp Ile Tyr Lys Thr Thr
 145 150 155 160
 Arg Tyr Asn Ala Pro Ser Ile Glu Gly Thr Arg Thr Phe Asp Gln Tyr
 165 170 175
 Trp Ser Val Arg Gln Ser Lys Arg Thr Ser Gly Thr Ile Thr Ala Gly
 180 185 190
 Asn His Phe Asp Ala Trp Ala Arg His Gly Met His Leu Gly Thr His
 195 200 205
 Asp Tyr Met Ile Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser
 210 215 220
 Asn Val Thr Leu Gly Thr Ser Gly Gly Gly Asn Pro Gly Gly Gly Asn
 225 230 235 240

<210> 43
 <211> 241
 <212> PRT
 <213> C. pachnodae

<400> 43

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

- 52DD -

Tyr Tyr Ile Val Asp Ser Trp Gly Thr Tyr Arg Pro Thr Gly Thr Phe
 130 135 140
 Met Gly Thr Val Asn Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Lys Thr
 145 150 155 160
 Thr Arg Thr Asn Ala Pro Ser Ile Glu Gly Thr Ala Thr Phe Thr Gln
 165 170 175
 Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr Thr
 180 185 190
 Ala Asn His Phe Asn Ala Trp Ala Ser His Gly Met Asn Leu Gly Arg
 195 200 205
 His Asp Tyr Gln Ile Leu Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser
 210 215 220
 Ser Asn Ile Thr Ile Gly Ser Thr Ser Gly Gly Gly Gly Ser Gly Gly
 225 230 235 240

Gly

<210> 44
 <211> 221
 <212> PRT
 <213> A. oryzae

<400> 44

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

- 52EE -

Thr Tyr Lys Ile Tyr Lys Thr Thr Arg Glu Asn Ala Pro Ser Ile Glu
 145 150 155 160
 Gly Thr Ser Thr Phe Asn Gln Tyr Trp Ser Val Arg Gln Ser Gly Arg
 165 170 175
 Val Gly Gly Thr Ile Thr Ala Gln Asn His Phe Asp Ala Trp Ala Asn
 180 185 190
 Val Gly Leu Gln Leu Gly Thr His Asn Tyr Met Ile Leu Ala Thr Glu
 195 200 205
 Gly Tyr Lys Ser Ser Gly Ser Ala Thr Ile Thr Val Glu
 210 215 220

<210> 45
 <211> 216
 <212> PRT
 <213> C. purpurea

<400> 45

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

- 52FF -

Gly Thr Tyr Asp Tyr Met Ile Val Ala Thr Glu Gly Phe Arg Ser Ser
 195 200 205

Gly Ser Ala Ser Ile Thr Val Ser
 210 215

<210> 46
 <211> 236
 <212> PRT
 <213> C. mixtus

<400> 46

Met Lys Phe Pro Leu Ile Gly Lys Ser Thr Leu Ala Ala Leu Phe Cys
 1 5 10 15

Ser Ala Leu Leu Gly Val Asn Asn Thr Gln Ala Gln Thr Leu Thr Asn
 20 25 30

Asn Ala Thr Gly Thr His Asn Gly Phe Tyr Tyr Thr Phe Trp Lys Asp
 35 40 45

Ser Gly Asp Ala Ser Met Gly Leu Gln Ala Gly Gly Arg Tyr Thr Ser
 50 55 60

Gln Trp Ser Asn Gly Thr Asn Asn Trp Val Gly Gly Lys Gly Trp Asn
 65 70 75 80

Pro Gly Gly Pro Lys Val Val Thr Tyr Ser Gly Ser Tyr Asn Val Asp
 85 90 95

Asn Ser Gln Asn Ser Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Ser Pro
 100 105 110

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

Ser Cys Ser Gly Gly Thr Asp Tyr Gly Ser Phe Gln Ser Asp Gly Ala
 130 135 140

Thr Tyr Asn Val Arg Arg Cys Gln Arg Val Gln Gln Pro Ser Ile Asp
 145 150 155 160

Gly Thr Gln Thr Phe Tyr Gln Tyr Phe Ser Val Arg Ser Pro Lys Lys
 165 170 175

Gly Phe Gly Gln Ile Ser Gly Thr Ile Thr Thr Ala Asn His Phe Asn
 180 185 190

Phe Trp Ala Ser Lys Gly Leu Asn Leu Gly Asn His Asp Tyr Met Val
 195 200 205

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

Ser Glu Gly Thr Gly Gly Thr Thr Ser Ser Ser Val
 225 230 235

<210> 47

- 52GG -

<211> 237
 <212> PRT
 <213> P. fluorescens cellulosa

<400> 47

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

<210> 48
 <211> 217
 <212> PRT
 <213> P. cochleariae

<400> 48

- 52HH -

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

<210> 49
 <211> 182
 <212> PRT
 <213> A. kawachi

<400> 49

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

- 52II -

Asn Ala Ile Thr Tyr Ser Ala Glu Tyr Ser Ala Ser Gly Ser Ser Ser
 50 55 60
 Tyr Leu Ala Val Tyr Gly Trp Val Asn Tyr Pro Gln Ala Glu Tyr Tyr
 65 70 75 80
 Ile Val Glu Asp Tyr Gly Asp Tyr Asn Pro Cys Ser Ser Ala Thr Ser
 85 90 95
 Leu Gly Thr Val Tyr Ser Asp Gly Ser Thr Tyr Gln Val Cys Thr Asp
 100 105 110
 Thr Arg Thr Asn Glu Pro Ser Ile Thr Gly Thr Ser Thr Phe Thr Gln
 115 120 125
 Tyr Phe Ser Val Arg Glu Ser Thr Arg Thr Ser Gly Thr Val Thr Val
 130 135 140
 Ala Asn His Phe Asn Phe Trp Ala Gln His Gly Phe Gly Asn Ser Asp
 145 150 155 160
 Phe Asn Tyr Gln Val Met Ala Val Glu Ala Trp Ser Gly Ala Gly Ser
 165 170 175
 Ala Ser Val Thr Ile Ser
 180

<210> 50
 <211> 211
 <212> PRT
 <213> A. niger

<400> 50

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

- 52JJ -

Tyr Ser Asp Gly Ser Thr Tyr Gln Val Cys Thr Asp Thr Arg Thr Asn
 130 135 140

Glu Pro Ser Ile Thr Gly Thr Ser Thr Phe Thr Gln Tyr Phe Ser Val
 145 150 155 160

Arg Glu Ser Thr Arg Thr Ser Gly Thr Val Thr Val Ala Asn His Phe
 165 170 175

Asn Phe Trp Ala Gln His Gly Phe Gly Asn Ser Asp Phe Asn Tyr Gln
 180 185 190

Val Met Ala Val Glu Ala Trp Ser Gly Ala Gly Ser Ala Ser Val Thr
 195 200 205

Ile Ser Ser
 210

<210> 51
 <211> 210
 <212> PRT
 <213> A .tubigensis

<400> 51

Met Lys Val Thr Ala Ala Phe Ala Gly Leu Leu Val Thr Ala Phe Ala
 1 5 10 15

Ala Pro Ala Pro Glu Pro Asp Leu Val Ser Arg Ser Ala Gly Ile Asn
 20 25 30

Tyr Val Gln Asn Tyr Asn Gly Asn Leu Gly Asp Phe Thr Tyr Asp Glu
 35 40 45

Ser Ala Gly Thr Phe Ser Met Tyr Trp Glu Asp Gly Val Ser Ser Asp
 50 55 60

Phe Val Val Gly Leu Gly Trp Thr Thr Gly Ser Ser Thr Ile Thr Tyr
 65 70 75 80

Ser Ala Glu Tyr Ser Ala Ser Gly Ser Ala Ser Tyr Leu Ala Val Tyr
 85 90 95

Gly Trp Val Asn Tyr Pro Gln Ala Glu Tyr Tyr Ile Val Glu Asp Tyr
 100 105 110

Gly Asp Tyr Asn Pro Cys Ser Ser Ala Thr Ser Leu Gly Thr Val Tyr
 115 120 125

Ser Asp Gly Ser Thr Tyr Gln Val Cys Thr Asp Thr Arg Thr Asn Glu
 130 135 140

Pro Ser Ile Thr Gly Thr Ser Thr Phe Thr Gln Tyr Phe Ser Val Arg
 145 150 155 160

Glu Ser Thr Arg Thr Ser Gly Thr Val Thr Val Ala Asn His Phe Asn
 165 170 175

- 52KK -

Phe Trp Ala His His Gly Phe Gly Asn Ser Asp Phe Asn Tyr Gln Val
 180 185 190

Val Ala Val Glu Ala Trp Ser Gly Ala Gly Ser Ala Ser Val Thr Ile
 195 200 205

Ser Ser
 210

<210> 52
 <211> 208
 <212> PRT
 <213> P. purpurogenum

<400> 52

Met Lys Val Thr Ala Ala Phe Ala Gly Leu Leu Ala Arg His Ser Pro
 1 5 10 15

Pro Leu Ser Thr Glu Leu Val Thr Arg Ser Ile Asn Tyr Val Gln Asn
 20 25 30

Tyr Asn Gly Asn Leu Gly Ala Phe Ser Tyr Asn Glu Gly Ala Gly Thr
 35 40 45

Phe Ser Met Tyr Trp Gln Gln Gly Val Ser Asn Asp Phe Val Val Gly
 50 55 60

Leu Gly Arg Ser Thr Gly Ser Ser Asn Pro Ile Thr Tyr Ser Ala Ser
 65 70 75 80

Tyr Ser Ala Ser Gly Gly Ser Tyr Leu Ala Val Tyr Gly Trp Val Asn
 85 90 95

Ser Pro Gln Ala Glu Tyr Tyr Val Val Glu Ala Tyr Gly Asn Tyr Asn
 100 105 110

Pro Cys Ser Ser Gly Ser Ala Thr Asn Leu Gly Thr Val Ser Ser Asp
 115 120 125

Gly Gly Thr Tyr Gln Val Cys Thr Asp Thr Arg Val Asn Gln Pro Ser
 130 135 140

Ile Thr Gly Thr Ser Thr Phe Thr Gln Phe Phe Ser Val Arg Gln Gly
 145 150 155 160

Ser Arg Thr Ser Gly Thr Val Thr Ile Ala Asn His Phe Asn Phe Trp
 165 170 175

Ala Asn Asp Gly Phe Gly Asn Ser Asn Phe Asn Tyr Gln Val Val Ala
 180 185 190

Val Glu Ala Trp Ser Gly Thr Gly Thr Ala Ser Val Thr Val Ser Ala
 195 200 205

<210> 53
 <211> 233
 <212> PRT
 <213> P. purpurogenum

- 52LL -

<400> 53

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Met Lys Val Thr Ala Ala Phe Ala Gly Leu Leu Ala Arg His Ser Pro
1          5          10          15

Pro Leu Ser Thr Glu Leu Val Thr Arg Ser Ile Asn Tyr Val Gln Asn
          20          25          30

Tyr Asn Gly Asn Leu Gly Ala Phe Ser Tyr Asn Glu Gly Ala Gly Thr
          35          40          45

Phe Ser Met Tyr Trp Gln Gln Gly Val Ser Asn Asp Phe Val Val Gly
          50          55          60

Leu Gly Arg Ser Thr Gly Ser Ser Asn Pro Ile Thr Tyr Ser Ala Ser
65          70          75          80

Tyr Ser Ala Ser Gly Gly Ser Tyr Leu Ala Val Tyr Gly Trp Val Asn
          85          90          95

Ser Pro Gln Ala Glu Tyr Tyr Val Val Glu Ala Tyr Gly Asn Tyr Asn
          100          105          110

Pro Cys Ser Ser Gly Ser Ala Thr Asn Leu Gly Thr Val Ser Ser Asp
          115          120          125

Gly Gly Thr Tyr Gln Val Cys Thr Asp Thr Arg Val Asn Gln Pro Ser
          130          135          140

Ile Thr Gly Thr Ser Thr Phe Thr Gln Phe Phe Ser Val Arg Gln Gly
145          150          155          160

Ser Arg Thr Ser Gly Thr Val Thr Ile Ala Asn His Phe Asn Phe Trp
          165          170          175

Ala Asn Asp Gly Phe Gly Asn Ser Asn Phe Asn Tyr Gln Val Val Ala
          180          185          190

Val Glu Ala Trp Ser Gly Thr Gly Thr Ala Ser Val Thr Val Ser Ala
          195          200          205

Asn Phe Asn Tyr Gln Val Leu Ala Val Glu Gly Phe Ser Gly Ser Gly
          210          215          220

Asn Ala Asn Met Lys Leu Ile Ser Gly
225          230

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<210> 54

<211> 229

<212> PRT

<213> T. reesei

<400> 54

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

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- 52MM -

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

<210> 55
 <211> 227
 <212> PRT
 <213> B. pumilus

<400> 55

Met Asn Leu Lys Arg Leu Arg Leu Leu Phe Val Met Cys Ile Gly Phe
 1 5 10 15
 Val Leu Thr Leu Thr Ala Val Pro Ala His Ala Glu Thr Ile Tyr Asp
 20 25 30
 Asn Arg Ile Gly Thr His Ser Gly Tyr Asp Phe Glu Leu Trp Lys Asp
 35 40 45

- 52NN -

Tyr Gly Asn Thr Ser Met Thr Leu Asn Asn Gly Gly Ala Phe Ser Ala
50 55 60

Ser Trp Asn Asn Ile Gly Asn Ala Leu Phe Arg Lys Gly Lys Lys Phe
65 70 75 80

Asp Ser Thr Lys Thr His His Gln Leu Gly Asn Ile Ser Ile Asn Tyr
85 90 95

Asn Ala Ala Phe Asn Pro Gly Gly Asn Ser Tyr Leu Cys Val Tyr Gly
100 105 110

Trp Thr Gln Ser Pro Leu Ala Glu Tyr Tyr Ile Val Glu Ser Trp Gly
115 120 125

Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Ser Phe Tyr Ala Asp Gly
130 135 140

Gly Thr Tyr Asp Ile Tyr Glu Thr Leu Arg Val Asn Gln Pro Ser Ile
145 150 155 160

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

Arg Thr Ser Gly Thr Ala Ser Val Ser Glu His Phe Lys Lys Trp Glu
180 185 190

Ser Leu Gly Met Pro Met Gly Lys Met Tyr Glu Thr Ala Leu Thr Val
195 200 205

Glu Gly Tyr Arg Ser Asn Gly Ser Ala Asn Val Met Thr Asn Gln Leu
210 215 220

Met Ile Arg
225

<210> 56
<211> 228
<212> PRT
<213> B. pumilus

<400> 56

Met Asn Leu Arg Lys Leu Arg Leu Leu Phe Val Met Cys Ile Gly Leu
1 5 10 15

Thr Leu Ile Leu Thr Ala Val Pro Ala His Ala Arg Thr Ile Thr Asn
20 25 30

Asn Glu Met Gly Asn His Ser Gly Tyr Asp Tyr Glu Leu Trp Lys Asp
35 40 45

Tyr Gly Asn Thr Ser Met Thr Leu Asn Asn Gly Gly Ala Phe Ser Ala
50 55 60

Gly Trp Asn Asn Ile Gly Asn Ala Leu Phe Arg Lys Gly Lys Lys Phe
65 70 75 80

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Asp Ser Thr Arg Thr His His Gln Leu Gly Asn Ile Ser Ile Asn Tyr
85 90 95

Asn Ala Ser Phe Asn Pro Gly Gly Asn Ser Tyr Leu Cys Val Tyr Gly
100 105 110

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

Thr Tyr Arg Pro Thr Gly Ala Tyr Lys Gly Ser Phe Tyr Ala Asp Gly
130 135 140

Gly Thr Tyr Asp Ile Tyr Glu Thr Thr Arg Val Asn Gln Pro Ser Ile
145 150 155 160

Ile Gly Ile Ala Thr Phe Lys Gln Tyr Trp Ser Val Arg Gln Thr Lys
165 170 175

Arg Thr Ser Gly Thr Val Ser Val Ser Ala His Phe Arg Lys Trp Glu
180 185 190

Ser Leu Gly Met Pro Met Gly Lys Met Tyr Glu Thr Ala Phe Thr Val
195 200 205

Glu Gly Tyr Gln Ser Ser Gly Ser Ala Asn Val Met Thr Asn Gln Leu
210 215 220

Phe Ile Gly Asn
225

<210> 57
<211> 261
<212> PRT
<213> C. acetobutylicum

<400> 57

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

Thr Ser Leu Thr Ile Val Asp Asn Thr Ala Phe Ala Ala Thr Asn Leu
20 25 30

Asn Thr Thr Glu Ser Thr Phe Ser Lys Glu Val Leu Ser Thr Gln Lys
35 40 45

Thr Tyr Ser Ala Phe Asn Thr Gln Ala Ala Pro Lys Thr Ile Thr Ser
50 55 60

Asn Glu Ile Gly Val Asn Gly Gly Tyr Asp Tyr Glu Leu Trp Lys Asp
65 70 75 80

Tyr Gly Asn Thr Ser Met Thr Leu Lys Asn Gly Gly Ala Phe Ser Cys
85 90 95

Gln Trp Ser Asn Ile Gly Asn Ala Leu Phe Arg Lys Gly Lys Lys Phe
100 105 110

- 52PP -

Asn Asp Thr Gln Thr Tyr Lys Gln Leu Gly Asn Ile Ser Val Asn Tyr
 115 120 125

Asp Cys Asn Tyr Gln Pro Tyr Gly Asn Ser Tyr Leu Cys Val Tyr Gly
 130 135 140

Trp Thr Ser Ser Pro Leu Val Glu Tyr Tyr Ile Val Asp Ser Trp Gly
 145 150 155 160

Ser Trp Arg Pro Pro Gly Gly Thr Ser Lys Gly Thr Ile Thr Val Asp
 165 170 175

Gly Gly Ile Tyr Asp Ile Tyr Glu Thr Thr Arg Ile Asn Gln Pro Ser
 180 185 190

Ile Gln Gly Asn Thr Thr Phe Lys Gln Tyr Trp Ser Val Arg Arg Thr
 195 200 205

Lys Arg Thr Ser Gly Thr Ile Ser Val Ser Lys His Phe Ala Ala Trp
 210 215 220

Glu Ser Lys Gly Met Pro Leu Gly Lys Met His Glu Thr Ala Phe Asn
 225 230 235 240

Ile Glu Gly Tyr Gln Ser Ser Gly Lys Ala Asp Val Asn Ser Met Ser
 245 250 255

Ile Asn Ile Gly Lys
 260

<210> 58
 <211> 234
 <212> PRT
 <213> C. thermocellum

<400> 58

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

Val Ser Leu Thr Leu Phe Pro Val Asn Val Arg Ala Asp Val Val Ile
 20 25 30

Thr Ser Asn Gln Thr Gly Thr Gly Gly Gly Tyr Asn Phe Glu Tyr Trp
 35 40 45

Lys Asp Thr Gly Asn Gly Thr Met Val Leu Lys Asp Gly Gly Ala Phe
 50 55 60

Ser Cys Glu Trp Ser Asn Ile Asn Asn Ile Leu Phe Arg Lys Gly Phe
 65 70 75 80

Lys Tyr Asp Glu Thr Lys Thr His Asp Gln Leu Gly Tyr Ile Thr Val
 85 90 95

Thr Tyr Ser Cys Asn Tyr Gln Pro Asn Gly Asn Ser Tyr Leu Gly Val
 100 105 110

- 52QQ -

Tyr Gly Trp Thr Ser Asn Pro Leu Val Glu Tyr Tyr Ile Ile Glu Ser
 115 120 125
 Trp Gly Thr Trp Arg Pro Pro Gly Ala Thr Pro Lys Gly Thr Ile Thr
 130 135 140
 Val Asp Gly Gly Thr Tyr Glu Ile Tyr Glu Thr Thr Arg Val Asn Gln
 145 150 155 160
 Pro Ser Ile Lys Gly Thr Ala Thr Phe Gln Gln Tyr Trp Ser Val Arg
 165 170 175
 Thr Ser Lys Arg Thr Ser Gly Thr Ile Ser Val Thr Glu His Phe Lys
 180 185 190
 Ala Trp Glu Arg Leu Gly Met Lys Met Gly Lys Met Tyr Glu Val Ala
 195 200 205
 Leu Val Val Glu Gly Tyr Gln Ser Ser Gly Lys Ala Asp Val Thr Ser
 210 215 220
 Met Thr Ile Thr Val Gly Asn Ala Pro Ser
 225 230
 <210> 59
 <211> 230
 <212> PRT
 <213> Bacillus sp 41M-1
 <400> 59
 Met Lys Gln Val Lys Ile Met Phe Leu Met Thr Met Phe Leu Gly Ile
 1 5 10 15
 Gly Leu Leu Phe Phe Ser Glu Asn Ala Glu Ala Ala Ile Thr Ser Asn
 20 25 30
 Glu Ile Gly Thr His Asp Gly Tyr Asp Tyr Glu Phe Trp Lys Asp Ser
 35 40 45
 Gly Gly Ser Gly Ser Met Thr Leu Asn Ser Gly Gly Thr Phe Ser Ala
 50 55 60
 Gln Trp Ser Asn Val Asn Asn Ile Leu Phe Arg Lys Gly Lys Lys Phe
 65 70 75 80
 Asp Glu Thr Gln Thr His Gln Gln Ile Gly Asn Met Ser Ile Asn Tyr
 85 90 95
 Gly Ala Thr Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Thr Val Tyr Gly
 100 105 110
 Trp Thr Val Asp Pro Leu Val Glu Phe Tyr Ile Val Asp Ser Trp Gly
 115 120 125
 Thr Trp Arg Pro Pro Gly Gly Thr Pro Lys Gly Thr Ile Asn Val Asp
 130 135 140

- 52RR -

Gly Gly Thr Tyr Gln Ile Tyr Glu Thr Thr Arg Tyr Asn Gln Pro Ser
145 150 155 160

Ile Lys Gly Thr Ala Thr Phe Gln Gln Tyr Trp Ser Val Arg Thr Ser
165 170 175

Lys Arg Thr Ser Gly Thr Ile Ser Val Ser Glu His Phe Arg Ala Trp
180 185 190

Glu Ser Leu Gly Met Asn Met Gly Asn Met Tyr Glu Val Ala Leu Thr
195 200 205

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

Leu Thr Ile Gly Gly Gln
225 230

<210> 60

<211> 217

<212> PRT

<213> P. multivesiculatum

<400> 60

Glu Lys Val Ile Cys Leu Leu Ile Ala Leu Phe Gly Leu Ile Glu Ala
1 5 10 15

Gln Thr Phe Tyr Asn Asn Ala Gln Gly Gln Ile Asp Gly Leu Asp Tyr
20 25 30

Glu Leu Trp Lys Asp Thr Gly Thr Thr Ser Met Thr Leu Leu Gly Gly
35 40 45

Gly Lys Phe Ser Cys Ser Trp Ser Asn Ile Asn Asn Cys Leu Phe Arg
50 55 60

Ile Gly Lys Lys Trp Asn Cys Gln Tyr Glu Trp Trp Glu Leu Gly Thr
65 70 75 80

Val Leu Val Asn Tyr Asp Val Asp Tyr Asn Pro Asn Gly Asn Ser Tyr
85 90 95

Leu Cys Ile Tyr Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Ile
100 105 110

Val Glu Ser Trp Gly Ser Trp Arg Pro Pro Gly Gly Ser Pro Met Asn
115 120 125

Thr Met Tyr Val Asp Asp Gly Gln Tyr Asp Val Tyr Val Thr Asp Arg
130 135 140

Ile Asn Gln Pro Ser Ile Asp Gly Asn Thr Asn Phe Lys Gln Tyr Trp
145 150 155 160

Ser Val Arg Thr Gln Lys Lys Thr Arg Gly Thr Val His Val Asn His
165 170 175

- 52SS -

His Phe Tyr Asn Trp Gln Glu Met Gly Leu Lys Val Gly Lys Val Tyr
 180 185 190

Glu Ala Ser Leu Asn Ile Glu Gly Tyr Gln Ser Ala Gly Ser Ala Thr
 195 200 205

Val Asn Lys Asn Glu Val Val Gln Thr
 210 215

<210> 61
 <211> 175
 <212> PRT
 <213> P multivesiculatum

<400> 61

Met Thr Leu Leu Gly Gly Gly Lys Phe Ser Cys Asn Trp Ser Asn Ile
 1 5 10 15

Gly Asn Ala Leu Phe Arg Ile Gly Lys Lys Trp Asp Cys Thr Lys Thr
 20 25 30

Trp Gln Gln Leu Gly Thr Ile Ser Val Ala Tyr Asn Val Asp Tyr Arg
 35 40 45

Pro Asn Gly Asn Ser Tyr Met Cys Val Tyr Gly Trp Thr Arg Ser Pro
 50 55 60

Leu Ile Glu Tyr Tyr Ile Val Asp Ser Trp Gly Ser Trp Arg Pro Pro
 65 70 75 80

Gly Ser Asn Ser Met Gly Thr Ile Asn Val Asp Gly Gly Thr Tyr Asp
 85 90 95

Ile Tyr Val Thr Asp Arg Ile Asn Gln Pro Ser Ile Asp Gly Thr Thr
 100 105 110

Thr Phe Lys Gln Phe Trp Ser Val Arg Thr Gln Lys Lys Thr Ser Gly
 115 120 125

Val Ile Ser Val Ser Lys His Phe Glu Ala Trp Thr Ser Lys Gly Leu
 130 135 140

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

Ser Ser Gly Ser Ala Thr Val Asn Gln Asn Asp Val Thr Gly Gly
 165 170 175

<210> 62
 <211> 265
 <212> PRT
 <213> R. albus

<400> 62

Met Arg Asn Asn Phe Lys Met Arg Val Met Ala Gly Val Ala Ala Val
 1 5 10 15

- 52TT -

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

<210> 63

<211> 226

<212> PRT

<213> Caldicellulosiruptor sp

<400> 63

Met Cys Val Val Leu Ala Asn Pro Phe Tyr Ala Gln Ala Ala Met Thr
 1 5 10 15

- 52UU -

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

Asn Gly
225

<210> 64
 <211> 232
 <212> PRT
 <213> D. thermophilum

<400> 64

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

- 52VV -

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

<210> 65
 <211> 255
 <212> PRT
 <213> R. flavefaciens

<400> 65

Met	Lys	Leu	Ser	Lys	Ile	Lys	Lys	Val	Leu	Ser	Gly	Thr	Val	Ser	Ala
1				5					10					15	
Leu	Met	Ile	Ala	Ser	Ala	Ala	Pro	Val	Val	Ala	Ser	Ala	Ala	Asp	Gln
			20					25					30		
Gln	Thr	Arg	Gly	Asn	Val	Gly	Gly	Tyr	Asp	Tyr	Glu	Met	Trp	Asn	Gln
		35					40					45			
Asn	Gly	Gln	Gly	Gln	Ala	Ser	Met	Asn	Pro	Gly	Ala	Gly	Ser	Phe	Thr
	50					55					60				
Cys	Ser	Trp	Ser	Asn	Ile	Glu	Asn	Phe	Leu	Ala	Arg	Met	Gly	Lys	Asn
65					70					75					80

- 52WW -

Tyr Asp Ser Gln Lys Lys Asn Tyr Lys Ala Phe Gly Asn Ile Val Leu
 85 90 95
 Thr Tyr Asp Val Glu Tyr Thr Pro Arg Gly Asn Ser Tyr Met Cys Val
 100 105 110
 Tyr Gly Trp Thr Arg Asn Pro Leu Met Glu Tyr Tyr Ile Val Glu Gly
 115 120 125
 Trp Gly Asp Trp Arg Pro Pro Gly Asn Asp Gly Glu Val Lys Gly Thr
 130 135 140
 Val Ser Ala Asn Gly Asn Thr Tyr Asp Ile Arg Lys Thr Met Arg Tyr
 145 150 155 160
 Asn Gln Pro Ser Leu Asp Gly Thr Ala Thr Phe Pro Gln Tyr Trp Ser
 165 170 175
 Val Arg Gln Thr Ser Gly Ser Ala Asn Asn Gln Thr Asn Tyr Met Lys
 180 185 190
 Gly Thr Ile Asp Val Thr Lys His Phe Asp Ala Trp Ser Ala Ala Gly
 195 200 205
 Leu Asp Met Ser Gly Thr Leu Tyr Glu Val Ser Leu Asn Ile Glu Gly
 210 215 220
 Tyr Arg Ser Asn Gly Ser Ala Asn Val Lys Ser Val Ser Val Thr Gln
 225 230 235 240
 Gly Gly Ser Ser Asp Asn Gly Gly Gln Gln Gln Asn Asn Asp Trp
 245 250 255

<210> 66
 <211> 280
 <212> PRT
 <213> P. stipitis

<400> 66

Met Thr Val Tyr Lys Arg Lys Ser Arg Val Leu Ile Ala Val Val Thr
 1 5 10 15
 Leu Leu His Val Leu Ser His Ala Pro Thr Lys Met Leu Thr Thr Asp
 20 25 30
 Val Leu Leu Thr Arg Cys Met His Leu Cys His Phe Arg Thr Ser Asp
 35 40 45
 Ser Val Tyr Thr Asn Glu Thr Ser Glu Glu Arg Ser Met Ser Asp Arg
 50 55 60
 Leu Asn Ile Thr Arg Val Met Ser Tyr Asp Arg Trp Thr Asp Leu Val
 65 70 75 80
 Gly Glu Leu Glu Val Arg Glu Leu Lys His Val Met Ser His Arg Thr
 85 90 95

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Tyr	Ser	Leu	Cys	Asp	Leu	Ser	Cys	Ser	Thr	Val	Leu	Asp	Ser	Asn	Ser			
			100					105					110					
Met	Phe	Ser	Leu	Gly	Lys	Gly	Trp	Gln	Ala	Ile	Ser	Ser	Arg	Gln	Gly			
		115					120					125						
Val	Gly	Ala	Thr	Val	Tyr	Gly	Trp	Thr	Arg	Ser	Pro	Leu	Leu	Ile	Glu			
	130					135					140							
Tyr	Tyr	Val	Val	Asp	Ser	Trp	Gly	Ser	Tyr	His	Pro	Ser	Asn	Thr	Ile			
145					150					155					160			
Thr	Gly	Thr	Phe	Val	Thr	Val	Lys	Cys	Asp	Gly	Gly	Thr	Tyr	Asp	Ile			
				165					170					175				
Tyr	Thr	Ala	Val	Arg	Val	Asn	Ala	Pro	Ser	Ile	Glu	Gly	Thr	Thr	Phe			
			180					185					190					
Thr	Gln	Tyr	Trp	Ser	Val	Arg	Gln	Ser	Ala	Thr	Ile	Gln	Leu	Ala	Val			
	195						200					205						
Ile	Lys	Pro	Leu	Thr	Leu	Gln	Asn	Ala	Thr	Ile	Thr	Phe	Thr	Phe	Ser			
	210					215					220							
Asn	His	Phe	Asp	Ala	Trp	Lys	Thr	Met	Thr	Leu	Glu	Ala	Thr	His	Ser			
225					230					235					240			
Thr	Glu	Gly	Tyr	Phe	Ser	Ser	Gly	Ile	Thr	Tyr	Glu	Gln	Pro	His	Gln			
				245				250						255				
Pro	His	Arg	Asn	Thr	Trp	Ala	Thr	Ser	Leu	Thr	Ser	Gln	Thr	Lys	His			
			260					265					270					
Thr	Ala	Arg	Ser	Leu	Pro	Ile	Asn											
		275					280											

CLAIMS:

1. A variant polypeptide having xylanase activity, wherein the variant polypeptide is a family 11 xylanase, comprising a mutation selected from D11Y, D11F, D11K, D11F/R122D and D11F/G34D with reference to the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other Family 11 xylanases, such that the variant polypeptide has reduced sensitivity to a xylanase inhibitor as compared with a polypeptide having the amino acid sequence shown as SEQ. ID. No. 1.
2. The variant polypeptide according to claim 1 comprising mutation D11F.
3. The variant polypeptide according to claim 1 or claim 2 comprising mutation D11F/R122D.
4. The variant polypeptide according to any one of claims 1 to 3, wherein said inhibitor is an inhibitor found naturally in plant tissues.
5. A composition comprising the variant polypeptide according to any one of claims 1 to 4 and flour.
6. A method of degrading or reducing the viscosity derived from the presence of hemicellulose or arabinoxylan in plant cell wall material which method comprises contacting said plant cell wall with the polypeptide according to any one of claims 1 to 4 or the composition according to claim 5.
7. A method of processing a plant material which method comprises contacting said plant material with the polypeptide according to any one of claims 1 to 4, or the composition according to claim 5.
8. A nucleic acid molecule encoding the variant polypeptide according to any one of claims 1 to 4.
9. A construct comprising the nucleic acid molecule of claim 8.

10. Use of the variant polypeptide according to any one of claims 1 to 4 in a method of reducing the viscosity derived from the presence of hemicellulose or arabinoxylan in plant cell wall material.

11. Use of the variant polypeptide according to any one of claims 1 to 4, in one or more of: bakery, animal feed, starch production, flour separation or, paper and pulp production.

12. Use of the variant polypeptide according to any one of claims 1 to 4, in one or more of: processing cereals, starch production, animal feed, in processing wood, or enhancing the bleaching process of wood pulp.

13. A method of selecting a xylanase polypeptide with altered sensitivity to an inhibitor, which method comprises:

modifying one or more amino acid residues of a parent xylanase polypeptide; and

measuring the sensitivity of the modified xylanase polypeptide to a xylanase inhibitor; wherein the modified xylanase polypeptide has reduced sensitivity to a xylanase inhibitor as compared with the parent xylanase polypeptide;

measuring the effect of said modified xylanase polypeptide on viscosity of flour slurry; wherein the modified xylanase polypeptide decreases viscosity of flour slurry more than the parent xylanase polypeptide;

wherein said modified xylanase polypeptide has at least one of said amino acids modifications at any one of positions 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 or 175 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other homologous xylanases, wherein at least one of said amino acid modifications is at least at position 11, 12 or 13 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1 or at equivalent position(s) in other homologous xylanases and wherein said modified xylanase polypeptide is a family 11 xylanase.

14. A method of selecting a xylanase polypeptide with altered sensitivity to an inhibitor, which method comprises:

modifying two or more amino acid residues of a parent xylanase polypeptide;

wherein the modified xylanase polypeptide has reduced sensitivity to a xylanase inhibitor as compared with the parent xylanase polypeptide;

measuring the effect of said modified xylanase polypeptide on viscosity of flour slurry; wherein the modified xylanase polypeptide decreases viscosity of flour slurry more than the parent xylanase polypeptide;

wherein said modifications are to two or more of positions 11, 12, 13, 15, 17, 29, 31, 32, 34, 113, 114, 119, 120, 121, 122, 123, 124 and 175 of the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, or at equivalent position(s) in other homologous xylanases, wherein one of said amino acid modifications is at position 11 and wherein said modified xylanase polypeptide is a family 11 xylanase.

15. The method of claim 14, further comprising measuring the sensitivity of said modified xylanase polypeptide to a xylanase inhibitor;

16. A method according to any one of claims 13 to 15, wherein said amino acid modification is carried out by site-directed mutagenesis.

17. A method according to any one of claims 13 to 16, wherein one of said amino acids modifications is any of: D11Y, D11N, D11F, D11K, D11S, D11W, G12F, G13F, I15K, N17K, N17Y, N17D, N29K, N29Y, N29D, S31K, S31Y, S31D, N32K, G34D, G34F, G34T, Y113A, Y113D, Y113K, N114A, N114D, N114F, N114K, D119K, D119Y, D119N, G120K, G120D, G120F, G120Y, G120N, D121N, D121K, D121F, D121A, R122D, R122F, R122A, T123K, T123Y, T123D, T124K, T124Y, T124D, Q175E, Q175S and Q175L.

18. A method according to any one of claims 13 to 17, wherein one of said amino acids modifications is any of: D11Y, D11N, D11F, D11K, D11S or D11W.

19. A method according to any one of claims 13 to 18, wherein one of said amino acids modifications is D11F.

20. A method according to any one of claims 13 to 19, wherein two of said amino acid modifications are D11F and R122D.

21. A variant xylanase polypeptide selected by the method according to any one of claims 13 to 20 wherein said variant xylanase polypeptide comprises a mutation selected from D11Y, D11F, D11K, D11F/R122D and D11F/G34D with reference to the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, and wherein said variant xylanase polypeptide is a family 11 xylanase.

22. Use of a variant xylanase polypeptide selected by the method according to any one of claims 13 to 20 or a variant xylanase polypeptide according to claim 21 in one or more of: bakery, animal feed, starch production, flour separation or, paper and pulp production, wherein said variant xylanase polypeptide comprises a mutation selected from D11Y, D11F, D11K, D11F/R122D and D11F/G34D with reference to the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, and wherein said variant xylanase polypeptide is a family 11 xylanase.

23. Use of a variant xylanase polypeptide selected by the method according to any one of claims 13 to 20 or a variant xylanase polypeptide according to claim 21 in one or more of: processing cereals, starch production, animal feed, in processing wood, or enhancing the bleaching process of wood pulp, wherein said variant xylanase polypeptide comprises a mutation selected from D11Y, D11F, D11K, D11F/R122D and D11F/G34D with reference to the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, and wherein said variant xylanase polypeptide is a family 11 xylanase.

24. Use of a variant xylanase polypeptide selected by the method according to any one of claims 13 to 20 or a variant xylanase polypeptide according to claim 21 to reduce the viscosity derived from the presence of hemicellulose or arabinoxylan in a solution or system comprising plant cell wall material, wherein said variant xylanase polypeptide comprises a mutation selected from D11Y, D11F, D11K, D11F/R122D and D11F/G34D with reference to the *B. subtilis* amino acid sequence shown as SEQ. ID. No. 1, and wherein said variant xylanase polypeptide is a family 11 xylanase.

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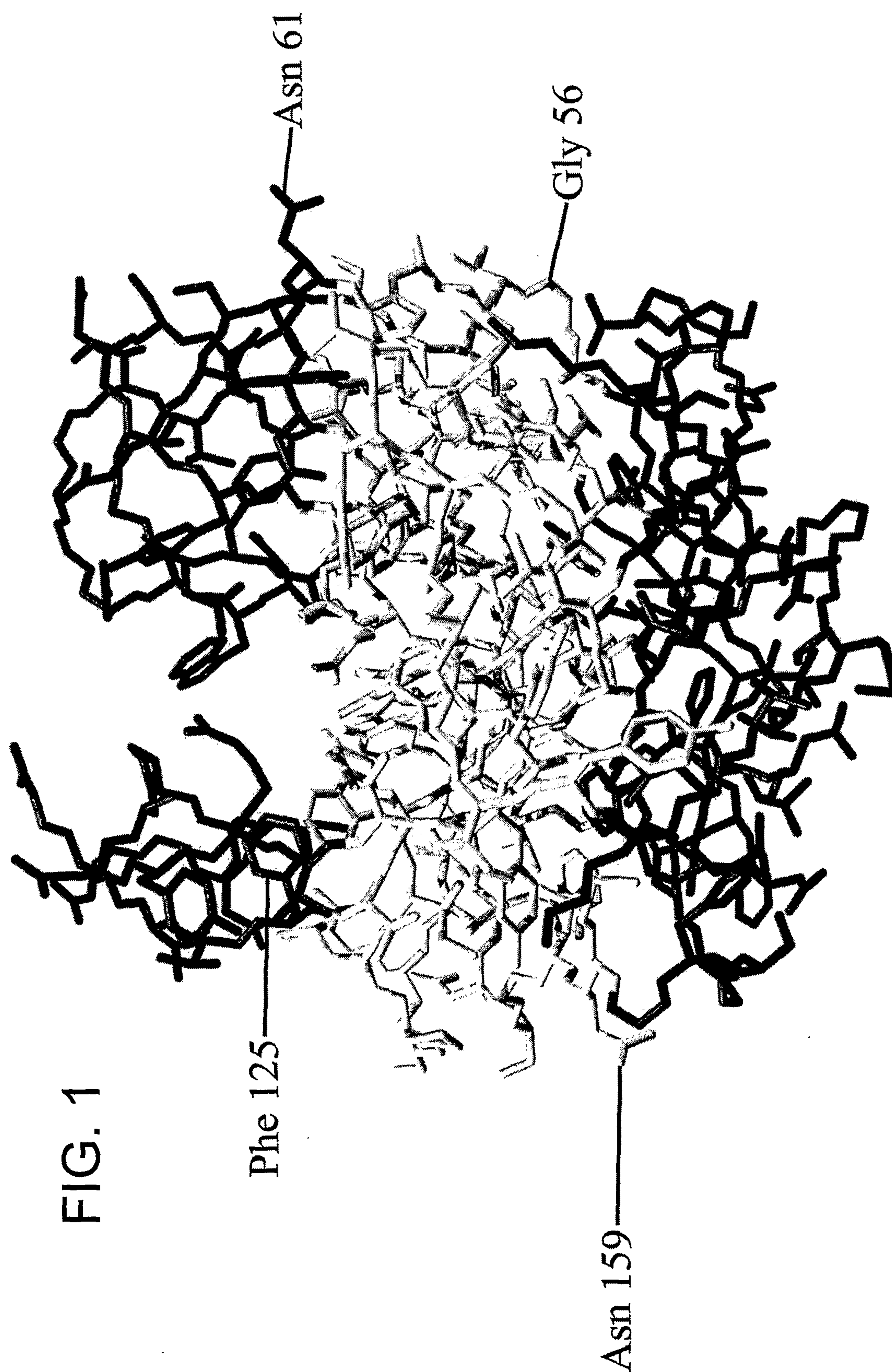


FIG. 2

B. subtilis xyla	1	15	16	30	31	45	46	60	61	75	76	90	33
B. circulans xyla	---	---	---	---	---	---	---	---	---	---	---	---	33
B. stearothermophilus xyla	---	---	---	---	---	---	---	---	---	---	---	---	31
A. caviae xyla	---	---	---	---	---	---	---	---	---	---	---	---	32
C. carbonum xyl1	---	---	---	---	---	---	---	---	---	---	---	---	44
H. turcicum xyl1	---	---	---	---	---	---	---	---	---	---	---	---	50
A. pisi xyl	---	---	---	---	---	---	---	---	---	---	---	---	50
S. commune xyla	---	---	---	---	---	---	---	---	---	---	---	---	16
T. lanuginosus xyla	---	---	---	---	---	---	---	---	---	---	---	---	45
C. carbonum xyl2	---	---	---	---	---	---	---	---	---	---	---	---	54
C. sativus xyl2	---	---	---	---	---	---	---	---	---	---	---	---	54
H. insolens xyl1	---	---	---	---	---	---	---	---	---	---	---	---	50
M. grisea xyl22	---	---	---	---	---	---	---	---	---	---	---	---	53
C. gracile cgrA	---	---	---	---	---	---	---	---	---	---	---	---	44
T. reesei xyl2(2)	---	---	---	---	---	---	---	---	---	---	---	---	47
T. reesei, ALKO2721 xyl2	---	---	---	---	---	---	---	---	---	---	---	---	47
T. reesei xyl1	---	---	---	---	---	---	---	---	---	---	---	---	46
T. harzianum xylD	---	---	---	---	---	---	---	---	---	---	---	---	14
T. viride xyl	---	---	---	---	---	---	---	---	---	---	---	---	47
C. gracile cgrxB	---	---	---	---	---	---	---	---	---	---	---	---	45
A. niger xyl2	---	---	---	---	---	---	---	---	---	---	---	---	50
Penicillium sp 40 xyla	---	---	---	---	---	---	---	---	---	---	---	---	46
Streptomyces sp xyl	---	---	---	---	---	---	---	---	---	---	---	---	64
Streptomyces sp xyl1	---	---	---	---	---	---	---	---	---	---	---	---	54
S. thermocyaneoviolaceus xylB	---	---	---	---	---	---	---	---	---	---	---	---	55
S. viridosporus T7A svxA	---	---	---	---	---	---	---	---	---	---	---	---	58
T. fusca xyl	---	---	---	---	---	---	---	---	---	---	---	---	56
C. pachnodae xyl11A	---	---	---	---	---	---	---	---	---	---	---	---	57
A. oryzae xylG1	---	---	---	---	---	---	---	---	---	---	---	---	46
C. purpurea xyl1	---	---	---	---	---	---	---	---	---	---	---	---	42
C. mixtus xyl	---	---	---	---	---	---	---	---	---	---	---	---	42
P. fluorescens cellulosa xyl	---	---	---	---	---	---	---	---	---	---	---	---	33
P. cochleariae xyl	---	---	---	---	---	---	---	---	---	---	---	---	4
A. kawachi xylC	---	---	---	---	---	---	---	---	---	---	---	---	32
A. niger xyl1	---	---	---	---	---	---	---	---	---	---	---	---	32
A. tubigenis xyl1	---	---	---	---	---	---	---	---	---	---	---	---	28
P. purpurogenum xylB	---	---	---	---	---	---	---	---	---	---	---	---	28
Cryptococcus sp S-2 xyl-CS2	---	---	---	---	---	---	---	---	---	---	---	---	55
T. reesei xyl2	---	---	---	---	---	---	---	---	---	---	---	---	42
B. pumilus xyla(1)	---	---	---	---	---	---	---	---	---	---	---	---	42
B. pumilus xyla(2)	---	---	---	---	---	---	---	---	---	---	---	---	74
C. acetobutylicum xylB	---	---	---	---	---	---	---	---	---	---	---	---	44
C. thermocellum xylB	---	---	---	---	---	---	---	---	---	---	---	---	41
Bacillus sp 41M-1 xylJ	---	---	---	---	---	---	---	---	---	---	---	---	31
P. multivesiculatum xyla	---	---	---	---	---	---	---	---	---	---	---	---	0
P. multivesiculatum xyl	---	---	---	---	---	---	---	---	---	---	---	---	69
R. albus xyla	---	---	---	---	---	---	---	---	---	---	---	---	29
Caldicellulosiruptor sp xylD	---	---	---	---	---	---	---	---	---	---	---	---	42
D. thermophilum xynB	---	---	---	---	---	---	---	---	---	---	---	---	42
R. flavefaciens xyla	---	---	---	---	---	---	---	---	---	---	---	---	72
P. stipitis xyla	---	---	---	---	---	---	---	---	---	---	---	---	

FIG. 2CONT'D

- B. subtilis xyla
B. circulans xyla
B. stearothermophilus xyla
A. caviae xyla
C. carbonum xyl1
H. turcicum xyl1
A. pisi xyl
S. commune xyla
T. lanuginosus xyla
C. carbonum xyl2
C. sativus xyl2
H. insolens xyl1
M. grisea xyl22
C. gracile cgxA
T. reesei xyl2(2)
T. reesei, ALKO2721 xyl2
T. reesei xyl1
T. harzianum xylD
T. viride xyl
C. gracile cgxB
A. niger xyl2
Penicillium sp 40 xyla
Streptomyces sp xyl
Streptomyces sp xyl1
S. thermocyanoeviolaceus xylB
S. viridosporus T7A svxA
T. fusca xyl
C. pachnodae xyl11A
A. oryzae XylG1
C. purpurea xyl1
C. mixtus xyl
P. fluorescens cellulosa xyl
P. cochleariae xyl
A. kawachi xylC
A. niger xyl1
A. tubigenis xyl1
P. purpurogenum xylB
Cryptococcus sp S-2 xyl1-CS2
T. reesei xyl2
B. pumilus xyla(1)
B. pumilus xyla(2)
C. acetobutylicum xylB
C. thermocellum XylB
Bacillus sp 41M-1 xylJ
P. multivesiculatum xyla
P. multivesiculatum xyl
R. albus xyla
Caldicellulosiruptor sp xylD
D. thermophilum xynB
R. flavefaciens xyla
P. stipitis xyla

91 105 106 120 121 135 136 150 151 165 166 180
WQNTDGGGIWNAVN GSGENYSVNWS--NT GNFVVGKGTG--- --SPERTINYNAGVW APNGNG--YLTLYG TRS-PLIEYYVVDWSW 113
WQNTDGGGIWNAVN GSGENYSVNWS--NT GNFVVGKGTG--- --SPERTINYNAGVW APNGNG--YLTLYG TRS-PLIEYYVVDWSW 113
WQYTDGGGMVNAVN GPGENYSVTWQ--NT GNFVVGKGTG--- --SPNRVINYNAGIW EPSGNG--YLTLYG TRN-ALIEYYVVDWSW 111
WQNTDGGGTWNAVN GSGENYSVSQW--NT GNFVVGKGTG--- --TPNRVVNYNAGVW APSGNG--YLTLYG TRN-ALIEYYVVDWSW 112
WSWSDGGEARATYTN GAGGSYSVSWG--SG GNLVGGKGNP----- --GTARTITYSGTY NYNGNS--YLAIVG TRN-PLVEYYVVENE 122
YSWSDGGEARATYTN GAGGSYSVSWG--TG GNLVGGKGNP----- --GTARTITYSGQY NPNGNS--YLAIVG TRN-PLVEYYVVENE 128
YSWTDGGAQATYTN GAGGSYSVNWK--TG GNLVGGKGNP----- --GAARTITYSGTY SPSCNS--YLAIVG TRN-PLIEYYVVENE 128
YSWTDGAGDATYQN NGGSYTLTWSG--NN GNLVGGKGNP----- --GAASRSISYSGTY QPNCNS--YLSVYG TRS-SLIEYYIVESY 96
YSWSDGGAQATYTN LEGGTYEISWG--DG GNLVGGKGNP----- --GLNARAIHFEVY QPNCNS--YLAIVG TRN-PLVEYYIVENE 124
YSWTDGGSQAQYTM GEGSRYSVTWR--NT GNFVGGKGNP----- --GSGRVINYGGAF NPQNG--YLAIVG TRN-PLVEYYVIESY 132
YSWTDGGSQAQYTM GEGSRYSVTWR--NT GNFVGGKGNP----- --GTGRVINYGGAF NPQNG--YLAIVG TRN-PLVEYYVIESY 132
YSWSDGGGQVQYTN LEGSRYQVRWR--NT GNFVGGKGNP----- --GTGRITINYGGEY NPQNG--YLAIVG TRN-PLVEYYVIESY 128
YSWTDGASPVQYQN GNGGSYSVQWQ--SG GNFVGGKGNP----- --GSKSITYSGTF NPVNNGNAYLCIYG TQN-PLVEYYILLENY 133
YSFTDGGGTWNYQN GAGGSYSVQWQ--NC GNFVGGKGNP----- --GAARTINESGTF SPQNG--YLAIVG TQN-PLVEYYIVESF 122
HSYWNDDGGGTWNYTN GPGGQFSVNWS--NS GNFVGGKGNP----- --GTKNKVINFESSY NPNGNS--YLSVYG SRN-PLIEYYIVGNE 126
YSYWNDDGGGTWNYTN GPGGQFSVNWS--NS GNFVGGKGNP----- --GTKNKVINFESSY NPNGNS--YLSVYG SRN-PLIEYYIVENE 126
YSYWNDDGGGTWNYTN GPGGQFSVNWS--NS GNFVGGKGNP----- --GTKNKVINFESSY NPNGNS--YLSVYG SRN-PLIEYYIVENE 125
YSYWNDDHAGVTYTN GGGGSFTVNWS--NS GNFVAGKGNP----- --GTKNKVINFESSY NPNGNS--YLSIYG SRN-PLIEYYIVENE 93
YSYWNDDHSGVTYTN GAGGSFSVNWA--NS GNFVGGKGNP----- --GSSSRVINFESSY NPNGNS--YLSVYG SKN-PLIEYYIVENE 126
YSFTDGGQNVQYTN EAGGQYSVTWS--GN GNFVGGKGNP----- --GS-ARTINYTANY NPNGNS--YLAIVG TRN-PLIEYYVVENE 123
YSFTDGGGDVTYTN GDAGATYVWS--NV GNFVGGKGNP----- --GSAQDITYSGTF TPSCNG--YLSVYG TTD-PLIEYYIVESY 128
YSFTWNGGGTVQYTN GAAGEYSVTWE--NC GDFSCKGWST----- --GSARDITFEGTF NPSCNA--YLAIVG TTS-PLVEYYILEDY 124
YSFTDGGGSVSMNL NGGSYSYQWQ--NC GNFVAGKGNW----- --GGRRTVRYSGYF NPSCNG--YGCILYG TSN-PLVEYYIVDNW 142
YSFTDGGGSVSMNL ASGSYGTSTWT--NC GNFVAGKGNW----- --GARRTVNYSGSF NPSCNA--YLTLYG TAN-PLVEYYIVDNW 132
YSFTDAPGTVMNT GAGGNYSTQWS--NT GNFVAGKGNW----- --GRRRTVRYSGTF NPSCNA--YLAIVG SQN-PLVEYYIVDNW 133
YSFTDAPGTVMNT GSGGTYSTQWR--NT GNFVAGKGNW----- --GRRRTVRYSGTF NPSCNA--YLTLYG TTG-PLIEYYIVDNW 136
YSFTDAPGTVMNT GSGGNYSTSWR--NT GNFVAGKGNW----- --GRRRTVRYSGTF NPSCNA--YLTLYG TRN-PLVEYYIVESW 134
YSFTDAPGTVMNT GSGGNYSTSWR--NT GNFVAGKGNW----- --GRRRTVRYSGTF NPSCNA--YLAIVG SHD-PLVEYYIVDSW 135
YSFTWNGGGDVEYTN GNGGQYSVKWT--NC DNFVAGKGNP----- --GSARTISYSGTF NPSCNA--YLAIVG TQN-PLVEYYIVDKY 124
SSYWDYG-NTRYSC GAGGHYDLSWG--NG GNVVAGRWKP----- --ASPRAVTYSGSW QCNNGC--YLSVYG TIN-PLVEYYIVENE 119
YTFWKDSC-DASMGL QAGGRTYSQWSN-GT NNWVGKGNP----- --GG-PKVVTYSGSY NVDNSQNSYLALYG TRS-PLIEYYVIESY 122
YTFWKDSC-DASMTL LSGGRTYSQWSN-ST NNWVGKGNP----- --GNSRVISYSGSY GVDSSQNSYLALYG TRS-PLIEYYVIESY 123
YAFWKDSC-SATFTL ESGGRTYAGNWT--ST NNWVGKGNP----- --GNSWRTVNYSGY GINEYANSYLSLYG TTN-PLIEYYVIESY 114
YVQNYNGNLGDFTYD ESAGTFSMYWEDGVS SDFVVGIGWT----- --GS-SNATITYSAEY SASGSS-SYLAIVG VNY-PQAEYYIVEDY 85
YVQNYNGNLGDFTYD ESAGTFSMYWEDGVS SDFVVGIGWT----- --GS-SKAITITYSAEY SASGSS-SYLAIVG VNY-PQAEYYIVEDY 113
YVQNYNGNLGDFTYD ESAGTFSMYWEDGVS SDFVVGIGWT----- --G--SSTITYSAEY SASGSS-SYLAIVG VNY-PQAEYYIVEDY 112
YVQNYNGNLGDFTYD ESAGTFSMYWEDGVS SDFVVGIGWT----- --GS-SNPTITYSAEY SASGG--SYLAIVG VNS-PQAEYYVVEAY 108
YVQNYNGNLGDFTYD ESAGTFSMYWEDGVS SDFVVGIGWT----- --GT-GRITITYSGSY NPQYSG-SYQALYG TQGSLSSEYXVIDNY 108
YVQNYNGNLGDFTYD PSNTGFSVNWT--Q DDFVVGIGWT----- --GS-SAPINFESSF SVNSGT-GLLSVYG STN-PLVEYYIMEDN 133
FELWKDYG-NTSMTL NNGGAFSASWNNIGN ALFRKGGKFDSTKTH HQ-LGNISINXNAAF NPNGNS--YLCVYG TQS-PLAEYYIVESW 127
YELWKDYG-NTSMTL NNGGAFSAGWNNIGN ALFRKGGKFDSTKTH HQ-LGNISINXNAAF NPNGNS--YLCVYG TQS-PLAEYYIVDSW 127
YELWKDYG-NTSMTL KNGGAFSCQWSNIGN ALFRKGGKFDSTKTH KQ-LGNISVNDYCN QPYGNS--YLCVYG TSS-PLVEYYIVDSW 159
FEYWKDYG-NTSMTL KNGGAFSCQWSNIGN ALFRKGGKFDSTKTH DQ-LGYITVTYSCNY QPNCNS--YLCVYG TSN-PLVEYYIIIESW 129
YEFWKDSCGGSMTL NSGGTFSQWSNIGN ALFRKGGKFDSTKTH QQ-IGNMSINYGATY NPNGNS--YLTVYG TVD-PLVEFFIVDSW 127
YELWKDYG-TTSMTL LGGKFSQWSNIGN ALFRKGGKFDSTKTH WE-LGTVLVNDYD VNPNGNS--YLCIYG TRN-PLVEYYIVESW 116
-----MTL LGGKFSQWSNIGN ALFRKGGKFDSTKTH QQ-LGTISVXNDY RPNNGNS--YMCVYG TRS-PLIEYYIVDSW 74
YELWKDYG-DTEMTI NEGGTFSQWSNIGN ALFRKGGKFDSTKTH KE-LGNISVXNDY QPDGNS--YMCVYG TID-PLVEFFIVDSW 154
YELWKDYG-NTTMTV DTGGRFSCQWSNIGN ALFRKGGKFDSTKTH NQ-LGTVKITYSATY NPNGNS--YLCIYG SRN-PLVEFFIVDSW 112
YELWKDYG-NTTMTV YTGGRFSCQWSNIGN ALFRKGGKFDSTKTH QS-LGTIRITYSATY NPNGNS--YLCIYG STN-PLVEFFIVDSW 125
YEMWQNGQGOASMN PGAGSFTCSWSNIGN FLARMGKNYDSQKN YKAFGNIVLTYDVEY TPRGNS--YMCVYG TRN-PLMEYYIVGEG 129
YDRWTDLVGELEVRE LKHVMSHRTYSLCDL SCSTVLDNSNS----- --MFSLGKGNQWQALS SRQGVG--ATVYG TRSPLLEYYVVDWSW 151

FIG. 2CONT'D

- B. subtilis xyla
B. circulans xyla
B. stearothermophilus xyla
A. caviae xyla
C. carbonum xyl1
H. turcicum xyl1
A. pisi xyl
S. commune xyla
T. lanuginosus xyla
C. carbonum xyl2
C. sativus xyl2
H. insolens xyl1
M. grisea xyl22
C. gracile cgsA
T. reesei xyl2(2)
T. reesei, ALK02721 xyl2
T. reesei xyl1
T. harzianum xylD
T. viride xyl
C. gracile cgsB
A. niger xyl2
Penicillium sp 40 xyla
Streptomyces sp xyl
Streptomyces sp xyl1
S. thermocyanoeviolaceus xylB
S. viridosporus T7A svxA
T. fusca xyl
C. pachnodae xyl11A
A. oryzae xylG1
C. purpurea xyl1
C. mixtus xyl
P. fluorescens cellulosa xyl
P. cochleariae xyl
A. kawachi xylC
A. niger xyl1
A. tubigenis xyl1
P. purpurogenum xylB
Cryptococcus sp S-2 xyl-CS2
T. reesei xyl2
B. pumilus xyla(1)
B. pumilus xyla(2)
C. acetobutylicum xylB
C. thermocellum xylB
Bacillus sp 41M-1 xylJ
P. multivesiculatum xyla
P. multivesiculatum xyl
R. albus xyla
Caldicellulosiruptor sp xylD
D. thermophilum xynB
R. flavefaciens xyla
P. stipitis xyla

181	195	196	210	211	225	226	240	241	255	256	270	189
GTYPRTGTYKG----	TVKSDGGTYDYITTT	RYNAPSIDGDRITFT	QYWSVRQSKRPTG--	-----SNATITF	SNHVNAWKSHGMNLG	189						
GTYPRTGTYKG----	TVKSDGGTYDYITTT	RYNAPSIDGDRITFT	QYWSVRQSKRPTG--	-----SNATITF	TNHVNAWKSHGMNLG	189						
GTYRATGNYESG---	TVNSDGGTYDYITTM	RYNAPSIDG-TQTFF	QFWSVRQSKRPTG--	-----SNVSITF	SNHVNAWRSKGMNLG	187						
GTYPRTGTYKG----	TVNSDGGTYDYITTM	RYNAPSIDG-TQTFF	QYWSVRQSKRPTG--	-----VNSTITF	SNHVNAWPSKGMYL	187						
GTYPDPSSQS--QNK	TVTSDGSSYKIAQST	RTNQPSIDG-TRTFQ	QYWSVRQNKRRSSG--	-----SVNM	KTHFDAWASKGMNLG	196						
GTYPDPSSQA--QNK	TVTSDGSSYKIAQST	RTNQPSIDG-TRTFQ	QYWSVRQNKRRSSG--	-----SVNM	KTHFDAWASKGMNLG	202						
GTYPDPSSQA--TVK	SVTADGSSYKIAQTQ	RTNQPSIDG-TQTFF	QYWSVRQNKRRSSG--	-----SVNM	KTHFDAWASKGMNLG	202						
GSYDPSSAA--SHK	SVTCNGATYDILSTW	RYNAPSIDG-TQTFF	QFWSVRNPKKAPGG-	S-----ISGTVDV	QCHFDAWKGLGMNLG	175						
GTYPDPSSGA--TDL	TVECDGSIYRLGKTT	RVNAPSIDG-TQTFF	QYWSVRQDKRTSG--	-----TVQT	GCHFDAWARAGLNVN	198						
GTYNPSSGA--QIK	SFQTDGGTYNVAVST	RYNQPSIDG-TRTFQ	QYWSVRTQKRVGG--	-----SVNM	QNHFNAWSRYGLNLG	206						
GTYNPSSGA--QVK	SFQTDGGTYNVAVST	RYNQPSIDG-TRTFQ	QYWSVRQDKRVGG--	-----SVNM	QNHFNAWSRYGLNLG	206						
GTYNPGSQA--QYK	TFYTDGDQYDIFVST	RYNQPSIDG-TRTFQ	QYWSIRKNKRVGG--	-----SVNM	QNHFNAWQHGMPLG	202						
GEYNPGNSA--QSR	TLQAAAGGTYTLHEST	RVNQPSIEG-TRTFQ	QYWAIRQKRNSSG--	-----TVNT	GEFFQAWERAGMRMG	207						
GTYPDPSSQA--SKF	TIQDGGSTYTIKTT	RVNQPSIEG-TSTFD	QFWSVRQNKRRSSG--	-----SVNV	AAHFNAWAQAAGLKL	196						
GTYNPSTGA--TKL	EVTSDEGSVDIYRTQ	RVNQPSIIG-TATFY	QYWSVRNHRSSG--	-----SVNT	ANHFNAWAQAAGLKL	200						
GTYNPSTGA--TKL	EVTSDEGSVDIYRTQ	RVNQPSIIG-TATFY	QYWSVRNHRSSG--	-----SVNT	ANHFNAWAQAAGLKL	200						
GTYNPSTGA--TKL	EVTSDEGSVDIYRTQ	RVNQPSIIG-TATFY	QYWSVRNHRSSG--	-----SVNT	ANHFNAWAQAAGLKL	199						
GTYNPSTGA--TKL	EVTSDEGSVDIYRTQ	RVNQPSIIG-TATFY	QYWSVRNHRSSG--	-----SVNT	ANHFNAWAQAAGLKL	167						
GTYNPSTGA--TKL	EVTSDEGSVDIYRTQ	RVNQPSIIG-TATFY	QYWSVRNHRSSG--	-----SVNT	ANHFNAWAQAAGLKL	200						
GTYNPSTGT--TKL	EVTSDEGSVDIYRTQ	RVNQPSIIG-TATFY	QYWSVRQNKRRSSG--	-----RSRL	RTTNSAWRNLGLTLG	197						
GTYNPSTGA--TKL	SVTTDGSVCXYDIYRTQ	RVNQPSIEG-TSTFY	QFWSVRQNKRRSSG--	-----SVNM	AAHFNAWAQAAGLQJG	200						
GDYNPGSGG--TYK	TVTSDGSVCXYDIYAT	RTNAAISIQ-TATFT	QYWSVRQNKRVG--	-----GTVTT	SNHFNAWAKLGMNLG	202						
GDYNPGNSM--TYK	TVTSDGSVCXYDIYHQ	QVNQPSISG-TATFN	QYWSIRQNRSS--	-----GTVTT	SNHFNAWAKLGMNLG	198						
GSYRPTG---EYR	TVYSDGGTYDYIKTT	RYNAPSEVG-TRTFD	QYWSVRQSKVIGS--	-----GTITT	GNHFDAAWARGMNLG	215						
GTYPRTG---TYK	TVYSDGGTYDYIYQTT	RYNAPSEVG-TKTEN	QYWSVRQSKRTG--	-----GSITA	GNHFDAAWARYGMPLG	204						
GTYPRTG---TYK	TVYSDGGTYDYIKTT	RYNAPSEVG-TKTEN	QYWSVRQNKRTG--	-----GTITT	GNHFDAAWAHGMPLG	205						
GTYPRTG---KYK	TVYSDGGTYDYIKTT	RYNAPSEVG-TKTEN	QYWSVRQSKRTG--	-----GTITS	GNHFDAAWARGMNLG	208						
GTYPRTG---TYM	TVYSDGGTYDYIKTT	RYNAPSEVG-TRTFD	QYWSVRQSKRTS--	-----GTITA	GNHFDAAWARGHMLG	206						
GTYPRTG---TFM	TVYSDGGTYDYIKTT	RYNAPSEVG-TATFT	QYWSVRQSKRVG--	-----GTITT	ANHFNAWASHGMNLG	207						
GDYDPSTGA--TEL	TVYSDGGTYDYIKTT	RENAPSEVG-TSTFN	QYWSVRQSKRVG--	-----GTITA	QNHFDAAWANVGLQJG	198						
GNYNPSAGA--QRR	QVTADGSIYDIYST	QHNQPSILG-TNTEH	QYWSIRRNKRVGG--	-----TVST	GVHFNAWRSGLMPLG	193						
GSYNPASCSGGTDY	SFQSDGATYNVRRCQ	RVQQPSIDG-TQTFF	QYFSVRSPKKGFG--	Q-----ISGTTT	ANHFNFWASKGLNLG	202						
GSYNPASCSGGTDY	SFQSDGATYNVRRCQ	RVNQPSIDG-TQTFF	QYFSVRNPKKGG--	N-----ISGTTT	ANHNVFWASKGLNLG	203						
GSYPLNCPGGTDEG	SFTSGGATYQVRKCR	RTNAPSIIG-TQSEF	QYFSVRTPKKGG--	Q-----VSGSVNF	ADHVQYWASKGLPLG	194						
GDYNPCSS--ATSL	TVYSDGSTYQVCTDT	RTNEPSITG-TSTFT	QYFSVRESTRTSG--	-----TVTV	ANHFNFWAQHGFNGS	159						
GDYNPCSS--ATSL	TVYSDGSTYQVCTDT	RTNEPSITG-TSTFT	QYFSVRESTRTSG--	-----TVTV	ANHFNFWAQHGFNGS	187						
GDYNPCSS--ATSL	TVYSDGSTYQVCTDT	RTNEPSITG-TSTFT	QYFSVRESTRTSG--	-----TVTV	ANHFNFWAHGFGNS	186						
GNYNPCSSGSATNLG	TVSSDGGTYQVCTDT	RVNQPSITG-TSTFT	QFFSVRQSGRTSG--	-----TVTI	ANHFNFWANDGFGNS	184						
GGYNPCTGSGVTQLG	SLYSDGSSYQVCTHT	QYNQPSIVG-TTTFP	QYFSVRQNKRRSSG--	-----SVNM	QNHFNFWAQHGFPPNR	184						
--HNYPAQG---TVK	TVTSDGATYTIWENT	RVNEPSIQG-TATFN	QYISVRNPSRTSG--	-----TVTV	QNHFNAWASLGLHLG	205						
GTYRPTG---TYK	SFYADGGTYDIYETL	RVNQPSIIG-DATFK	QYWSVRQTKRTS---	-----GTASV	SEHFKKWESLGNPDMG	199						
GTYRPTG---AYK	SFYADGGTYDIYETT	RVNQPSIIG-TATEK	QYWSVRQTKRTS---	-----GTVSV	SAHFRKWESLGNPDMG	199						
GSWRPPGG---TSK	TIYVDDGGTYDIYETT	RVNQPSIQG-NTEFK	QYWSVRRTKRTS---	-----GTISV	SKHFAAWESKGMPLG	232						
GTYRPPGA---TPK	TIYVDDGGTYDIYETT	RVNQPSIKG-TATFQ	QYWSVRTSKRTS---	-----GTISV	TEHFKAWERLGMKMG	202						
GTYRPPGG---TPK	TINYDDGGTYQIYETT	RVNQPSIKG-TATFQ	QYWSVRTSKRTS---	-----GTISV	SEHFRAWESLGNMNG	200						
GSWRPPGGS--P-MN	TMVYDDGQYDVYVTD	RVNQPSIDG-NTEFK	QYWSVRTQKKT---	-----RGTVHV	NHFFYNWQEMGLKVG	189						
GSWRPPGSN--S-MG	TINYDDGGTYDIYVTD	RVNQPSIDG-TTTFK	QFWSVRTQKKT---	-----SGVISV	SKHFEAWTSKGLNLG	147						
GSWRPPCAA--ESL	TVYVDDGGTYDIYKTT	RYEQPSIDG-TKTFD	QYWSVRQDKPTDGT	K-----LEGTISI	SKHFDaweQVGLTLG	234						
GSWRPPG-A--TSL	TVYIDGATYDIYKTT	RVNQPSIEG-TRTFD	QYWSVRTSKRTS---	-----GTVTV	TDHFKAWAAKGLNLG	185						
GNWRPPG-A--TSL	QVTIDGGTYDIYRTT	RVNQPSIVG-TATFD	QYWSVRTSKRTS---	-----GTVTV	TDHFRAWANRGLNLG	198						
GDWRPPGND-GEVKG	TVSANGNTYDIRKTM	RYNQPSLDG-TATFP	QYWSVRQTSGSANNO	TN-----YMKGTIDV	TKHFDAWSAAGLDMS	212						
GSYHPSNTIT-GTFV	TVKCDGGTYDIYTAV	RVNAPSIIEG--TTFT	QYWSVRQSATIQIYAV	IKPLITLQNTITFFF	SNHFDAAWKTMTEAT	238						

FIG. 2CONT'D

B. subtilis xyla	271	285	286	300	301	315	316	330	331	345	346	360	213
B. circulans xyla	SNWAYQVMATEGYQS	SGSSNVTWV	---	---	---	---	---	---	---	---	---	---	213
B. stearothermophilus xyla	SSWAYQVLATEGYQS	SGSSNVTWV	---	---	---	---	---	---	---	---	---	---	211
A. caviae xyla	NSWSYQVMATEGYQS	SGNANVTWV	---	---	---	---	---	---	---	---	---	---	211
C. carbonum xyl1	-QHYIQIVATEGYFS	TGNAQITVNCP	---	---	---	---	---	---	---	---	---	---	221
H. turcicum xyl1	-SHYIQIVATEGYFS	SGSASITVNCP	---	---	---	---	---	---	---	---	---	---	227
A. pisi xyl	-THNYQIVATEGYFS	SGSAQITVNCA	---	---	---	---	---	---	---	---	---	---	227
S. commune xyla	SEHNYQIVATEGYQS	SGTATITVTAS	---	---	---	---	---	---	---	---	---	---	201
T. lanuginosus xyla	GDHYIQIVATEGYFS	SGYARITVADVQ	---	---	---	---	---	---	---	---	---	---	225
C. carbonum xyl2	-QHYIQIVATEGYQS	SGSSDIYVQTQ	---	---	---	---	---	---	---	---	---	---	231
C. sativus xyl2	-QHYIQIVATEGYQS	SGSSDIYVQTQ	---	---	---	---	---	---	---	---	---	---	231
H. insolens xyl1	-QHYIQVVATEGYQS	SGESDIYVQTH	---	---	---	---	---	---	---	---	---	---	227
M. grisea xyl22	-NHNMYIVATEGYRS	AGNSNINVQTPA	---	---	---	---	---	---	---	---	---	---	233
C. gracile cgxA	-SHNYQIVATEGYQS	SGSSSITVS	---	---	---	---	---	---	---	---	---	---	219
T. reesei xyl2(2)	-TMDYQIVAVEGYFS	SGSASITVS	---	---	---	---	---	---	---	---	---	---	223
T. reesei, ALKO2721 xyl2	-TMDYQIVAVEGYFS	SGSASITVS	---	---	---	---	---	---	---	---	---	---	223
T. reesei xyl1	-TMDYQIVAVEGYFS	SGSASITVS	---	---	---	---	---	---	---	---	---	---	222
T. harzianum xylD	-TMDYQIVAVEGYFS	SGSASITVS	---	---	---	---	---	---	---	---	---	---	190
T. viride xyl	-TLDYQIIAVEGYFS	SGNANINVS	---	---	---	---	---	---	---	---	---	---	223
C. gracile cgxB	-THDYQIVATEGYYS	SGSATVNVGASSDGS	TGGG	STGGG	STNVSF	---	---	---	---	---	---	---	241
A. niger xyl2	THN-YQIVATEGYQS	SGSSSITVQ	---	---	---	---	---	---	---	---	---	---	225
Penicillium sp 40 xyla	SFN-YQIVSTEGYES	SGSSSITVS	---	---	---	---	---	---	---	---	---	---	221
Streptomyces sp xyl	QFYIMMATEGYQS	SGSSNITVSG	---	---	---	---	---	---	---	---	---	---	240
Streptomyces sp xyl1	SFNYIMMATEGYQS	SGSSSISVS	---	---	---	---	---	---	---	---	---	---	228
S. thermocyanoeviolaceus xylB	TFN-YMILATEGYQS	SGSSNITVGDGSDN	GGGG	---	---	---	---	---	---	---	---	---	292
S. viridosporus T7A svxA	NHN-YMIMATEGYQS	SGSSSITVSESGSG	GGGG	---	---	---	---	---	---	---	---	---	286
T. fusca xyl	THD-YMIMATEGYQS	SGSSNVTLGTSGGN	PGGN	---	---	---	---	---	---	---	---	---	295
C. pachnodae xyl11A	RHD-YQILLATEGYQS	SGSSNITIGTSGGG	GGGG	---	---	---	---	---	---	---	---	---	293
A. oryzae XylG1	THN-YMILATEGYKS	SGSATITVE	---	---	---	---	---	---	---	---	---	---	221
C. purpurea xyl1	-TYDYIMIVATEGFRS	SGSASITVS	---	---	---	---	---	---	---	---	---	---	216
C. mixtus xyl	-NHDYMLATEGYQS	RGSSDITVSEGTGGT	TSSSV	---	---	---	---	---	---	---	---	---	290
P. fluorescens cellulosa xyl	-NHNQVLATEGYQS	RGSSDITVSEGTSGG	GTSSV	---	---	---	---	---	---	---	---	---	292
P. cochleariae xyl	-THAQIIFATEGYQS	SGFADITVS	---	---	---	---	---	---	---	---	---	---	217
A. kawachi xylC	DFN-YQVMAVEAWSG	AGSASVTIS	---	---	---	---	---	---	---	---	---	---	182
A. niger xyl1	DFN-YQVMAVEAWSG	AGSASVTISS	---	---	---	---	---	---	---	---	---	---	211
A. tubigenis xyl1	DFN-YQVVAVEAWSG	AGSASVTISS	---	---	---	---	---	---	---	---	---	---	210
P. purpurogenum xylB	NFN-YQVMAVEAWSG	TGTASVTVSA	---	---	---	---	---	---	---	---	---	---	208
Cryptococcus sp S-2 xyl-CS2	NFN-YQVLAVEGFSG	SGNANMKLISG	---	---	---	---	---	---	---	---	---	---	209
T. reesei xyl2	QMN-YQVVAVEGWGG	SGSASQSVSN	---	---	---	---	---	---	---	---	---	---	229
B. pumilus xyla(1)	-KMYETALTVEGYRS	NGSANVMTNQLMIR	---	---	---	---	---	---	---	---	---	---	227
B. pumilus xyla(2)	-KMYETALTVEGYQS	SGSANVMTNQLFIGN	---	---	---	---	---	---	---	---	---	---	228
C. acetobutylicum xylB	-KMHETAFNIEGYQS	SGKADVNSMSINICK	---	---	---	---	---	---	---	---	---	---	261
C. thermocellum xylB	-KMYEVALVVEGYQS	SGKADVTSMITVGN	APS	---	---	---	---	---	---	---	---	---	277
Bacillus sp 4IM-1 xylJ	-NMYEVALTVEGYQS	SGSANVSNITLFIGG	Q	---	---	---	---	---	---	---	---	---	268
P. multivesiculatum xyla	-KVYEASLNIEGYQS	AGSATVNKNEVVQT	---	---	---	---	---	---	---	---	---	---	217
P. multivesiculatum xyl	-LMYEASLTIEGYQS	SGSATVNQNDVTGG	---	---	---	---	---	---	---	---	---	---	175
R. albus xyla	-NMYEVALNIEGYQS	NGQATIYENELTVDG	NY	---	---	---	---	---	---	---	---	---	303
Caldicellulosiruptor sp xylD	-TIDQITLCVEGYQS	SGSANITQNTFTIGG	SSSGSSNGSNNG	---	---	---	---	---	---	---	---	---	274
D. thermophilum xynB	-TIDQITLCVEGYQS	SGSANITQNTFSQG	---	---	---	---	---	---	---	---	---	---	273
R. flavofaciens xyla	GTLIEVSLNIEGYRS	NGSANKSVSVTQGG	SSDNGGQQQNDW	---	---	---	---	---	---	---	---	---	255
P. stipitis xyla	HSTEGYFSSGITVEQ	PHQPHRNTWATSLTS	QTKHTARSLPIN	---	---	---	---	---	---	---	---	---	328