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(71) Applicant *(for all designated States except US)*: **NOVOZYMES A/S** [DK/DK]; Krogshoejvej 36, DK-2880 Bagsvaerd (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **VIKSOE-NIELSEN, Anders** [DK/DK]; Lindevej 12, Joerlunde, DK-3550 Slangerup (DK). **PEDERSEN, Sven** [DK/DK]; Emil Reesens Vej 9, DK-2820 Gentofte (DK).

(74) Common Representative: **NOVOZYMES A/S**; Patents, Krogshoejvej 36, DK-2880 Bagsvaerd (DK).

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(54) Title: STARCH PROCESS

(57) Abstract: The present invention relates, inter alia, to the use of a glucoamylase derived from Talaromyces sp. and an acid alpha-amylase comprising a carbohydrate-binding module in a starch saccharification process in which starch is degraded to glucose.

STARCH PROCESS

FIELD OF THE INVENTION

The present invention relates, inter alia, to the use of a glucoamylase derived from *Talaromyces* sp. and an acid alpha-amylase comprising a carbohydrate-binding module ("CBM") in a starch saccharification process comprising degrading starch to glucose.

BACKGROUND OF THE INVENTION

A thermostable glucoamylase from *Talaromyces emersonii* is disclosed in WO9928448A1. The purified enzyme shows markedly enhanced stability and a 3-4 fold higher specific activity compared to *Aspergillus niger* glucoamylase and has optimal activity at pH 4.5 and at 70 °C and thus appears suited for industrial saccharification for production of glucose. The yield of glucose during industrial saccharification with *Talaromyces emersonii* glucoamylase, however, is 1-2% lower than for *Aspergillus niger* glucoamylase thereby reducing the enzymes profitability in a process for production of high DX glucose syrups and/or high fructose syrups.

SUMMARY OF THE INVENTION

Now the inventors of the present invention have surprisingly discovered that in a saccharification process using the *Talaromyces* glucoamylase a high DX can be reached by the addition of an acid alpha amylase comprising a carbohydrate binding domain (CBM).

Thus the invention provides in a first aspect a process for saccharifying a starch comprising contacting a liquefied starch substrate with a glucoamylase derived from *Talaromyces* sp. and an acid alpha-amylase comprising a CBM.

In a second aspect the invention provides a process for producing a starch hydrolysate comprising (a) liquefaction, e.g. by jet cooking, with the addition of a thermostable alpha-amylase and (b) subsequently contacting the liquefied starch with an acid alpha-amylase comprising a CBM, and a glucoamylase derived from *Talaromyces* sp.

The invention provides further embodiments of the two aspects comprising (a) the process wherein the DX (free glucose %) of the hydrolysate following saccharification reaches a value of at least 94.00%, at least 94.50%, at least 94.75% at least 95%, at least 95.25%, at least 95.5%, at least 95.75% or even at least 96%, (b) the process wherein the at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or preferably at least 99% of the dry solids starch is converted into a soluble hydrolysate, such as e.g. glucose, (c) the process wherein the glucoamylase is a polypeptide having at least 50% homology to the amino acid sequence shown in SEQ ID NO:1, (d) the process wherein the glucoamylase is derived from *Talaromyces emersonii*, (e) the process wherein the acid

alpha-amylase comprising a CBM is a wild type, a variant and/or a hybrid, (f) the process wherein the acid alpha-amylase comprising a CBM is a polypeptide having at least 50% homology to any of the amino acid sequence in the group consisting of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, the process wherein the acid alpha-amylase comprising a CBM is present in amounts of 0.05 to 1.0 mg EP/g DS, more preferably from 0.1 to 0.5 mg EP/g DS, even more preferably 0.2 to 0.5 mg EP/g DS of starch, (g) the process wherein the acid alpha-amylase comprising a CBM is present in an amount of 10-10000 AFAU/kg of DS, in an amount of 500-2500 AFAU/kg of DS, or more preferably in an amount of 100-1000 AFAU/kg of DS, such as approximately 500 AFAU/kg DS, (h) the process wherein the glucoamylase is present in amounts of 0.001 to 2.0 AGU/g DS, preferably from 0.01 to 1.5 AGU/g DS, more preferably from 0.05 to 1.0 AGU/g DS, and most preferably from 0.01 to 0.5 AGU/g DS of starch, (i) the process wherein the activities of acid alpha-amylase and glucoamylase are present in a ratio of at least 0.1, at least 0.2, at least 0.25, at least 0.3, at least 0.35, at least 0.40, at least 0.50, at least 0.60, at least 0.7, at least 0.8, at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.85, or even at least 1.9 AFAU/AGU, (j) the process wherein the thermostable alpha-amylase is a bacterial alpha-amylase, preferably derived from a species within *Bacillus* sp., preferably from a strain of *Bacillus licheniformis*, (k) the process further comprising adding a debranching enzyme, e.g. a pullulanase or an isoamylase, (l) the process further comprising saccharification to a DX of at least 95 at a temperature from 60°C to 75°C, preferably from 62°C to 68°C, more preferably from 64°C to 66°C, and most preferably 65°C, (m) the process further comprising saccharification to a DX of at least 95 at a temperature from 64°C to 72°C, preferably from 66°C to 74°C, more preferably from 68°C to 72°C, and most preferably 70 °C. In a particular embodiment the process further comprises contacting the hydrolysate with a fermenting organism, said fermenting organism preferably a yeast to produce a fermentation product, said fermentation product preferably ethanol, wherein said ethanol is optionally recovered. The saccharification and fermentation may carried out as a simultaneous saccharification and fermentation process (SSF process).

DETAILED DESCRIPTION OF THE INVENTION

In an embodiment the process of the invention is applied for production of glucose- and/or fructose-containing syrups from starch. The starch may be derived from grain or other starch rich plant parts, preferably corn, wheat, barley, rice, potato. The process may comprise the consecutive enzymatic step; (a) a liquefaction step followed by (b) a saccharification step and optionally (c) (for production of fructose-containing syrups) an isomerization step. During the liquefaction process, starch (initially in the form starch suspension in aqueous medium) is degraded to dextrins (oligo- and polysaccharide fragments of starch), preferably by an thermostable alpha-amylase (EC 3.2.1.1), e.g. a bacterial thermostable alpha-amylase, e.g. a

Bacillus licheniformis alpha-amylase (Termamyl™ or Liquozyme X™ available from Novozymes, Denmark), typically at pH values between 5.5 and 6.2 and at temperatures of 95-160°C for a period of approximately 2 hours. After the liquefaction step and before the saccharification step the pH of the medium may be reduced to a value below 4.5 (e.g approximately pH 4.3), maintaining the high temperature (above 95°C), whereby the liquefying alpha-amylase activity is denatured.

During saccharification the temperature is then normally lowered to below 65°C, such as to 60°C, and the dextrins are converted into dextrose (D-glucose) in the presence of (a) a glucoamylase which according to the invention is derived from *Talaromyces* and (b) an acid alpha-amylase comprising a CBM. In an embodiment an additional enzyme may be present, preferably a debranching enzyme, such as an isoamylase (EC 3.2.1.68) and/or a pullulanase (EC 3.2.1.41). Preferably the saccharification process allowed to proceed for 24-72 hours until the DX of the hydrolysate reaches a value of at least 94.00%, at least 94.50%, at least 94.75% at least 95%, at least 95.25%, at least 95.5%, at least 95.75% or even at least 96%. Optionally the resulting high DX glucose syrups is converted into high fructose syrup using, e.g., an immobilized "glucose isomerase" (xylose isomerase, EC 5.3.1.5)).

Alignment and identity

For purposes of the present invention, alignments of amino acid sequences and calculation of identity scores were done using the software Align, a Needleman-Wunsch alignment (i.e. global alignment), useful for both protein and DNA alignments. The default scoring matrices BLOSUM50 and the identity matrix are used for protein and DNA alignments respectively. The penalty for the first residue in a gap is -12 for proteins and -16 for DNA, while the penalty for additional residues in a gap is -2 for proteins and -4 for DNA. Align is from the FASTA package version v20u6 (W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448, and W. R. Pearson (1990) "Rapid and Sensitive Sequence Comparison with FASTP and FASTA", Methods in Enzymology, 183:63-98). The relevant part of the amino acid sequence for the identity determination is the mature polypeptide, i.e. without the signal peptide.

Enzymes

Glucoamylases

Preferred for the invention is any glucoamylase derived from a strain of *Talaromyces* sp. and in particular derived from *Talaromyces leycettanus* such as the glucoamylase disclosed in US patent no. Re. 32,153, *Talaromyces duponti* and/or *Talaromyces thermopiles* such as the glucoamylases disclosed in US patent no. 4,587,215 and more preferably derived from *Talaromyces emersonii,* and most preferably the glucoamylase derived from strain CBS 793.97 and/or disclosed as SEQ ID NO: 7 in WO 99/28448 and as SEQ ID NO:1

herein. Further preferred is a glucoamylase which has an amino acid sequence having at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or even at least 95% identity to the aforementioned amino acid sequence. A commercial *Talaromyces* glucoamylase preparation is supplied by Novozymes A/S as Spirizyme Fuel.

Enzymes having acid alpha-amylase activity and comprising a CBM

Preferably the CBM is a starch binding domain (SBD), and preferably the acid alpha-amylase activity is derived from an acid alpha-amylase within EC 3.2.1.1. The enzyme having acid alpha-amylase activity and comprising a CBM to be used in the invention may be a hybrid enzyme or the polypeptide may be a wild type enzyme which already comprises a catalytic module having alpha-amylase activity and a carbohydrate-binding module. The polypeptide to be used in the process of the invention may also be a variant of such a wild type enzyme. The hybrid may be produced by fusion of a first DNA sequence encoding a first amino acid sequences and a second DNA sequence encoding a second amino acid sequences, or the hybrid may be produced as a completely synthetic gene based on knowledge of the amino acid sequences of suitable CBMs, linkers and catalytic domains. The term "hybrid enzyme" is used herein to characterize polypeptides, i.e. enzymes, having acid alpha-amylase activity and comprising a CBM that comprises a first amino acid sequence comprising a catalytic module having alpha-amylase activity and a second amino acid sequence comprising at least one carbohydrate-binding module wherein the first and the second are derived from different sources. The term "source" being understood as e.g. but not limited to a parent polypeptide, e.g. an enzyme, e.g. an amylase or glucoamylase, or other catalytic activity comprising a suitable catalytic module and/or a suitable CBM and/or a suitable linker. The parent polypeptides of the CBM and the acid alpha-amylase activity may be derived from the same strain, and/or the same species or it may be derived from different stains of the same species or from strains of different species. CBM-containing hybrid enzymes, as well as detailed descriptions of the preparation and purification thereof, are known in the art [see, e.g. WO 90/00609, WO 94/24158 and WO 95/16782, as well as Greenwood et al. Biotechnology and Bioengineering 44 (1994) pp. 1295-1305].

Preferred for the invention is any enzyme having acid alpha-amylase activity and comprising a CBM including but not limited to the hybrid enzymes and wild type variants disclosed in PCT/US2004/020499 (NZ10490), and in Danish patent application from Novozymes A/S internal number NZ10729 filed on the same day as the present application. More preferred is an enzyme having acid alpha-amylase activity and comprising a CBM which enzyme has the amino acid sequence disclosed as SEQ ID NO:2 (A.niger+CBM), SEQ ID NO:3 (JA126) or SEQ ID NO:4 (JA129) or any enzyme having acid alpha-amylase activity and comprising a CBM which enzyme which has an amino acid sequence having at

least 50%, 60%, 70%, 80%, 90% or even at least 95% identity to any of the aforementioned amino acid sequences.

Preferably the activities of acid alpha-amylase and glucoamylase are present in a ratio of between 0.3 and 5.0 AFAU/AGU. More preferably the ratio between acid alpha-amylase activity and glucoamylase activity is at least 0.35, at least 0.40, at least 0.50, at least 0.60, at least 0.7, at least 0.8, at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.85, or even at least 1.9 AFAU/AGU. However, the ratio between acid alpha-amylase activity and glucoamylase activity should preferably be less than 4.5, less than 4.0, less than 3.5, less than 3.0, less than 2.5, or even less than 2.25 AFAU/AGU.

Methods

MATERIALS AND METHODS

Determination of acid alpha-amylase activity

When used according to the present invention the activity of any acid alpha-amylase may be measured in AFAU (Acid Fungal Alpha-amylase Units), which are determined relative to an enzyme standard. 1 AFAU is defined as the amount of enzyme which degrades 5.260 mg starch dry matter per hour under the below mentioned standard conditions.

Acid alpha-amylase, i.e., acid stable alpha-amylase, an endo-alpha-amylase (1,4-alpha-D-glucan-glucano-hydrolase, E.C. 3.2.1.1) hydrolyzes alpha-1,4-glucosidic bonds in the inner regions of the starch molecule to form dextrins and oligosaccharides with different chain lengths. The intensity of color formed with iodine is directly proportional to the concentration of starch. Amylase activity is determined using reverse colorimetry as a reduction in the concentration of starch under the specified analytical conditions.

$$\text{STARCH} + \text{IODINE} \xrightarrow[40^\circ,\ \text{pH}\,2{,}5]{\text{ALPHA-AMYLASE}} \text{DEXTRINS} + \text{OLIGOSACCHARIDES}$$

$\lambda = 590\ \text{nm}$

blue/violet t = 23 sec. decoloration

Standard conditions/reaction conditions:

Substrate:	Soluble starch, approx. 0.17 g/L
Buffer:	Citrate, approx. 0.03 M
Iodine (I2):	0.03 g/L
CaCl2:	1.85 mM
pH:	2.50 ± 0.05
Incubation temperature:	40°C
Reaction time:	23 seconds
Wavelength:	590nm
Enzyme concentration:	0.025 AFAU/mL
Enzyme working range:	0.01-0.04 AFAU/mL

A folder EB-SM-0259.02/01 describing this analytical method in more detail is available upon request to Novozymes A/S, Denmark, which folder is hereby included by reference.

Glucoamylase activity

Glucoamylase (AMG) activity may be measured in AmyloGlucosidase Units (AGU). The AGU is defined as the amount of enzyme, which hydrolyzes 1 micromole maltose per minute under the standard conditions 37°C, pH 4.3, substrate: maltose 23.2 mM, buffer: acetate 0.1 M, reaction time 5 minutes.

An autoanalyzer system may be used. Mutarotase is added to the glucose dehydrogenase reagent so that any alpha-D-glucose present is turned into beta-D-glucose. Glucose dehydrogenase reacts specifically with beta-D-glucose in the reaction mentioned above, forming NADH which is determined using a photometer at 340 nm as a measure of the original glucose concentration.

AMG incubation:

Substrate:	maltose 23.2 mM
Buffer:	acetate 0.1 M
pH:	4.30 ± 0.05
Incubation temperature:	37°C ± 1
Reaction time:	5 minutes
Enzyme working range:	0.5-4.0 AGU/mL

Color reaction:

GlucDH:	430 U/L
Mutarotase:	9 U/L
NAD:	0.21 mM
Buffer:	phosphate 0.12 M; 0.15 M NaCl
pH:	7.60 ± 0.05
Incubation temperature:	37°C ± 1
Reaction time:	5 minutes
Wavelength:	340 nm

A folder (EB-SM-0131.02/01) describing this analytical method in more detail is available on request from Novozymes A/S, Denmark, which folder is hereby included by reference.

Example 1

Substrates for saccharification were prepared by dissolving a DE 11 maltodextrin prepared from corn starch liquefied with thermostable bacterial alpha-amylase (LIQUOZYME X™, Novozymes A/S) in Milli-Q™ water, and adjusting the dry solid matter content (DS) to 30%. The saccharification experiments were carried out in sealed 2 ml glass vials at 60°C and initial pH of 4.3 under continuous stirring. The following enzymes were used: a *Talaromyces emersonii* composition (T-AMG), a wild type *Aspergillus niger* acid alpha-amylase and JA001, which is an alpha-amylase with the same catalytic domain as the wild type *A.niger* acid alpha-amylase but also comprising a CBM.

Samples were taken at set intervals and heated in boiling water for 15 minutes to inactivate the enzymes. After cooling, the samples were diluted to 5% DS and filtered (Sartorius MINISART™ NML 0.2 µm), before being analysed by HPLC. The glucose levels as a % of total soluble carbohydrate are given in table 1 below.

Table 1. The performance of the CBM amylase variant JA001 at two glucoamylase levels compared with the wild type *A.niger* acid alpha-amylase, having the same catalytic module as JA001. Results shown as glucose pct. after 24, 32, 48 and 70 hrs.

Enzyme dosage			DP1% (glucose)			
			24 hrs	32 hrs	48 hrs	70 hrs
AGU/g DS	AFAU/g DS					
0.35	JA001	0.0000	88.2	90.3	92.2	93.4
		0.0875	92.0	93.6	94.9	95.5
		0.1750	93.8	94.9	95.4	95.3
0.15	JA001	0.0000	73.8	77.4	81.1	84.0
		0.0875	79.2	85.8	91.4	93.9
		0.1750	88.0	92.0	94.3	95.2
0.35	WT *A.niger*	0.0875	89.8	91.9	93.5	94.4
	Alpha-amylase	0.1750	91.0	93.0	94.2	94.9

The results show that the addition of *A.niger* acid alpha-amylase with *Talaromyces emersonii* glucoamylase gave a higher glucose yield than with the AMG alone. However the largest effect was seen when the CBM containing acid alpha-amylase variant was added with the T-AMG. The use of the CBM containing acid alpha-amylase variant furthermore allowed reducing the AMG level and still maintaining a high glucose yield.

CLAIMS

1) A process for saccharifying of a starch comprising contacting a liquefied starch substrate with a glucoamylase derived from *Talaromyces* sp. and an acid alpha-amylase comprising a carbohydrate-binding module.

2) A process for producing a starch hydrolysate comprising;

   a) liquefaction. e.g. by jet cooking, with the addition of a thermostable alpha-amylase and;
   b) subsequently contacting the liquefied starch with;
      i) an acid alpha-amylase comprising a carbohydrate-binding module, and;
      ii) a glucoamylase derived from *Talaromyces* sp.

3) The process of any of claims 1-2 wherein the DX of the hydrolysate following saccharification reaches a value of at least 94.00%, at least 94.50%, at least 94.75% at least 95%, at least 95.25%, at least 95.5%, at least 95.75% or even at least 96%.

4) The process of any of claims 1-3 wherein at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or even at least 99% of the dry solids starch is converted into a soluble hydrolysate, such as e.g. glucose.

5) The process of any of claims 1-4 wherein the glucoamylase is a polypeptide having at least 50% homology to the amino acid sequence shown in SEQ ID NO:1

6) The process of any of claims 1-5 wherein the glucoamylase is derived from *Talaromyces emersonii.*

7) The process of any of claims 1-6 wherein the acid alpha-amylase comprising a carbohydrate-binding module is a wild type, a variant and/or a hybrid.

8) The process of any of claims 1-7 wherein the acid alpha-amylase comprising a carbohydrate-binding module is a polypeptide having at least 50% homology to any of the amino acid sequence in the group consisting of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

9) The process of any of claims 1-8 wherein the acid alpha-amylase comprising a carbohydrate-binding module is present in amounts of 0.05 to 1.0 mg EP/g DS, more

preferably from 0.1 to 0.5 mg EP/g DS, even more preferably 0.2 to 0.5 mg EP/g DS of starch.

10) The process of any of claims 1-9 wherein the acid alpha-amylase comprising a carbohydrate-binding module is present in an amount of 10-10000 AFAU/kg of DS, in an amount of 500-2500 AFAU/kg of DS, or more preferably in an amount of 100-1000 AFAU/kg of DS, such as approximately 500 AFAU/kg DS.

11) The process of any of claims 1-10 wherein the glucoamylase is present in amounts of 0.001 to 2.0 AGU/g DS, preferably from 0.01 to 1.5 AGU/g DS, more preferably from 0.05 to 1.0 AGU/g DS, and most preferably from 0.01 to 0.5 AGU/g DS of starch.

12) The process of any of claims 1-11 wherein the activities of acid alpha-amylase and glucoamylase are present in a ratio of at least 0.1, at least 0.2, at least 0.25, at least 0.3, at least 0.35, at least 0.40, at least 0.50, at least 0.60, at least 0.7, at least 0.8, at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.85, or even at least 1.9 AFAU/AGU.

13) The process of any of claims 1-12 wherein the thermostable alpha-amylase is a bacterial alpha-amylase, preferably derived from a species within *Bacillus* sp., preferably from a strain of *Bacillus licheniformis*.

14) The process of any of claims 1-13 further comprising adding a debranching enzyme, e.g. a pullulanase or an isoamylase.

15) The process of any of claim 1-14 comprising saccharification to a DX of at least 95 at a temperature from 60°C to 75°C, preferably from 62°C to 68°C, more preferably from 64°C to 66°C, and most preferably 65°C

16) The process of any of claim 1-15 comprising saccharification to a DX of at least 95 at a temperature from 64°C to 72°C, preferably from 66°C to 74°C, more preferably from 68°C to 72°C, and most preferably 70 °C.

17) The process of any of claims 1-16 further comprising contacting the hydrolysate with a fermenting organism, said fermenting organism preferably a yeast to produce a fermentation product., said fermentation product preferably ethanol, wherein said ethanol is optionally recovered.

18) The process of any of claims 1-17 wherein saccharification and fermentation may be carried out as a simultaneous saccharification and fermentation process (SSF process).

Sequences 10735.204-WO.ST25.txt
SEQUENCE LISTING

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Pro Glu Leu Gly Asn Trp Ser Thr Ala Ser Ala Ile Pro Leu Arg Ala
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Asp Ala Tyr Thr Asn Ser Asn Pro Leu Trp Tyr Val Thr Val Asn Leu
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Pro Pro Gly Thr Ser Phe Glu Tyr Lys Phe Phe Lys Asn Gln Thr Asp
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Ile Gln Gly Met Gly Phe Thr Ala Ile Trp Ile Ser Pro Ile Thr Glu
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Gln Leu Pro Gln Asp Thr Ala Asp Gly Glu Ala Tyr His Gly Tyr Trp

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Gln Gln Lys Ile Tyr Asp Val Asn Ser Asn Phe Gly Thr Ala Asp Asp
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Leu Lys Ser Leu Ser Asp Ala Leu His Ala Arg Gly Met Tyr Leu Met
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Val Asp Val Val Pro Asn His Met Gly Tyr Ala Gly Asn Gly Asn Asp
115 120 125

Val Asp Tyr Ser Val Phe Asp Pro Phe Asp Ser Ser Ser Tyr Phe His
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Cys Trp Glu Gly Asp Thr Ile Val Ser Leu Pro Asp Leu Asn Thr Thr
165 170 175

Glu Thr Ala Val Arg Thr Ile Trp Tyr Asp Trp Val Ala Asp Leu Val
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Ser Asn Tyr Ser Val Asp Gly Leu Arg Ile Asp Ser Val Leu Glu Val
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Glu Pro Asp Phe Phe Pro Gly Tyr Gln Glu Ala Ala Gly Val Tyr Cys
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Val Gly Glu Val Asp Asn Gly Asn Pro Ala Leu Asp Cys Pro Tyr Gln
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Lys Val Leu Asp Gly Val Leu Asn Tyr Pro Ile Tyr Trp Gln Leu Leu
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Tyr Ala Phe Glu Ser Ser Ser Gly Ser Ile Ser Asn Leu Tyr Asn Met
260 265 270

Ile Lys Ser Val Ala Ser Asp Cys Ser Asp Pro Thr Leu Leu Gly Asn
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Phe Ile Glu Asn His Asp Asn Pro Arg Phe Ala Ser Tyr Thr Ser Asp
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Tyr Ser Gln Ala Lys Asn Val Leu Ser Tyr Ile Phe Leu Ser Asp Gly
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Ile Pro Ile Val Tyr Ala Gly Glu Glu Gln His Tyr Ser Gly Gly Lys
325 330 335

Val Pro Tyr Asn Arg Glu Ala Thr Trp Leu Ser Gly Tyr Asp Thr Ser

340 345 350

Ala Glu Leu Tyr Thr Trp Ile Ala Thr Thr Asn Ala Ile Arg Lys Leu
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Gln Val Ile Thr Val Leu Ser Asn Lys Gly Ser Ser Gly Ser Ser Tyr
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Thr Leu Thr Leu Ser Gly Ser Gly Tyr Thr Ser Gly Thr Lys Leu Ile
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Pro Val Pro Met Ala Ser Gly Leu Pro Arg Val Leu Leu Pro Ala Ser
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Thr Thr Leu Pro Ile Thr Phe Glu Glu Leu Val Thr Thr Thr Tyr Gly
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Pro Glu Trp Ser Val Thr Val Ser Leu Pro Val Gly Thr Thr Phe Glu
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Tyr Lys Phe Ile Lys Val Asp Glu Gly Gly Ser Val Thr Trp Glu Ser
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Met Gly Phe Asp Ala Ile Trp Ile Ser Pro Ile Pro Lys Asn Ser Asp
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Gly Gly Tyr His Gly Tyr Trp Ala Thr Asp Phe Tyr Gln Leu Asn Ser
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Asn Phe Gly Asp Glu Ser Gln Leu Lys Ala Leu Ile Gln Ala Ala His
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115 120 125

Pro Thr Ser Asn Gly Tyr Ser Gly Tyr Thr Phe Gly Asp Ala Ser Leu
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Tyr His Pro Lys Cys Thr Ile Asp Tyr Asn Asp Gln Thr Ser Ile Glu
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Asp Asn Val Ala Ile Leu Asn Asp Ile Val Ser Gly Trp Val Gly Asn
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Tyr Ser Phe Asp Gly Ile Arg Ile Asp Thr Val Lys His Ile Arg Lys
195 200 205

Asp Phe Trp Thr Gly Tyr Ala Glu Ala Ala Gly Val Phe Ala Thr Gly
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Glu Val Phe Asn Gly Asp Pro Ala Tyr Val Gly Pro Tyr Gln Lys Tyr
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Phe Val Ser Lys Ser Lys Gly Phe Ser Arg Ile Ser Glu Met Leu Gly
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Ser Asn Arg Asn Ala Phe Glu Asp Thr Ser Val Leu Thr Thr Phe Val
275 280 285

Asp Asn His Asp Asn Pro Arg Phe Leu Asn Ser Gln Ser Asp Lys Ala
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Leu Phe Lys Asn Ala Leu Thr Tyr Val Leu Leu Gly Glu Gly Ile Pro
305 310 315 320

Ile Val Tyr Tyr Gly Ser Glu Gln Gly Phe Ser Gly Gly Ala Asp Pro
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Ala Asn Arg Glu Val Leu Trp Thr Thr Asn Tyr Asp Thr Ser Ser Asp
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Leu Tyr Gln Phe Ile Lys Thr Val Asn Ser Val Arg Met Lys Ser Asn
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Lys Ala Val Tyr Met Asp Ile Tyr Val Gly Asp Asn Ala Tyr Ala Phe
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Lys His Gly Asp Ala Leu Val Val Leu Asn Asn Tyr Gly Ser Gly Ser
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Ser Leu Met Asp Ile Val Ser Asn Ile Thr Thr Thr Val Ser Ser Asp
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Thr Phe Asp Val Tyr Ala Thr Thr Val Tyr Gly Gln Asn Ile Tyr Ile
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Thr Gly Asp Val Ser Glu Leu Gly Asn Trp Thr Pro Ala Asn Gly Val
485 490 495

Ala Leu Ser Ser Ala Asn Tyr Pro Thr Trp Ser Ala Thr Ile Ala Leu
500 505 510

Pro Ala Asp Thr Thr Ile Gln Tyr Lys Tyr Val Asn Ile Asp Gly Ser
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35 40 45

Gly Tyr Gly Phe Val Gln Val Ser Pro Pro Gln Glu Thr Ile Gln Gly
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Ala Gln Trp Trp Thr Asp Tyr Gln Pro Val Ser Tyr Thr Leu Thr Gly
65 70 75 80

Lys Arg Gly Asp Arg Ser Gln Phe Ala Asn Met Ile Thr Thr Cys His
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Ala Ala Gly Val Gly Val Ile Val Asp Thr Ile Trp Asn His Met Ala
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Gly Val Asp Ser Gly Thr Gly Thr Ala Gly Ser Ser Phe Thr His Tyr
115 120 125

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Glu Pro Gly Asp Asp Ile Val Asn Tyr Asp Asn Ala Val Glu Val Gln
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Thr Cys Glu Leu Val Asn Leu Ala Asp Leu Ala Thr Asp Thr Glu Tyr
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Val Arg Gly Arg Leu Ala Gln Tyr Gly Asn Asp Leu Leu Ser Leu Gly
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Ala Asp Gly Leu Arg Leu Asp Ala Ser Lys His Ile Pro Val Gly Asp
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Ile Ala Asn Ile Leu Ser Arg Leu Ser Arg Ser Val Tyr Ile Thr Gln
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Glu Val Ile Phe Gly Ala Gly Glu Pro Ile Thr Pro Asn Gln Tyr Thr
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Gly Asn Gly Asp Val Gln Glu Phe Arg Tyr Thr Ser Ala Leu Lys Asp
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Gly Trp Val Pro Gly Ser Gly Ala Asn Val Phe Val Val Asn His Asp
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Thr Glu Arg Asn Gly Ala Ser Leu Asn Asn Asn Ser Pro Ser Asn Thr
    290                 295                 300

Tyr Val Thr Ala Thr Ile Phe Ser Leu Ala His Pro Tyr Gly Thr Pro
305                 310                 315                 320

Thr Ile Leu Ser Ser Tyr Asp Gly Phe Thr Asn Thr Asp Ala Gly Ala
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Pro Asn Asn Asn Val Gly Thr Cys Ser Thr Ser Gly Gly Ala Asn Gly
            340                 345                 350

Trp Leu Cys Gln His Arg Trp Thr Ala Ile Ala Gly Met Val Gly Phe
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Arg Asn Asn Val Gly Ser Ala Ala Leu Asn Asn Trp Gln Ala Pro Gln
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Asp Gly Ser Tyr Cys Asp Val Ile Ser Gly Lys Ala Ser Gly Ser Ser
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Cys Thr Gly Ser Ser Phe Thr Val Ser Gly Gly Lys Leu Thr Ala Thr
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Val Pro Ala Arg Ser Ala Ile Ala Val His Thr Gly Gln Lys Gly Ser
450 455 460

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485 490 495

Thr Gly Asp Val Ser Glu Leu Gly Asn Trp Thr Pro Ala Asn Gly Val
500 505 510

Ala Leu Ser Ser Ala Asn Tyr Pro Thr Trp Ser Ala Thr Ile Ala Leu
515 520 525

Pro Ala Asp Thr Thr Ile Gln Tyr Lys Tyr Val Asn Ile Asp Gly Ser
530 535 540

Thr Val Ile Trp Glu Asp Ala Ile Ser Asn Arg Glu Ile Thr Thr Pro
545 550 555 560

Ala Ser Gly Thr Tyr Thr Glu Lys Asp Thr Trp Asp Glu Ser
565 570

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2005/000783

A. CLASSIFICATION OF SUBJECT MATTER
C12P19/14 C12P19/20 C12N9/28 C12N9/34

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P C12N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/068976 A (NOVOZYMES A/S; NORMAN, BARRIE, EDMUND; VIKSOE-NIELSEN, ANDERS; OLSEN,) 21 August 2003 (2003-08-21) the whole document	1-18
A	WO 02/38787 A (NOVOZYMES A/S; NOVOZYMES NORTH AMERICA, INC; VEIT, CHRISTOPHER; FELBY,) 16 May 2002 (2002-05-16) abstract page 6, line 6 - line 23 page 14, line 14 - line 37	1-18
A	WO 99/28448 A (NOVO NORDISK A/S) 10 June 1999 (1999-06-10) cited in the application abstract example 11	1-18

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2006	16/03/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Huse, I

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2005/000783

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/055178 A (NOVOZYMES A/S; TANG, LAN; WU, WENPING; DUAN, JUNXIN; JOHANNESEN, PIA,) 1 July 2004 (2004-07-01) abstract page 17, line 27 - page 18, line 9	1-18
A	JANECEK S ET AL: "The evolution of starch-binding domain" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 456, no. 1, 30 July 1999 (1999-07-30), pages 119-125, XP004260050 ISSN: 0014-5793 table 1	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2005/000783

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 2474082 A1	21-08-2003
		CN 1633503 A	29-06-2005
		EP 1476556 A2	17-11-2004
		MX PA04007811 A	15-10-2004
		US 2005107332 A1	19-05-2005
WO 0238787 A	16-05-2002	AU 1384102 A	21-05-2002
		EP 1335982 A2	20-08-2003
WO 9928448 A	10-06-1999	AU 1434299 A	16-06-1999
		CN 1284129 A	14-02-2001
		EP 1032654 A1	06-09-2000
		JP 2001525167 T	11-12-2001
WO 2004055178 A	01-07-2004	AU 2003287900 A1	09-07-2004
		EP 1576152 A1	21-09-2005