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(54) Title: ALPHA-AMYLASE MUTANTS WITH ALTERED PROPERTIES

(57) Abstract: The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered stability, in particular at high temperatures and/or at low pH relative, and/or low Ca2+ to the parent alpha-amylase.

Alpha-amylase mutants with altered properties

FIELD OF THE INVENTION

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: stability under, e.g., high temperature and/or low pH conditions, in particular at low calcium concentrations. The variant of the invention are suitable for starch conversion, ethanol production, laundry wash, dish wash, hard surface cleaning, textile desizing, and/or sweetner production.

BACKGROUND OF THE INVENTION

Alpha-Amylases (alpha-1,4-glucan-4-glucanohydrolases, E.C. 3.2.1.1) constitute a group of enzymes, which catalyze hydrolysis of starch and other linear and branched 1,4-glucosidic oligo- and polysaccharides.

BRIEF DISCLOSURE OF THE INVENTION

The object of the present invention is to provide Termamyl-like amylases which variants in comparison to the corresponding parent alpha-amylase, i.e., un-mutated alpha-amylase, has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: stability under, e.g., high temperature and/or low pH conditions, in particular at low calcium concentrations.

Nomenclature

In the present description and claims, the conventional one-letter and three-letter codes for amino acid residues are

used. For ease of reference, alpha-amylase variants of the invention are described by use of the following nomenclature:
Original amino acid(s): position(s): substituted amino acid(s)

According to this nomenclature, for instance the substitution of alanine for asparagine in position 30 is shown as:

Ala30Asn or A30N

a deletion of alanine in the same position is shown as:

Ala30* or A30*

and insertion of an additional amino acid residue, such as lysine, is shown as:

Ala30AlaLys or A30AK

A deletion of a consecutive stretch of amino acid residues, such as amino acid residues 30-33, is indicated as (30-33)* or Δ(A30-N33).

Where a specific alpha-amylase contains a "deletion" in comparison with other alpha-amylases and an insertion is made in such a position this is indicated as:

*36Asp or *36D

for insertion of an aspartic acid in position 36.
Multiple mutations are separated by plus signs, i.e.:

Ala30Asp + Glu34Ser or A30N+E34S

representing mutations in positions 30 and 34 substituting alanine and glutamic acid for asparagine and serine, respectively.

When one or more alternative amino acid residues may be inserted in a given position it is indicated as

A30N,E or

A30N or A30E

Furthermore, when a position suitable for modification is identified herein without any specific modification being suggested, it is to be understood that any amino acid residue may be substituted for the amino acid residue present in the

position. Thus, for instance, when a modification of an alanine in position 30 is mentioned, but not specified, it is to be understood that the alanine may be deleted or substituted for any other amino acid, i.e., any one of:

R,N,D,A,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V.

Further, "A30X" means any one of the following substitutions: A30R, A30N, A30D, A30C, A30Q, A30E, A30G, A30H, A30I, A30L, A30K, A30M, A30F, A30P, A30S, A30T, A30W, A30Y, or A30 V; or in short: A30R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V.

If the parent enzyme - used for the numbering - already has the amino acid residue in question suggested for substitution in that position the following nomenclature is used:

"X30N" or "X30N,V" in the case where for instance one or N or V is present in the wildtype.

Thus, it means that other corresponding parent enzymes are substituted to an "Asn" or "Val" in position 30.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an alignment of the amino acid sequences of five parent Termamyl-like alpha-amylases. The numbers on the extreme left designate the respective amino acid sequences as follows:

1: SEQ ID NO: 4 (SP722)

2: SEQ ID NO: 2 (SP690)

3: SEQ ID NO: 10 (BAN)

4: SEQ ID NO: 8 (BLA)

5: SEQ ID NO: 6 (BSG).

DETAILED DISCLOSURE OF THE INVENTION

The object of the present invention is to provide Termamyl-like amylases, which variants have alpha-amylase activity and exhibits altered stability at high temperatures and/or at low pH, in particular at low calcium concentrations.

Termamyl-like alpha-amylases

A number of alpha-amylases produced by Bacillus spp. are highly homologous (identical) on the amino acid level. The identity of a number of known Bacillus alpha-amylases can be found in the below Table 1:

Table 1

Percent identity	707	AP1378	BAN	BSG	SP690	SP722	AA560	Termamyl
707	100.0	86.4	66.9	66.5	87.6	86.2	95.5	68.1
AP1378	86.4	100.0	67.1	68.1	95.1	86.6	86.0	69.4
BAN	66.9	67.1	100.0	65.6	67.1	68.8	66.9	80.7
BSG	66.5	68.1	65.6	100.0	67.9	67.1	66.3	65.4
SP690	87.6	95.1	67.1	67.9	100.0	87.2	87.0	69.2
SP722	86.2	86.6	68.8	67.1	87.2	100.0	86.8	70.8
AA560	95.5	86.0	66.9	66.3	87.0	86.8	100.0	68.3
Termamyl	68.1	69.4	80.7	65.4	69.2	70.8	68.3	100.0

For instance, the B. licheniformis alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 8 (commercially available as Termamyl™) has been found to be about 81% homologous with the B. amyloliquefaciens alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 10 and about 65% homologous with the B. stearothermophilus alpha-amylase (BSG) comprising the amino acid sequence shown in SEQ ID NO: 6. Further homologous alpha-amylases include SP690 and SP722 disclosed in WO 95/26397 and further depicted in SEQ ID NO: 2 and SEQ ID NO: 4, respectively, herein. Other amylases are the AA560 alpha-amylase derived from Bacillus sp. and shown in SEQ ID NO: 12, and the #707 alpha-amylase derived from Bacillus sp., shown in SEQ ID NO: 13 and described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31.

The KSM AP1378 alpha-amylase is disclosed in WO 97/00324 (from KAO Corporation).

Still further homologous alpha-amylases include the alpha-amylase produced by the B. licheniformis strain described in EP 0252666 (ATCC 27811), and the alpha-amylases identified in WO 91/00353 and WO 94/18314. Other commercial Termamyl-like alpha-amylases are comprised in the products sold under the following tradenames: Optitherm™ and Takatherm™ (Solvay); Maxamyl™ (available from Gist-brocades/Genencor), Spezym AA™ and Spezyme Delta AATM (available from Genencor), and Keistase™ (available from Daiwa), Dex lo, GC 521 (available from Genencor) and Ultraphlow (from Enzyme Biosystems).

Because of the substantial homology found between these alpha-amylases, they are considered to belong to the same class of alpha-amylases, namely the class of "Termamyl-like alpha-amylases".

Accordingly, in the present context, the term "Termamyl-like" alpha-amylase" is intended to indicate an alpha-amylase, in particular Bacillus alpha-amylase, which, at the amino acid level, exhibits a substantial identity to Termamyl™, i.e., the B. licheniformis alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8, herein.

In other words, all the following alpha-amylases, which has the amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12 and 13 herein are considered to be "Termamyl-like alpha-amylase". Other Termamyl-like alpha-amylases are alpha-amylases i) which displays at least 60%, such as at least 70%, e.g., at least 75%, or at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% homology (identity) with at least one of said amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, and 13, and/or is encoded by a DNA sequence which hybridizes to the DNA

sequences encoding the above-specified alpha-amylases which are apparent from SEQ ID NOS: 1, 3, 5, 7, 9, and of the present specification (which encoding sequences encode the amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10 and 12 herein, respectively).

Homology

The homology may be determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (described above). Thus, Gap GCGv8 may be used with the default scoring matrix for identity and the following default parameters: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, respectively for nucleic acidic sequence comparison, and GAP creation penalty of 3.0 and GAP extension penalty of 0.1, respectively, for protein sequence comparison. GAP uses the method of Needleman and Wunsch, (1970), J.Mol. Biol. 48, p.443-453, to make alignments and to calculate the identity.

A structural alignment between Termamyl (SEQ ID NO: 8) and, e.g., another alpha-amylase may be used to identify equivalent/corresponding positions in other Termamyl-like alpha-amylases. One method of obtaining said structural alignment is to use the Pile Up programme from the GCG package using default values of gap penalties, i.e., a gap creation penalty of 3.0 and gap extension penalty of 0.1. Other structural alignment methods include the hydrophobic cluster analysis (Gaboriaud et al., (1987), FEBS LETTERS 224, pp. 149-155) and reverse threading (Huber, T; Torda, AE, PROTEIN SCIENCE Vol. 7, No. 1 pp. 142-149 (1998).

Hybridisation

The oligonucleotide probe used in the characterisation of the Termamyl-like alpha-amylase above may suitably be prepared on the basis of the full or partial nucleotide or amino acid sequence of the alpha-amylase in question.

Suitable conditions for testing hybridisation involve pre-soaking in 5xSSC and prehybridizing for 1 hour at 40°C in a solution of 20% formamide, 5xDenhardt's solution, 50mM sodium phosphate, pH 6.8, and 50mg of denatured sonicated calf thymus DNA, followed by hybridisation in the same solution supplemented with 100 mM ATP for 18 hours at 40°C, followed by three times washing of the filter in 2xSSC, 0.2% SDS at 40°C for 30 minutes (low stringency), preferred at 50°C (medium stringency), more preferably at 65°C (high stringency), even more preferably at 75°C (very high stringency). More details about the hybridisation method can be found in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989.

In the present context, "derived from" is intended not only to indicate an alpha-amylase produced or producible by a strain of the organism in question, but also an alpha-amylase encoded by a DNA sequence isolated from such strain and produced in a host organism transformed with said DNA sequence. Finally, the term is intended to indicate an alpha-amylase, which is encoded by a DNA sequence of synthetic and/or cDNA origin and which has the identifying characteristics of the alpha-amylase in question. The term is also intended to indicate that the parent alpha-amylase may be a variant of a naturally occurring alpha-amylase, i.e., a variant, which is the result of a modification (insertion, substitution, deletion) of one or more amino acid residues of the naturally occurring alpha-amylase.

Parent Termamyl-like Alpha-amylases

According to the invention all Termamy-like alpha-amylases, as defined above, may be used as the parent (i.e., backbone) alpha-amylase. In a preferred embodiment of the invention the parent alpha-amylase is derived from B. licheniformis, e.g., one of those referred to above, such as the B. licheniformis alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

Parent hybrid Termamyl-like Alpha-amylases

The parent alpha-amylase (i.e., backbone alpha-amylase) may also be a hybrid alpha-amylase, i.e., an alpha-amylase, which comprises a combination of partial amino acid sequences derived from at least two alpha-amylases.

The parent hybrid alpha-amylase may be one, which on the basis of amino acid homology (identity) and/or DNA hybridization (as defined above) can be determined to belong to the Termamyl-like alpha-amylase family. In this case, the hybrid alpha-amylase is typically composed of at least one part of a Termamyl-like alpha-amylase and part(s) of one or more other alpha-amylases selected from Termamyl-like alpha-amylases or non-Termamyl-like alpha-amylases of microbial (bacterial or fungal) and/or mammalian origin.

Thus, the parent hybrid alpha-amylase may comprise a combination of partial amino acid sequences deriving from at least two Termamyl-like alpha-amylases, or from at least one Termamyl-like and at least one non-Termamyl-like bacterial alpha-amylase, or from at least one Termamyl-like and at least one fungal alpha-amylase. The Termamyl-like alpha-amylase from which a partial amino acid sequence derives, may be any of the specific Termamyl-like alpha-amylase referred to herein.

For instance, the parent alpha-amylase may comprise a C-terminal part of an alpha-amylase derived from a strain of B. licheniformis, and a N-terminal part of an alpha-amylase derived from a strain of B. amyloliquefaciens or from a strain of B. stearothermophilus. For instance, the parent alpha-amylase may comprise at least 430 amino acid residues of the C-terminal part of the B. licheniformis alpha-amylase, and may, e.g., comprise a) an amino acid segment corresponding to the 37 N-terminal amino acid residues of the B. amyloliquefaciens alpha-amylase having the amino acid sequence shown in SEQ ID NO: 10 and an amino acid segment corresponding to the 445 C-terminal amino acid residues of the B. licheniformis alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8, or a hybrid Termamyl-like alpha-amylase being identical to the Termamyl sequence, i.e., the Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8, except that the N-terminal 35 amino acid residues (of the mature protein) has been replaced by the N-terminal 33 residues of BAN (mature protein), i.e., the Bacillus amyloliquefaciens alpha-amylase shown in SEQ ID NO: 10; or b) an amino acid segment corresponding to the 68 N-terminal amino acid residues of the B. stearothermophilus alpha-amylase having the amino acid sequence shown in SEQ ID NO: 6 and an amino acid segment corresponding to the 415 C-terminal amino acid residues of the B. licheniformis alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

Another suitable parent hybrid alpha-amylase is the one previously described in WO 96/23874 (from Novo Nordisk) constituting the N-terminus of BAN, Bacillus amyloliquefaciens alpha-amylase (amino acids 1-300 of the mature protein) and the C-terminus from Termamyl (amino acids 301-483 of the mature protein).

In a preferred embodiment of the invention the parent Termamyl-like alpha-amylase is a hybrid alpha-amylase of SEQ ID NO: 8 and SEQ ID NO: 10. Specifically, the parent hybrid Termamyl-like alpha-amylase may be a hybrid alpha-amylase comprising the 445 C-terminal amino acid residues of the B. licheniformis alpha-amylase shown in SEQ ID NO: 8 and the 37 N-terminal amino acid residues of the alpha-amylase derived from B. amyloliquefaciens shown in SEQ ID NO: 10, which may suitably further have the following mutations: H156Y+A181T+N190F+A209V+Q264S (using the numbering in SEQ ID NO: 8). The latter mentioned hybrid is used in the examples below and is referred to as LE174.

Other specifically contemplated parent alpha-amylase include LE174 with fewer mutations, i.e., the right above mentioned hydrid having the following mutations: A181T+N190F+A209V+Q264S; N190F+A209V+Q264S; A209V+Q264S; Q264S; H156Y+N190F+A209V+Q264S; H156Y+A209V+Q264S; H156Y+Q264S; H156Y+A181T+A209V+Q264S; H156Y+A181T+Q264S; H156Y+Q264S; H156Y+A181T+N190F+Q264S; H156Y+A181T+N190F; H156Y+A181T+N190F+A209V. These hybrids are also considered to be part of the invention.

In a preferred embodiment the parent Termamyl-like alpha amylase is LE174, SP722, or AA560 including any of LE174+G48A+T49I+G107A+I201F; LE174+M197L; LE174+G48A+T49I+G107A+M197L+I201F, or SP722+D183*+G184*; SP722+D183*+G184*+N195F; SP722+D183*+G184*+M202L; SP722+D183*+G184*+N195F+M202L; BSG+I181*+G182*; BSG+I181*+G182*+N193F; BSG+I181*+G182*+M200L; BSG+I181*+G182*+N193F+M200L; AA560+D183*+G184*; AA560+D183*+G184*+N195F; AA560+D183*+G184*+M202L; AA560+D183*+G184*+N195F+M202L.

Other parent alpha-amylases contemplated include LE429, which is LE174 with an additional substitution in I201F.

According to the invention LE335 is the alpha-amylase, which in comparison to LE429 has additional substitutions in T49I+G107A; LE399 is LE335+G48A, i.e., LE174, with G48A+T49I+G107A+I201F.

Altered properties

The following section discusses the relationship between mutations, which are present in variants of the invention, and desirable alterations in properties (relative to those of a parent Termamyl-like alpha-amylase), which may result therefrom.

As mentioned above the invention relates to Termamyl-like alpha-amylases with altered properties (as mentioned above), in particular at high temperatures and/or at low pH, in particular at low calcium concentrations.

In the context of the present invention "high temperature" means temperatures from 70-120°C, preferably 80-100°C, especially 85-95°C.

In the context of the present invention the term "low pH" means from a pH in the range from 4-6, preferably 4.2-5.5, especially 4.5-5.

In the context of the present invention the term "high pH" means from a pH in the range from 8-11, especially 8.5-10.6.

In the context of the present invention the term "low calcium concentration" means free calcium levels lower than 60 ppm, preferably 40 ppm, more preferably 25 ppm, especially 5 ppm calcium.

Parent Termamyl-like alpha-amylase specifically contemplated in connection with going through the specifically contemplated altered properties are the above mentioned parent Termamyl-like alpha-amylase and parent hydrid Termamyl-like alpha-amylases.

The Termamyl® alpha-amylase is used as the starting point, but corresponding positions in, e.g., the SP722, BSG, BAN, AA560, SP690, KSM AP1378, and #707 should be understood as disclosed and specifically comtemplated too.

In a preferred embodiment the variant of the invention has in particular at high temperatures and/or at low pH.

In an aspect the invention relates to variant with altered properties as mentioned above.

In the first aspect a variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions (using SEQ ID NO: 8 for the amino acid numbering) selected from the group of:

49, 60, 104, 132, 161, 170, 176, 179, 180, 181, 183, 200, 203, 204, 207, 212, 237, 239, 250, 280, 298, 318, 374, 385, 393, 402, 406, 427, 430, 440, 444, 447, 482,

wherein

(a) the alteration(s) are independently

(i) an insertion of an amino acid downstream of the amino acid which occupies the position,

(ii) a deletion of the amino acid which occupies the position, or

(iii) a substitution of the amino acid which occupies the position with a different amino acid,

(b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

In Termamyl® (SEQ ID NO: 8) such corresponding positions are:

T49; D60; N104; E132; D161; K170; K176; G179; K180; A181; D183; D200; Y203; D204; D207; I212; K237; S239; E250; N280; Q298; L318; Q374; E385; Q393; Y402; H406; L427 D430; V440; N444; E447; Q482.

In SP722 (SEQ ID NO: 4) the corresponding positions are: T51; D62; N106; D134; D163; Q172; K179; G184; K185; A186; D188; D205; M208; D209; X212; L217, K242, S244, N255, N285, S303, M323; D387, N395; Y404; H408; I429; D432; V442; K446; Q449; K484.

Corresponding positions in other parent alpha-amylases can be found by alignment as described above and shown in the alignment in Fig. 1.

In a preferred embodiment the variant of the invention (using SEQ ID NO: 8 (Termamyl™) for the numbering) has one or more of the following substitutions:

T49I; D60N; N104D; E132A,V,P; D161N; K170Q; K176R; G179N; K180T; A181N; D183N; D200N; X203Y; D204S; D207V,E,L,G; X212I; K237P; S239W; E250G,F; N280S; X298Q; L318M; Q374R; E385V; Q393R; Y402F; H406L,W; L427I D430N; V440A; N444R,K; E447Q,K; Q482K.

In a preferred embodiment the variant of the invention (using SEQ ID NO: 4 (SP722) for the numbering) has one or more of the following substitutions:

T51I; D62N; N106D; D134A,V,P; D163N; X172Q; K179R; G184N; K185T; A186N; D188N; D205N; M208Y; D209S; X212V,E,L,G; L217I, K242P, S244W, N255G,F, N285S, S303Q, X323M; D387V, N395R; Y404F; H408L,W; X429I; D432N; V442A; X446R,K; X449Q,K; X484K, using SEQ ID NO: 4 (SP722) for the numbering.

Preferred double, triple and multi-mutations - using SEQ ID NO: 8 as the basis for the numbering - are selected from the group consisting of:

T49I+D60N; T49I+D60N+E132A; T49I+D60N+E132V;
T49I+D60N+E132V+K170Q; T49I+D60N+E132A+K170Q;
T49I+D60N+E132V+K170Q+K176R; T49I+D60N+E132A+K170Q+K176R;
T49I+D60N+E132V+K170Q+K176R+D207V;
T49I+D60N+E132A+K170Q+K176R+D207V;
T49I+D60N+E132V+K170Q+K176R+D207E;
T49I+D60N+E132A+K170Q+K176R+D207E;

T49I+D60N+E132V+K170Q+K176R+D207V+E250G;
T49I+D60N+E132A+K170Q+K176R+D207V+E250G;
T49I+D60N+E132V+K170Q+K176R+D207E+E250G;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G;
T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S;
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E385V;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
E385V;
T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
E385V+Q393R;
T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
E385V+Q393R;
T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
E385V+Q393R;
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V+ Q393R;

T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
E385V+Q393R+Y402F;
T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F;
T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R+Y402F;
T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
E385V+Q393R+Y402F+H406L;
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E385V+Q393R+Y402F+H406L;
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E385V+Q393R+Y402F+H406L;
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V+ Q393R+Y402F+H406L;
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T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
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E385V+Q393R+Y402F+H406L+L427I+V440A;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R+Y402F+H406L+L427I+V440A;

D60N+E132A; D60N+E132V; D60N+E132V+K170Q; D60N+E132A+K170Q;
D60N+E132V+K170Q+K176R; T49I+D60N+E132A+K170Q+K176R;
D60N+E132V+K170Q+K176R+D207V;
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H406W+D430N; N444K+E447K+Q482K; E447K+Q482K;
N104D+D161N+A181N+D183N+D200N+D204S+K237P+S239W;
N104D+D161N+A181N+D183N+D200N+D204S+K237P;
N104D+D161N+A181N+D183N+D200N+D204S;
D161N+A181N+D183N+D200N+D204S+K237P+S239W;
D161N+A181N+D183N+D200N+D204S+K237P;
D161N+A181N+D183N+D200N+D204S; K237P+S239W, using SEQ ID NO: 8 for the numbering.

In a preferred embodiment the variant has the following substitutions: K170Q+D207V+N280S; E132A+D207V; D207E+E250G+H406L+L427I; D207V+L318M; D60N+D207V+L318M; T49I+E132V+V440A; T49I+K176R+D207V+Y402F; Q374R+E385V+Q393R; N190F+A209V+Q264S; G48A+T49I+G107A+I201F; T49I+G107A+I201F; G48A+T49I+I201F; G48A+T49I+G107A; T49I+I201F; T49I+G107A; G48A+T49I;
D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
D430N+N444K+E447Q+Q482K using SEQ ID NO: 8 for the numbering. Specific variant include: LE399; LE174+G48A+T49I+G107A; LE174+G48A+T49I+I201F; LE174+G48A+G107A+I201F; LE174+T49I+G107A+I201F; LE174+G48A+T49I; LE174+G48A; LE174+G107A+I201F; LE174+I201F, are specifically contemplated variants of the invention.

Stability

In the context of the present invention, mutations (including amino acid substitutionsa and deletion) of importance with respect to achieving altered stability, in particular improved stability (i.e., higher or lower), at

especially high temperatures (i.e., 70-120°C) and/or extreme pH (i.e. low or high pH, i.e, pH 4-6 or pH 8-11, respectively), in particular at free (i.e., unbound, therefore in solution) calcium concentrations below 60 ppm, include any of the mutations listed in the "Altered properties" section. The stability may be determined as described in the "Materials & Methods" section below.

General mutations in variants of the invention

A variant of the invention may in one embodiment comprise one or more modifications in addition to those outlined above. Thus, it may be advantageous that one or more Proline (Pro) residues present in the part of the alpha-amylase variant which is modified is/are replaced with a non-Proline residue which may be any of the possible, naturally occurring non-Proline residues, and which preferably is an Alanine, Glycine, Serine, Threonine, Valine or Leucine.

Analogously, in one embodiment one or more Cysteine residues present in the parent alpha-amylase may be replaced with a non-Cysteine residue such as Serine, Alanine, Threonine, Glycine, Valine or Leucine.

Furthermore, a variant of the invention may - either as the only modification or in combination with any of the above outlined modifications - be modified so that one or more Asp and/or Glu present in an amino acid fragment corresponding to the amino acid fragment 185-209 of SEQ ID NO: 10 is replaced by an Asn and/or Gln, respectively. Also of interest is the replacement, in the Termamyl-like alpha-amylase, of one or more of the Lys residues present in an amino acid fragment corresponding to the amino acid fragment 185-209 of SEQ ID NO: 10 by an Arg.

It is to be understood that the present invention encompasses variants incorporating two or more of the above outlined modifications.

Furthermore, it may be advantageous to introduce mutations in one or more of the following positions (using SEQ ID NO: 8 (Termamyl) for the numbering):

M15, V128, A111, H133, W138, T149, M197, N188, A209, A210, H405, T412, in particular the following single, double or triple or multi mutations:

M15X, in particular M15T,L;

V128X, in particular V128E;

H133X, in particular H133Y;

N188X, in particular N188S,T,P;

M197X, in particular M197T,L;

A209X, in particular A209V;

M197T/W138F; M197T/W138Y; M15T/H133Y/N188S; M15/V128E/H133Y/N188S; E119C/S130C; D124C/R127C; H133Y/T149I; G475R, H133Y/S187D; H133Y/A209V.

Methods for preparing alpha-amylase variants of the invention

Several methods for introducing mutations into genes are known in the art. After a brief description of cloning of alpha-amylase-encoding DNA sequences, methods for generating mutations at specific sites within the alpha-amylase-encoding sequence will be discribed.

Cloning a DNA sequence encoding an alpha-amylase

The DNA sequence encoding a parent alpha-amylase may be isolated from any cell or microorganism producing the alpha-amylase in question, using various methods well known in the art. First, a genomic DNA and/or cDNA library should be constructed using chromosomal DNA or messenger RNA from the organism that produces the alpha-amylase to be studied. Then,

if the amino acid sequence of the alpha-amylase is known, homologous, labeled oligonucleotide probes may be synthesized and used to identify alpha-amylase-encoding clones from a genomic library prepared from the organism in question. Alternatively, a labeled oligonucleotide probe containing sequences homologous to a known alpha-amylase gene could be used as a probe to identify alpha-amylase-encoding clones, using hybridization and washing conditions of lower stringency.

Yet another method for identifying alpha-amylase-encoding clones would involve inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming alpha-amylase-negative bacteria with the resulting genomic DNA library, and then plating the transformed bacteria onto agar containing a substrate for alpha-amylase, thereby allowing clones expressing the alpha-amylase to be identified.

Alternatively, the DNA sequence encoding the enzyme may be prepared synthetically by established standard methods, e.g., the phosphoroamidite method described by S.L. Beaucage and M.H. Caruthers, Tetrahedron Letters 22, 1981, pp. 1859-1869, or the method described by Matthes et al., The EMBO J. 3, 1984, pp. 801-805. In the phosphoroamidite method, oligonucleotides are synthesized, e.g., in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors.

Finally, the DNA sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate, the fragments corresponding to various parts of the entire DNA sequence), in accordance with standard techniques. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., Science 239, 1988, pp. 487-491.

Site-directed mutagenesis

Once an alpha-amylase-encoding DNA sequence has been isolated, and desirable sites for mutation identified, mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites; mutant nucleotides are inserted during oligonucleotide synthesis. In a specific method, a single-stranded gap of DNA, bridging the alpha-amylase-encoding sequence, is created in a vector carrying the alpha-amylase gene. Then the synthetic nucleotide, bearing the desired mutation, is annealed to a homologous portion of the single-stranded DNA. The remaining gap is then filled in with DNA polymerase I (Klenow fragment) and the construct is ligated using T4 ligase. A specific example of this method is described in Morinaga et al. (1984). US 4,760,025 disclose the introduction of oligonucleotides encoding multiple mutations by performing minor alterations of the cassette. However, an even greater variety of mutations can be introduced at any one time by the Morinaga method, because a multitude of oligonucleotides, of various lengths, can be introduced.

Another method for introducing mutations into alpha-amylase-encoding DNA sequences is described in Nelson and Long

(1989). It involves the 3-step generation of a PCR fragment containing the desired mutation introduced by using a chemically synthesized DNA strand as one of the primers in the PCR reactions. From the PCR-generated fragment, a DNA fragment carrying the mutation may be isolated by cleavage with restriction endonucleases and reinserted into an expression plasmid.

Alternative methods for providing variants of the invention include gene shuffling, e.g., as described in WO 95/22625 (from Affymax Technologies N.V.) or in WO 96/00343 (from Novo Nordisk A/S), or other corresponding techniques resulting in a hybrid enzyme comprising the mutation(s), e.g., substitution(s) and/or deletion(s), in question. Examples of parent alpha-amylases, which suitably may be used for providing a hybrid with the desired mutations(s) according to the invention include the KSM-K36 and KSM-K38 alpha-amylases disclosed in EP 1,022,334 (hereby incorporated by reference).

Expression of alpha-amylase variants

According to the invention, a DNA sequence encoding the variant produced by methods described above, or by any alternative methods known in the art, can be expressed, in enzyme form, using an expression vector which typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

The recombinant expression vector carrying the DNA sequence encoding an alpha-amylase variant of the invention may be any vector, which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of

which is independent of chromosomal replication, e.g., a plasmid, a bacteriophage or an extrachromosomal element, minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

The expression vector of the invention may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the alpha-amylase variant of the invention. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the dal genes from B. subtilis or B. licheniformis, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracyclin resistance. Furthermore, the vector may comprise Aspergillus selection markers such as amdS, argB, niaD and sC, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, e.g., as described in WO 91/17243.

While intracellular expression may be advantageous in some respects, e.g., when using certain bacteria as host cells, it is generally preferred that the expression is extracellular. In general, the Bacillus alpha-amylases mentioned herein comprise a preregion permitting secretion of the expressed protease into the culture medium. If desirable, this preregion may be replaced by a different preregion or signal sequence, conveniently accomplished by substitution of the DNA sequences encoding the respective preregions.

The procedures used to ligate the DNA construct of the invention encoding an alpha-amylase variant, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989).

The cell of the invention, either comprising a DNA construct or an expression vector of the invention as defined above, is advantageously used as a host cell in the

recombinant production of an alpha-amylase variant of the invention. The cell may be transformed with the DNA construct of the invention encoding the variant, conveniently by integrating the DNA construct (in one or more copies) in the host chromosome. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional methods, e.g., by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells.

The cell of the invention may be a cell of a higher organism such as a mammal or an insect, but is preferably a microbial cell, e.g., a bacterial or a fungal (including yeast) cell.

Examples of suitable bacteria are Gram-positive bacteria such as Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus, Bacillus megaterium, Bacillus thuringiensis, or Streptomyces lividans or Streptomyces murinus, or gramnegative bacteria such as E.coli. The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known per se.

The yeast organism may favorably be selected from a species of Saccharomyces or Schizosaccharomyces, e.g. Saccharomyces cerevisiae. The filamentous fungus may advantageously belong to a species of Aspergillus, e.g., Aspergillus oryzae or Aspergillus niger. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a

manner known per se. A suitable procedure for transformation of Aspergillus host cells is described in EP 238 023.

In a yet further aspect, the present invention relates to a method of producing an alpha-amylase variant of the invention, which method comprises cultivating a host cell as described above under conditions conducive to the production of the variant and recovering the variant from the cells and/or culture medium.

The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the alpha-amylase variant of the invention. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g., as described in catalogues of the American Type Culture Collection).

The alpha-amylase variant secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

Industrial Applications

The alpha-amylase variants of this invention possess valuable properties allowing for a variety of industrial applications. In particular, enzyme variants of the invention are applicable as a component in washing, dishwashing, and hard surface cleaning detergent compositions.

Variant of the invention with altered properties may be used for starch processes, in particular starch conversion, especially liquefaction of starch (see, e.g., US 3,912,590, EP

patent publications Nos. 252 730 and 63 909, WO 99/19467, and WO 96/28567 all references hereby incorporated by reference). Also contemplated are compositions for starch conversion purposes, which may beside the variant of the invention also comprise a AMG, pullulanase, and other alpha-amylases.

Further, variants of the invention are also particularly useful in the production of sweeteners and ethanol (see, e.g., US patent no. 5,231,017 hereby incorporated by reference), such as fuel, drinking and industrial ethanol, from starch or whole grains.

A variant of the invention may also be used for textile desizing (see, e.g., WO 95/21247, US patent 4,643,736, EP 119,920 hereby in corporate by reference).

Detergent compositions

As mentioned above, variants of the invention may suitably be incorporated in detergent compositions. Reference is made, for example, to WO 96/23874 and WO 97/07202 for further details concerning relevant ingredients of detergent compositions (such as laundry or dishwashing detergents), appropriate methods of formulating the variants in such detergent compositions, and for examples of relevant types of detergent compositions.

Detergent compositions comprising a variant of the invention may additionally comprise one or more other enzymes, such as a protease, a lipase, a peroxidase, another amylolytic enzyme, glucoamylase, maltogenic amylase, CGTase and/or a cellulase, mannanase (such as Mannaway™ from Novozymes, Denmark)), pectinase, pectine lyase, cutinase, laccase, and/or another alpha-amylase.

Alpha-amylase variants of the invention may be incorporated in detergents at conventionally employed concentrations. It is at present contemplated that a variant of the invention may be

incorporated in an amount corresponding to 0.00001-10 mg (calculated as pure, active enzyme protein) of alpha-amylase per liter of wash/dishwash liquor using conventional dosing levels of detergent.

Compositions

The invention also related to composition comprising a variant of the invention, and in a preferred embodiment also a B. stearothermophilus alpha-amylase (BSG), in particular a variant thereof.

In another embodient the composition comprises beside a variant of the invention a glucoamylase, in particular a glucoamylase originating from Aspergillus niger (e.g., the G1 or G2 A. niger AMG disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from Aspergillus niger are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102, or a variant therefore, in particular a variant disclosed in WO 00/04136 or WO 01/04273 or the Talaromyces emersonii AMG disclosed in WO 99/28448.

A specific combination is LE399 and a variant disclosed in WO 00/04136 or Wo 01/04273, in particular a variant with oe or more of the following substitutions:
N9A,S56A,V59A,S119P,A246T,N313G,E342T,A393R,S394R,Y402F,E408R,
in particular a variant with all mutation.

In an embodiment the composition of the invention also comprises a pullulanase, in particular a Bacillus pullulanase.

MATERIALS AND METHODS

Enzymes:

Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8 and also available from Novozymes.

AA560: SEQ ID NO: 12; disclosed in WO 00/60060; deposited on 25th January 1999 at DSMZ and assigned the DSMZ no. 12649. AA560 were deposited by the inventors under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at Deutshe Sammmlung von Microorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig DE.

LB medium (In 1 liter H2O: 10 g bacto-tryptone, 5 g bacto-yeast extract, 10 g NaCl, pH adjusted to 7.0 w. NaOH, autoclaved).

TY agar plates (In 1 liter H2O: 16 g bacto-tryptone, 10 g bacto-yeast extract, 5 g NaCl, pH adjusted to 7.0 w. NaOH, and 15 g bacto-agar is added prior to autoclaving).

10% Lugol solution (Iodine/Potassium iodine solution; made by 10-fold dil. in H2O of stock: Sigma Cat. no. L 6146).

Bacillus subtilis SHA273: see WO 95/10603

Plasmids

pDN1528 contains the complete gene encoding Termamyl, amyL, the expression of which is directed by its own promoter. Further, the plasmid contains the origin of replication, ori, from plasmid pUB110 and the cat gene from plasmid pC194 conferring resistance towards chloramphenicol. pDN1528 is shown in Fig. 9 of WO 96/23874.

Methods:

Low pH filter assay

Bacillus libraries are plated on a sandwich of cellulose acetate (OE 67, Schleicher & Schuell, Dassel, Germany) - and

nitrocellulose filters (Protran-Ba 85, Schleicher & Schuell, Dassel, Germany) on TY agar plates with 10 micro g/ml chloramphenicol at 37°C for at least 21 hours. The cellulose acetate layer is located on the TY agar plate.

Each filter sandwich is specifically marked with a needle after plating, but before incubation in order to be able to localize positive variants on the filter, and the nitrocellulose filter with bound variants is transferred to a container with citrate buffer, pH 4.5 and incubated at 80°C for 20 minutes (when screening for variants in the wild type backbone) or 85oC for 60 minutes (when screening for variants in the LE399 backbone). The cellulose acetate filters with colonies are stored on the TY-plates at room temperature until use. After incubation, residual activity is detected on assay plates containing 1% agarose, 0.2% starch in citrate buffer, pH 6.0. The assay plates with nitrocellulose filters are marked the same way as the filter sandwich and incubated for 2 hours at 50°C. After removal of the filters the assay plates are stained with 10% Lugol solution. Starch degrading variants are detected as white spots on dark blue background and then identified on the storage plates. Positive variants are re-screened twice under the same conditions as the first screen.

Secondary screening

Positive transformants after rescreening are picked from the storage plate and tested in a secondary plate assay. Positive transformants are grown for 22 hours at 37°C in 5 ml LB + chloramphenicol. The Bacillus culture of each positive transformant and as a control a clone expressing the corresponding backbone are incubated in citrate buffer, pH 4.5 at 90°C and samples are taken at 0, 10, 20, 30, 40, 60 and 80 minutes. A 3 micro liter sample is spotted on an assay plate.

The assay plate is stained with 10% Lugol solution. Improved variants are seen as variants with higher residual activity (detected as halos on the assay plate) than the backbone. The improved variants are determined by nucleotide sequencing.

Stability assay of unpurified variants:

Bacillus cultures expressing the variants to be analysed are grown for 21 hours at 37°C in 10 ml LB+chloramphenicol. 800 micro liter culture is mixed with 200 micro l citrate buffer, pH 4.5. A number of 70 micro l aliquots corresponding to the number of sample time points are made in PCR tubes and incubated at 70°C (for variants in the wt backbone) or 90°C (for variants in LE399) for various time points (typically 5, 10, 15, 20, 25 and 30 minutes) in a PCR machine. The 0 min sample is not incubated at high temperature. Activity in the sample is measured by transferring 20 micro l to 200 micro l of the alpha-amylase PNP-G7 substrate MPR3 ((Boehringer Mannheim Cat. no. 1660730) as described below under "Assays for Alpha-Amylase Activity". Results are plotted as percentage activity (relative to the 0 time point) versus time, or stated as percentage residual activity after incubation for a certain period of time.

Fermentation and purification of alpha-amylase variants

A B. subtilis strain harbouring the relevant expression plasmid is streaked on a LB-agar plate with 10 micro g/ml kanamycin from -80°C stock, and grown overnight at 37°C.
The colonies are transferred to 100 ml PS-1 media supplemented with 10 micro g/ml chloamphinicol in a 500 ml shaking flask.

Composition of PS-1 medium:

Pearl sugar	100 g/l
Soy Bean Meal	40 g/l
Na2HPO4, 12 H2O	10 g/l
PluronicTM PE 6100	0.1 g/l
CaCO3	5 g/l

The culture is shaken at 37°C at 270 rpm for 5 days.

Cells and cell debris are removed from the fermentation broth by centrifugation at 4500 rpm in 20-25 minutes. Afterwards the supernatant is filtered to obtain a completely clear solution. The filtrate is concentrated and washed on a UF-filter (10000 cut off membrane) and the buffer is changed to 20mM Acetate pH 5.5. The UF-filtrate is applied on a S-sepharose F.F. and elution is carried out by step elution with 0.2M NaCl in the same buffer. The eluate is dialysed against 10mM Tris, pH 9.0 and applied on a Q-sepharose F.F. and eluted with a linear gradient from 0-0.3M NaCl over 6 column volumes. The fractions that contain the activity (measured by the Phadebas assay) are pooled, pH was adjusted to pH 7.5 and remaining color was removed by a treatment with 0.5% W/vol. active coal in 5 minutes.

Stability determination of purified variants

All stability trials of purified variants are made using the same set up. The method is as follows:
The enzyme is incubated under the relevant conditions (1-4). Samples are taken at various time points, e.g., after 0, 5, 10, 15 and 30 minutes and diluted 25 times (same dilution for all taken samples) in assay buffer (0.1M 50mM Britton buffer pH 7.3) and the activity is measured using the Phadebas assay (Pharmacia) under standard conditions pH 7.3, 37°C.

The activity measured before incubation (0 minutes) is used as reference (100%). The decline in percent is calculated as a

function of the incubation time. The table shows the residual activity after, e.g., 30 minutes of incubation.

Specific activity determination

The specific activity is determined using the Phadebas assay (Pharmacia) as activity/mg enzyme. The manufactures instructions are followed (see also below under "Assay for α-amylase activity).

Assays for Alpha-Amylase Activity

1. Phadebas assay

Alpha-amylase activity is determined by a method employing Phadebas® tablets as substrate. Phadebas tablets (Phadebas® Amylase Test, supplied by Pharmacia Diagnostic) contain a cross-linked insoluble blue-colored starch polymer, which has been mixed with bovine serum albumin and a buffer substance and tabletted.

For every single measurement one tablet is suspended in a tube containing 5 ml 50 mM Britton-Robinson buffer (50 mM acetic acid, 50 mM phosphoric acid, 50 mM boric acid, 0.1 mM CaCl2, pH adjusted to the value of interest with NaOH). The test is performed in a water bath at the temperature of interest. The alpha-amylase to be tested is diluted in x ml of 50 mM Britton-Robinson buffer. 1 ml of this alpha-amylase solution is added to the 5 ml 50 mM Britton-Robinson buffer. The starch is hydrolyzed by the alpha-amylase giving soluble blue fragments. The absorbance of the resulting blue solution, measured spectrophotometrically at 620 nm, is a function of the alpha-amylase activity.

It is important that the measured 620 nm absorbance after 10 or 15 minutes of incubation (testing time) is in the range of 0.2 to 2.0 absorbance units at 620 nm. In this absorbance range there is linearity between activity and absorbance

(Lambert-Beer law). The dilution of the enzyme must therefore be adjusted to fit this criterion. Under a specified set of conditions (temp., pH, reaction time, buffer conditions) 1 mg of a given alpha-amylase will hydrolyze a certain amount of substrate and a blue colour will be produced. The colour intensity is measured at 620 nm. The measured absorbance is directly proportional to the specific activity (activity/mg of pure alpha-amylase protein) of the alpha-amylase in question under the given set of conditions.

2. Alternative method

Alpha-amylase activity is determined by a method employing the PNP-G7 substrate. PNP-G7 which is a abbreviation for p-nitrophenyl-alpha,D-maltoheptaoside is a blocked oligosaccharide which can be cleaved by an endo-amylase. Following the cleavage, the alpha-Glucosidase included in the kit digest the substrate to liberate a free PNP molecule which has a yellow colour and thus can be measured by visible spectophometry at λ=405nm (400-420 nm). Kits containing PNP-G7 substrate and alpha-Glucosidase is manufactured by Boehringer-Mannheim (cat. No.1054635).

To prepare the reagent solution 10 ml of substrate/buffer solution is added to 50 ml enzyme/buffer solution as recommended by the manufacturer. The assay is performed by transferring 20 micro l sample to a 96 well microtitre plate and incubating at 25°C. 200 micro l reagent solution pre-equilibrated to 25°C is added. The solution is mixed and pre-incubated 1 minute and absorption is measured every 30 sec. over 4 minutes at OD 405 nm in an ELISA reader.

The slope of the time dependent absorption-curve is directly proportional to the activity of the alpha-amylase in question under the given set of conditions.

EXAMPLES

Example 1.

Construction, by error-prone PCR mutagenesis, of Bacillus licheniformis alpha-amylase variants having an improved stability at low pH, high temperature and low calcium ion concentration compared to the parent enzyme.

Error-prone PCR mutagenesis and library construction

To improve the stability at low pH and low calcium concentration of the parent Bacillus licheniformis alpha-amylase, error-prone PCR mutagenesis was performed. The plasmid pDN1528 encoding the wild-type Bacillus licheniformis alpha-amylase gene was utilized as template to amplify this gene with primers: 22149: 5'-CGA TTG CTG ACG CTG TTA TTT GCG-3' (SEQID NO: 14) and 24814: 5'-GAT CAC CCG CGA TAC CGT C-3' (SEQ ID NO: 15) under PCR conditions where increased error rates leads to introduction of random point mutations. The PCR conditions utilized were: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM MgCl2, 0.3 mM MnCl2, 0.1mM dGTP/dATP, 0.5 mM dTTP/dCTP, and 2.5 units Taq polymerase per 100 micro l reaction.

The resultant PCR fragment was purified on gel and used in a PCR-based multimerization step with a gel purified vector fragment created by PCR amplification of pDN1528 with primers #24: 5'-GAA TGT ATG TCG GCC GGC AAA ACG CCG GTG A-3' (SEQ ID NO: 16) and #27: 5'-GCC GCC GCT GCT GCA GAA TGA GGC AGC AAG-3' (SEQ ID NO:17) forming an overlap to the insert fragment. The multimerization reaction was subsequently introduced into B. subtilis (Shafikhani et al., Biotechniques, 23 (1997), 304-310).

Screening

The error-prone library described above was screened in the low pH filter assay (see "Materials & Methods"). Clones

testing positive upon rescreening was submitted to secondary screening for stability in the liquid assay described in Materials and Methods.

Results:

Increased stability at pH 4.5, 5 ppm calcium incubated at 90°C

Name	wt	LE488	LE489	7.19.1	8.9.1
Mutations	-	D207V	K170Q D207V N280S	E132A D207V	D207E E250G H406L L427I
Stability1)	-	+	+	+	+

1) A "+" indicates significant increase in stability relative to wild type.

Increased stability at pH 4.5, 5 ppm calcium incubated at 90°C

Name	wt	LE491	LE492	LE493	LE494	19.3.1
Mutations	-	D60N D207V L318M	T49I E132V V440A	T49I K176R D207V Y402F	Q374R E385V Q393R	N190F A209V Q264S
Stability1)	-	+	+	+	+	+

1) A "+" indicates significant increase in stability relative to wt.

Increased stability at pH 4.5, 5 ppm calcium incubated at 90°C

Name	wt	E132-1	D207-7	D207-6	E250-8
Mutations	-	E132P	D207L	D207G	E250F
Stability1)	-	+	+	+	+

1) A "+" indicates significant increase in stability relative to wt.

Example 2

Transfer, by site-directed mutagenesis, of a selection of mutations from Example 1 to a new (non-wild type) backbone to improve stability at low pH and low calcium ion concentration compared to the parent enzyme.

Site-directed mutagenesis

Mutations from LE493 (K176R+D207V+Y402F) were transferred to LE399 yielding LE495. This was performed by the overlap PCR method (Kirchhoff and Desrosiers, PCR Methods and Applications, 2 (1993), 301-304). 2 overlapping PCR fragments were generated by amplification of the LE399 template with the primers: Fragment A: #312 Mut176 5'-CCC GAA AGC TGA ACC GCA TCT ATA GGT TTC AAG GGA AGA CTT GGG ATT-3' (SEQ ID NO: 18)(mutated codon indicated in bold) and #290 D207overlap 5'-AGG ATG GTC ATA ATC AAA GTC GG-3'(SEQ ID NO: 19); Fragment B: #313 Mut207 5'-CCG ACT TTG ATT ATG ACC ATC CTG TTG TCG TAG CAG AGA TTA AGA GAT GGG G-3' (SEQ ID NO: 20) and #314 Mut402 5'-CGA CAA TGT CAT GGT GGT CGA AAA AAT CAT GCT GTG CTC CGT ACG-3' (SEQ ID NO: 21). Fragments A and B were mixed in equimolar ratios and subsequently the full-length fragment was amplified with the external primers: #312 Mut176 and #314 Mut402. This fragment was used in a multimerization reaction with the vector PCR fragment created with the primers #296 Y402multi 5'-TTT CGA CCA CCA TGA CAT TGT CG-3' (SEQ ID NO: 22) and #305 399Multi176 5'-TAT AGA TGC GGT TCA GCT TTC GGG-3' (SEQ ID NO: 23) on template LE399 as described above. The multimerization reaction was subsequently transformed into B. subtilis. Clones were screened for stability in the assay mentioned above. The presence of the mutations from LE493 in several clones with increased stability was confirmed by sequencing.

LE 497 was obtained in a similar manner by amplifying the LE399 encoding template with primers #312 Mut176 and #314 Mut402 and using the resulting PCR fragment in a multimerization reaction with a vector fragment obtained by PCR amplification of the LE399 template with the primers #296 Y402multi and #305 399Multi176.

Results:

Stabilization of LE399 variant at pH 4.5, 5ppm calcium incubated at 90oC

Name	LE399	LE495	LE497
Mutations	- (backbone)	K176R D207V Y402F	K176R Y402F
Stability1)	-	+	+

1) A "+" indicates significant increase in stability relative to backbone.

CLAIMS

1. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of:

49, 60, 104, 132, 161, 170, 176, 179, 180, 181, 183, 200, 203, 204, 207, 212, 237, 239, 250, 280, 298, 318, 374, 385, 393, 402, 406, 427, 430, 440, 444, 447, 482,

wherein

(a) the alteration(s) are independently

(i) an insertion of an amino acid downstream of the amino acid which occupies the position,

(ii) a deletion of the amino acid which occupies the position, or

(iii) a substitution of the amino acid which occupies the position with a different amino acid,

(b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

2. The variant of claim 1, which variant has one or more of the following mutations: T49I; D60N; N104D; E132A,V,P; D161N; K170Q; K176R; G179N; K180T; A181N; D183N; D200N; X203Y; D204S; D207V,E,L,G; X212I; K237P; S239W; E250G,F; N280S; X298Q; L318M; Q374R; E385V; Q393R; Y402F; H406L,W; L427I D430N; V440A; N444R,K; E447Q,K; Q482K using SEQ ID NO: 8 for the numbering.

3. The variant of claim 1 or 2, wherein the variant has the following mutations: K170Q+D207V+N280S; E132A+D207V; D207E+E250G+H406L+L427I; D207V+L318M; D60N+D207V+L318M; T49I+E132V+V440A; T49I+K176R+D207V+Y402F; Q374R+E385V+Q393R; N190F+A209V+Q264S; G48A+T49I+G107A+I201F; T49I+G107A+I201F;

G48A+T49I+I201F; G48A+T49I+G107A; T49I+I201F; T49I+G107A; G48A+T49I;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444K+E447Q+Q482K;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444K+E447Q+Q482K;

D161N+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444K+E447Q+Q482K;

D161N+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+E447Q+Q482K;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+E447Q+Q482K;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+E447Q+Q482K;

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D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N;

H406W+D430N; N444K+E447Q+Q482K; E447Q+Q482K;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444R+N444K+E447K+Q482K;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444R+N444K+E447K+Q482K;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;

H406W+D430N; N444K+E447K+Q482K; E447K+Q482K;

N104D+D161N+A181N+D183N+D200N+D204S+K237P+S239W;

N104D+D161N+A181N+D183N+D200N+D204S+K237P;

N104D+D161N+A181N+D183N+D200N+D204S;

D161N+A181N+D183N+D200N+D204S+K237P+S239W;

D161N+A181N+D183N+D200N+D204S+K237P;

D161N+A181N+D183N+D200N+D204S; K237P+S239W, using SEQ ID NO: 8 for the numbering.

4. The variant of any of claims 1-3, wherein the parent Termamyl-like alpha-amylase is derived from a strain of B. licheniformis (SEQ ID NO: 8), B. amyloliquefaciens (SEQ ID NO: 10), B. stearothermophilus (SEQ ID NO: 6).

5. The variant of any of claims 1-4, wherein the parent Termamyl-like amylase is any of:
LE174; LE174+G48A+T49I+G107A+I201F; LE174+M197L;
LE174+G48A+T49I+G107A+M197L+I201F.

6. The variant of claim 1, wherein the variant is mutated in one or more of the following positions: T51I; D62N; N106D; D134A,V,P; D163N; X172Q; K179R; G184N; K185T; A186N; D188N; D205N; M208Y; D209S; X212V,E,L,G; L217I, K242P, S244W, N255G,F, N285S, S303Q, X323M; D387V, N395R; Y404F; H408L,W; X429I; D432N; V442A; X446R,K; X449Q,K; X484K, using SEQ ID NO: 4 (SP722) for the numbering.

7. The variant of claim 1 or 6, wherein the variant has the following mutations: E212V+N285S; D134A+E212V; N255G+H408L+X429I; E212V+X323M; D62N+E212V+X323M; T51I+D134V+V442A; T51I+K179R+E212V+Y404F; D387V+N395R; N195F+X212V+K269S, when using SEQ ID NO: 4 (SP722) for the numbering.

8. The variant of any of claims 1-7, wherein the parent Termamyl-like alpha-amylase is selected from the group comprising: SP690 (SEQ ID NO: 2); SP722 (SEQ ID NO: 4; AA560 (SEQ ID NO: 12); #707 alpha-amylase (SEQ ID NO: 13); KSM-AP1378.

9. The variant of any of claims 1-8, wherein the parent Termamyl-like amylase is any of: SP722+D183*+G184*; SP722+D183*+G184*+N195F; SP722+D183*+G184*+M202L; SP722+D183*+G184*+N195F+M202L; BSG+I181*+G182*; BSG+I181*+G182*+N193F; BSG+I181*+G182*+M200L; BSG+I181*+G182*+N193F+M200L; AA560+D183*+G184*; AA560+D183*+G184*+N195F; AA560+D183*+G184*+M202L; AA560+D183*+G184*+N195F+M202L.

10. The variant of any of claims 1-9, wherein the parent Termamyl-like alpha-amylase has an amino acid sequence which has a degree of identity to SEQ ID NO: 8 of at least 60%, preferably 70%, more preferably at least 80%, even more preferably at least about 90%, even more preferably at least 95%, even more preferably at least 97%, and even more preferably at least 99%.

11. The variant of any of claims 1-10, wherein the parent Termamyl-like alpha-amylase is encoded by a nucleic acid sequence, which hydridizes under low, preferably medium, preferred high stringency conditions, with the nucleic acid sequence of SEQ ID NO: 7.

12. The variant of any of claims 1-11, which variant has altered stability, in particular at high temperatures from 70-120°C and/or low pH in the range from pH 4-6

13. A DNA construct comprising a DNA sequence encoding an alpha-amylase variant according to any one of claims 1-12.

14. A recombinant expression vector which carries a DNA construct according to claim 13.

15. A cell which is transformed with a DNA construct according to claim 13 or a vector according to claim 14.

16. The cell according to claim 15, which is a microorganism, preferably a bacterium or a fungus.

17. The cell according to claim 16, which cell is a gram-positive bacterium, such as Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus or Bacillus thuringiensis.

18. A composition comprising an alpha-amylase variant of any of claims 1-12.

19. The composition of claim 18, further comprising a B. stearothermophilus (BSG) alpha-amylase, in particular SP961, particular in a ratio of 1:10 to 10:1, preferably 1:2.

20. The composition of claim 18 or 19, wherein the composition further comprises a glucoamylase, pullulanase and/or a phytase.

21. A detergent composition comprising an alpha-amylase variant according to any of claims 1-12.

22. A detergent composition of claim 21, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme, glucoamylase, maltogenic amylase, CGTase, mannanase, cutinase, laccase and/or a cellulase.

23. Use of an alpha-amylase variant according to any of claims 1-12 or a composition according to any of claims 18-20 for starch liquefaction.

24. Use of an alpha-amylase variant according to any of claims 1-12 or a composition according to claims 18-20 for ethanol production.

25. Use of an alpha-amylase variant according to any one of claims 1-12 or a composition according to claims 18-20 for washing and/or dishwashing.

26. Use of an alpha-amylase variant of any one of claims 1-12 or a composition according to claims 18-20 for textile desizing.

```
     1                                                              50
1    HHNGTNGTMM QYFEWHLPND GNHWNRLRDD ASNLRNRGIT AIWIPPAWKG
2    HHNGTNGTMM QYFEWYLPND GNHWNRLRDD AANLKSKGIT AVWIPPAWKG
3    ....VNGTLM QYFEWYTPND GQHWKRLQND AEHLSDIGIT AVWIPPAYKG
4    ..ANLNGTLM QYFEWYMPND GQHWRRLQND SAYLAEHGIT AVWIPPAYKG
5    .AAPFNGTMM QYFEWYLPDD GTLWTKVANE ANNLSSLGIT ALWLPPAYKG

     51                                                             100
1    TSQNDVGYGA YDLYDLGEFN QKGTVRTKYG TRSQLESAIH ALKNNGVQVY
2    TSQNDVGYGA YDLYDLGEFN QKGTVRTKYG TRNQLQAAVT SLKNNGIQVY
3    LSQSDNGYGP YDLYDLGEFQ QKGTVRTKYG TKSELQDAIG SLHSRNVQVY
4    TSQADVGYGA YDLYDLGEFH QKGTVRTKYG TKGELQSAIK SLHSRDINVY
5    TSRSDVGYGV YDLYDLGEFN QKGTVRTKYG TKAQYLQAIQ AAHAAGMQVY

     101                                                            150
1    GDVVMNHKGG ADATENVLAV EVNPNNRNQE ISGDYTIEAW TKFDFPGRGN
2    GDVVMNHKGG ADGTEIVNAV EVNRSNRNQE TSGEYAIEAW TKFDFPGRGN
3    GDVVLNHKAG ADATEDVTAV EVNPANRNQE TSEEYQIKAW TDFRFPGRGN
4    GDVVINHKGG ADATEDVTAV EVDPADRNRV ISGEHLIKAW THFHFPGRGS
5    ADVVFDHKGG ADGTEWVDAV EVNPSDRNQE ISGTYQIQAW TKFDFPGRGN

     151                                                            200
1    TYSDFKWRWY HFDGVDWDQS RQFQNRIYKF RGDGKAWDWE VDSENGNYDY
2    NHSSFKWRWY HFDGTDWDQS RQLQNKIYKF RGTGKAWDWE VDTENGNYDY
3    TYSDFKWHWY HFDGADWDES RKI.SRIFKF RGEGKAWDWE VSSENGNYDY
4    TYSDFKWHWY HFDGTDWDES RKL.NRIYKF ..QGKAWDWE VSNENGNYDY
5    TYSSFKWRWY HFDGVDWDES RKL.SRIYKF RGIGKAWDWE VDTENGNYDY
```

Fig. 1

```
    201                                                         250
1   LMYADVDMDH PEVVNELRRW GEWYTNTLNL DGFRIDAVKH IKYSFTRDWL
2   LMYADVDMDH PEVIHELRNW GVWYTNTLNL DGFRIDAVKH IKYSFTRDWL
3   LMYADVDYDH PDVVAETKKW GIWYANELSL DGFRIDAAKH IKFSFLRDWV
4   LMYADIDYDH PDVAAEIKRW GTWYANELQL DGFRLDAVKH IKFSFLRDWV
5   LMYADLDMDH PEVVTELKNW GKWYVNTTNI DGFRLDAVKH IKFSFFPDWL

    251                                                         300
1   THVRNATGKE MFAVAEFWKN DLGALENYLN KTNWNHSVFD VPLHYNLYNA
2   THVRNTTGKP MFAVAEFWKN DLGAIENYLN KTSWNHSAFD VPLHYNLYNA
3   QAVRQATGKE MFTVAEYWQN NAGKLENYLN KTSFNQSVFD VPLHFNLQAA
4   NHVREKTGKE MFTVAEYWQN DLGALENYLN KTNFNHSVFD VPLHYQFHAA
5   SYVRSQTGKP LFTVGEYWSY DINKLHNYIT KTDGTMSLFD APLHNKFYTA

    301                                                         350
1   SNSGGNYDMA KLLNGTVVQK HPMHAVTFVD NHDSQPGESL ESFVQEWFKP
2   SNSGGYYDMR NILNGSVVQK HPTHAVTFVD NHDSQPGEAL ESFVQQWFKP
3   SSQGGGYDMR RLLDGTVVSR HPEKAVTFVE NHDTQPGQSL ESTVQTWFKP
4   STQGGGYDMR KLLNGTVVSK HPLKSVTFVD NHDTQPGQSL ESTVQTWFKP
5   SKSGGAFDMR TLMTNTLMKD QPTLAVTFVD NHDTEPGQAL QSWVDPWFKP

    351                                                         400
1   LAYALILTRE QGYPSVFYGD YYGIPTHS.. .VPAMKAKID PILEARQNFA
2   LAYALVLTRE QGYPSVFYGD YYGIPTHG.. .VPAMKSKID PLLQARQTFA
3   LAYAFILTRE SGYPQVFYGD MYGTKGTSPK EIPSLKDNIE PILKARKEYA
4   LAYAFILTRE SGYPQVFYGD MYGTKGDSQR EIPALKHKIE PILKARKQYA
5   LAYAFILTRQ EGYPCVFYGD YYGIPQYN.. .IPSLKSKID PLLIARRDYA

    401                                                         450
1   YGTQHDYFDH HNIIGWTREG NTTHPNSGLA TIMSDGPGGE KWMYVGQNKA
2   YGTQHDYFDH HDIIGWTREG NSSHPNSGLA TIMSDGPGGN KWMYVGKNKA
3   YGPQHDYIDH PDVIGWTREG DSSAAKSGLA ALITDGPGGS KRMYAGLKNA
4   YGAQHDYFDH HDIVGWTREG DSSVANSGLA ALITDGPGGA KRMYVGRQNA
5   YGTQHDYLDH SDIIGWTREG GTEKPGSGLA ALITDGPGGS KWMYVGKQHA
```

Fig. 1 (continued)

```
    451                                                        500
1   GQVWHDITGN KPGTVTINAD GWANFSVNGG SVSIWVKR.. ..........
2   GQVWRDITGN RTGTVTINAD GWGNFSVNGG SVSVWVKQ.. ..........
3   GETWYDITGN RSDTVKIGSD GWGEFHVNDG SVSIYVQ... ..........
4   GETWHDITGN RSEPVVINSE GWGEFHVNGG SVSIYVQR.. ..........
5   GKVFYDLTGN RSDTVTINSD GWGEFKVNGG SVSVWVPRKT TVSTIARPIT

    501                519
1   .......... .........
2   .......... .........
3   .......... .........
4   .......... .........
5   TRPWTGEFVR WTEPRLVAW
```

Fig. 1 (continued)

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SEQUENCE LISTING
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<130>

<160> 28

<170> PatentIn Ver. 2.1

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<211> 1455
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<223> SP690

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His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
1 5 10 15

ttg cca aat gac ggg aat cat tgg aac agg ttg agg gat gac gca gct 96
Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ala
20 25 30

aac tta aag agt aaa ggg ata aca gct gta tgg atc cca cct gca tgg 144
Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp
35 40 45

aag ggg act tcc cag aat gat gta ggt tat gga gcc tat gat tta tat 192
Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
50 55 60

gat ctt gga gag ttt aac cag aag ggg acg gtt cgt aca aaa tat gga 240
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65 70 75 80

aca cgc aac cag cta cag gct gcg gtg acc tct tta aaa aat aac ggc 288
Thr Arg Asn Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly
85 90 95

att cag gta tat ggt gat gtc gtc atg aat cat aaa ggt gga gca gat 336
Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
100 105 110

ggt acg gaa att gta aat gcg gta gaa gtg aat cgg agc aac cga aac 384
Gly Thr Glu Ile Val Asn Ala Val Glu Val Asn Arg Ser Asn Arg Asn
115 120 125

cag gaa acc tca gga gag tat gca ata gaa gcg tgg aca aag ttt gat 432
Gln Glu Thr Ser Gly Glu Tyr Ala Ile Glu Ala Trp Thr Lys Phe Asp
130 135 140

ttt cct gga aga gga aat aac cat tcc agc ttt aag tgg cgc tgg tat 480
Phe Pro Gly Arg Gly Asn Asn His Ser Ser Phe Lys Trp Arg Trp Tyr
145 150 155 160

cat ttt gat ggg aca gat tgg gat cag tca cgc cag ctt caa aac aaa 528
His Phe Asp Gly Thr Asp Trp Asp Gln Ser Arg Gln Leu Gln Asn Lys
165 170 175

ata tat aaa ttc agg gga aca ggc aag gcc tgg gac tgg gaa gtc gat 576

SEQUENCE LISTING

```
Ile Tyr Lys Phe Arg Gly Thr Gly Lys Ala Trp Asp Trp Glu Val Asp
            180                 185                 190

aca gag aat ggc aac tat gac tat ctt atg tat gca gac gtg gat atg   624
Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met
        195                 200                 205

gat cac cca gaa gta ata cat gaa ctt aga aac tgg gga gtg tgg tat   672
Asp His Pro Glu Val Ile His Glu Leu Arg Asn Trp Gly Val Trp Tyr
    210                 215                 220

acg aat aca ctg aac ctt gat gga ttt aga ata gat gca gtg aaa cat   720
Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225                 230                 235                 240

ata aaa tat agc ttt acg aga gat tgg ctt aca cat gtg cgt aac acc   768
Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Thr
                245                 250                 255

aca ggt aaa cca atg ttt gca gtg gct gag ttt tgg aaa aat gac ctt   816
Thr Gly Lys Pro Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
            260                 265                 270

ggt gca att gaa aac tat ttg aat aaa aca agt tgg aat cac tcg gtg   864
Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Ser Trp Asn His Ser Val
        275                 280                 285

ttt gat gtt cct ctc cac tat aat ttg tac aat gca tct aat agc ggt   912
Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly
    290                 295                 300

ggt tat tat gat atg aga aat att tta aat ggt tct gtg gtg caa aaa   960
Gly Tyr Tyr Asp Met Arg Asn Ile Leu Asn Gly Ser Val Val Gln Lys
305                 310                 315                 320

cat cca aca cat gcc gtt act ttt gtt gat aac cat gat tct cag ccc  1008
His Pro Thr His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
                325                 330                 335

ggg gaa gca ttg gaa tcc ttt gtt caa caa tgg ttt aaa cca ctt gca  1056
Gly Glu Ala Leu Glu Ser Phe Val Gln Gln Trp Phe Lys Pro Leu Ala
            340                 345                 350

tat gca ttg gtt ctg aca agg gaa caa ggt tat cct tcc gta ttt tat  1104
Tyr Ala Leu Val Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
        355                 360                 365

ggg gat tac tac ggt atc cca acc cat ggt gtt ccg gct atg aaa tct  1152
Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
    370                 375                 380

aaa ata gac cct ctt ctg cag gca cgt caa act ttt gcc tat ggt acg  1200
Lys Ile Asp Pro Leu Leu Gln Ala Arg Gln Thr Phe Ala Tyr Gly Thr
385                 390                 395                 400

cag cat gat tac ttt gat cat cat gat att atc ggt tgg aca aga gag  1248
Gln His Asp Tyr Phe Asp His His Asp Ile Ile Gly Trp Thr Arg Glu
                405                 410                 415

gga aat agc tcc cat cca aat tca ggc ctt gcc acc att atg tca gat  1296
Gly Asn Ser Ser His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
            420                 425                 430

ggt cca ggt ggt aac aaa tgg atg tat gtg ggg aaa aat aaa gcg gga  1344
Gly Pro Gly Gly Asn Lys Trp Met Tyr Val Gly Lys Asn Lys Ala Gly
        435                 440                 445

caa gtt tgg aga gat att acc gga aat agg aca ggc acc gtc aca att  1392
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SEQUENCE LISTING

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Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465                 470                 475                 480

gtt tgg gtg aag caa                                           1455
Val Trp Val Lys Gln
                485
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<213> Bacillus sp.

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            20                  25                  30

Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp
        35                  40                  45

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

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65                  70                  75                  80

Thr Arg Asn Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly
                85                  90                  95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
            100                 105                 110

Gly Thr Glu Ile Val Asn Ala Val Glu Val Asn Arg Ser Asn Arg Asn
        115                 120                 125

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

Phe Pro Gly Arg Gly Asn Asn His Ser Ser Phe Lys Trp Arg Trp Tyr
145                 150                 155                 160

His Phe Asp Gly Thr Asp Trp Asp Gln Ser Arg Gln Leu Gln Asn Lys
                165                 170                 175

Ile Tyr Lys Phe Arg Gly Thr Gly Lys Ala Trp Asp Trp Glu Val Asp
            180                 185                 190

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

Asp His Pro Glu Val Ile His Glu Leu Arg Asn Trp Gly Val Trp Tyr
    210                 215                 220

Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225                 230                 235                 240

Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Thr
                245                 250                 255

Thr Gly Lys Pro Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
            260                 265                 270
```

SEQUENCE LISTING

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Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Ser Trp Asn His Ser Val
        275                 280                 285

Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly
    290                 295                 300

Gly Tyr Tyr Asp Met Arg Asn Ile Leu Asn Gly Ser Val Val Gln Lys
305                 310                 315                 320

His Pro Thr His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
                325                 330                 335

Gly Glu Ala Leu Glu Ser Phe Val Gln Gln Trp Phe Lys Pro Leu Ala
            340                 345                 350

Tyr Ala Leu Val Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
        355                 360                 365

Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
    370                 375                 380

Lys Ile Asp Pro Leu Leu Gln Ala Arg Gln Thr Phe Ala Tyr Gly Thr
385                 390                 395                 400

Gln His Asp Tyr Phe Asp His His Asp Ile Ile Gly Trp Thr Arg Glu
                405                 410                 415

Gly Asn Ser Ser His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
            420                 425                 430

Gly Pro Gly Gly Asn Lys Trp Met Tyr Val Gly Lys Asn Lys Ala Gly
        435                 440                 445

Gln Val Trp Arg Asp Ile Thr Gly Asn Arg Thr Gly Thr Val Thr Ile
    450                 455                 460

Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465                 470                 475                 480

Val Trp Val Lys Gln
                485
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<210> 3
<211> 1455
<212> DNA
<213> Bacillus sp.

<220>
<221> CDS
<222> (1)..(1455)
<223> SP722

<400> 3

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cat cat aat ggg aca aat ggg acg atg atg caa tac ttt gaa tgg cac   48
His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His
  1               5                  10                  15

ttg cct aat gat ggg aat cac tgg aat aga tta aga gat gat gct agt   96
Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser
            20                  25                  30

aat cta aga aat aga ggt ata acc gct att tgg att ccg cct gcc tgg   144
Asn Leu Arg Asn Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp
        35                  40                  45
```

SEQUENCE LISTING

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aaa ggg act tcg caa aat gat gtg ggg tat gga gcc tat gat ctt tat   192
Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
 50                  55                  60

gat tta ggg gaa ttt aat caa aag ggg acg gtt cgt act aag tat ggg   240
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65                  70                  75                  80

aca cgt agt caa ttg gag tct gcc atc cat gct tta aag aat aat ggc   288
Thr Arg Ser Gln Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly
                85                  90                  95

gtt caa gtt tat ggg gat gta gtg atg aac cat aaa gga gga gct gat   336
Val Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
            100                 105                 110

gct aca gaa aac gtt ctt gct gtc gag gtg aat cca aat aac cgg aat   384
Ala Thr Glu Asn Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn
        115                 120                 125

caa gaa ata tct ggg gac tac aca att gag gct tgg act aag ttt gat   432
Gln Glu Ile Ser Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
    130                 135                 140

ttt cca ggg agg ggt aat aca tac tca gac ttt aaa tgg cgt tgg tat   480
Phe Pro Gly Arg Gly Asn Thr Tyr Ser Asp Phe Lys Trp Arg Trp Tyr
145                 150                 155                 160

cat ttc gat ggt gta gat tgg gat caa tca cga caa ttc caa aat cgt   528
His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg
                165                 170                 175

atc tac aaa ttc cga ggt gat ggt aag gca tgg gat tgg gaa gta gat   576
Ile Tyr Lys Phe Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp
            180                 185                 190

tcg gaa aat gga aat tat gat tat tta atg tat gca gat gta gat atg   624
Ser Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met
        195                 200                 205

gat cat ccg gag gta gta aat gag ctt aga aga tgg gga gaa tgg tat   672
Asp His Pro Glu Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr
    210                 215                 220

aca aat aca tta aat ctt gat gga ttt agg atc gat gcg gtg aag cat   720
Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225                 230                 235                 240

att aaa tat agc ttt aca cgt gat tgg ttg acc cat gta aga aac gca   768
Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala
                245                 250                 255

acg gga aaa gaa atg ttt gct gtt gct gaa ttt tgg aaa aat gat tta   816
Thr Gly Lys Glu Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
            260                 265                 270

ggt gcc ttg gag aac tat tta aat aaa aca aac tgg aat cat tct gtc   864
Gly Ala Leu Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
        275                 280                 285

ttt gat gtc ccc ctt cat tat aat ctt tat aac gcg tca aat agt gga   912
Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly
    290                 295                 300

ggc aac tat gac atg gca aaa ctt ctt aat gga acg gtt gtt caa aag   960
Gly Asn Tyr Asp Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys
305                 310                 315                 320
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SEQUENCE LISTING

```
cat cca atg cat gcc gta act ttt gtg gat aat cac gat tct caa cct   1008
His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
                325                 330                 335

ggg gaa tca tta gaa tca ttt gta caa gaa tgg ttt aag cca ctt gct   1056
Gly Glu Ser Leu Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala
            340                 345                 350

tat gcg ctt att tta aca aga gaa caa ggc tat ccc tct gtc ttc tat   1104
Tyr Ala Leu Ile Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
        355                 360                 365

ggt gac tac tat gga att cca aca cat agt gtc cca gca atg aaa gcc   1152
Gly Asp Tyr Tyr Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala
    370                 375                 380

aag att gat cca atc tta gag gcg cgt caa aat ttt gca tat gga aca   1200
Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr
385                 390                 395                 400

caa cat gat tat ttt gac cat cat aat ata atc gga tgg aca cgt gaa   1248
Gln His Asp Tyr Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
                405                 410                 415

gga aat acc acg cat ccc aat tca gga ctt gcg act atc atg tcg gat   1296
Gly Asn Thr Thr His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
            420                 425                 430

ggg cca ggg gga gag aaa tgg atg tac gta ggg caa aat aaa gca ggt   1344
Gly Pro Gly Gly Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly
        435                 440                 445

caa gtt tgg cat gac ata act gga aat aaa cca gga aca gtt acg atc   1392
Gln Val Trp His Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile
    450                 455                 460

aat gca gat gga tgg gct aat ttt tca gta aat gga gga tct gtt tcc   1440
Asn Ala Asp Gly Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465                 470                 475                 480

att tgg gtg aaa cga                                              1455
Ile Trp Val Lys Arg
                485
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<210> 4
<211> 485
<212> PRT
<213> Bacillus sp.

<400> 4

```
His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His
  1               5                  10                  15

Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser
            20                  25                  30

Asn Leu Arg Asn Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp
        35                  40                  45

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

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65                  70                  75                  80

Thr Arg Ser Gln Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly
                85                  90                  95
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SEQUENCE LISTING

Val Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
100 105 110

Ala Thr Glu Asn Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn
115 120 125

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

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

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

Ile Tyr Lys Phe Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp
180 185 190

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

Asp His Pro Glu Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr
210 215 220

Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225 230 235 240

Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala
245 250 255

Thr Gly Lys Glu Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
260 265 270

Gly Ala Leu Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
275 280 285

Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly
290 295 300

Gly Asn Tyr Asp Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys
305 310 315 320

His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
325 330 335

Gly Glu Ser Leu Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala
340 345 350

Tyr Ala Leu Ile Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
355 360 365

Gly Asp Tyr Tyr Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala
370 375 380

Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr
385 390 395 400

Gln His Asp Tyr Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
405 410 415

Gly Asn Thr Thr His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
420 425 430

Gly Pro Gly Gly Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly
435 440 445

Gln Val Trp His Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile

SEQUENCE LISTING

```
450                 455                 460

Asn Ala Asp Gly Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465                 470                 475                 480

Ile Trp Val Lys Arg
                485
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<210> 5
<211> 1548
<212> DNA
<213> Bacillus stearothermophilus

<220>
<221> CDS
<222> (1)..(1548)
<223> BSG

<400> 5

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gcc gca ccg ttt aac ggc acc atg atg cag tat ttt gaa tgg tac ttg   48
Ala Ala Pro Phe Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu
  1               5                  10                  15

ccg gat gat ggc acg tta tgg acc aaa gtg gcc aat gaa gcc aac aac   96
Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn
             20                  25                  30

tta tcc agc ctt ggc atc acc gct ctt tgg ctg ccg ccc gct tac aaa  144
Leu Ser Ser Leu Gly Ile Thr Ala Leu Trp Leu Pro Pro Ala Tyr Lys
         35                  40                  45

gga aca agc cgc agc gac gta ggg tac gga gta tac gac ttg tat gac  192
Gly Thr Ser Arg Ser Asp Val Gly Tyr Gly Val Tyr Asp Leu Tyr Asp
     50                  55                  60

ctc ggc gaa ttc aat caa aaa ggg acc gtc cgc aca aaa tac gga aca  240
Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr
 65                  70                  75                  80

aaa gct caa tat ctt caa gcc att caa gcc gcc cac gcc gct gga atg  288
Lys Ala Gln Tyr Leu Gln Ala Ile Gln Ala Ala His Ala Ala Gly Met
                 85                  90                  95

caa gtg tac gcc gat gtc gtg ttc gac cat aaa ggc ggc gct gac ggc  336
Gln Val Tyr Ala Asp Val Val Phe Asp His Lys Gly Gly Ala Asp Gly
            100                 105                 110

acg gaa tgg gtg gac gcc gtc gaa gtc aat ccg tcc gac cgc aac caa  384
Thr Glu Trp Val Asp Ala Val Glu Val Asn Pro Ser Asp Arg Asn Gln
        115                 120                 125

gaa atc tcg ggc acc tat caa atc caa gca tgg acg aaa ttt gat ttt  432
Glu Ile Ser Gly Thr Tyr Gln Ile Gln Ala Trp Thr Lys Phe Asp Phe
    130                 135                 140

ccc ggg cgg ggc aac acc tac tcc agc ttt aag tgg cgc tgg tac cat  480
Pro Gly Arg Gly Asn Thr Tyr Ser Ser Phe Lys Trp Arg Trp Tyr His
145                 150                 155                 160

ttt gac ggc gtt gat tgg gac gaa agc cga aaa ttg agc cgc att tac  528
Phe Asp Gly Val Asp Trp Asp Glu Ser Arg Lys Leu Ser Arg Ile Tyr
                165                 170                 175

aaa ttc cgc ggc atc ggc aaa gcg tgg gat tgg gaa gta gac acg gaa  576
Lys Phe Arg Gly Ile Gly Lys Ala Trp Asp Trp Glu Val Asp Thr Glu
            180                 185                 190
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SEQUENCE LISTING

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aac gga aac tat gac tac tta atg tat gcc gac ctt gat atg gat cat   624
Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Leu Asp Met Asp His
        195                 200                 205

ccc gaa gtc gtg acc gag ctg aaa aac tgg ggg aaa tgg tat gtc aac   672
Pro Glu Val Val Thr Glu Leu Lys Asn Trp Gly Lys Trp Tyr Val Asn
    210                 215                 220

aca acg aac att gat ggg ttc cgg ctt gat gcc gtc aag cat att aag   720
Thr Thr Asn Ile Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys
225                 230                 235                 240

ttc agt ttt ttt cct gat tgg ttg tcg tat gtg cgt tct cag act ggc   768
Phe Ser Phe Phe Pro Asp Trp Leu Ser Tyr Val Arg Ser Gln Thr Gly
                245                 250                 255

aag ccg cta ttt acc gtc ggg gaa tat tgg agc tat gac atc aac aag   816
Lys Pro Leu Phe Thr Val Gly Glu Tyr Trp Ser Tyr Asp Ile Asn Lys
            260                 265                 270

ttg cac aat tac att acg aaa aca gac gga acg atg tct ttg ttt gat   864
Leu His Asn Tyr Ile Thr Lys Thr Asp Gly Thr Met Ser Leu Phe Asp
        275                 280                 285

gcc ccg tta cac aac aaa ttt tat acc gct tcc aaa tca ggg ggc gca   912
Ala Pro Leu His Asn Lys Phe Tyr Thr Ala Ser Lys Ser Gly Gly Ala
    290                 295                 300

ttt gat atg cgc acg tta atg acc aat act ctc atg aaa gat caa ccg   960
Phe Asp Met Arg Thr Leu Met Thr Asn Thr Leu Met Lys Asp Gln Pro
305                 310                 315                 320

aca ttg gcc gtc acc ttc gtt gat aat cat gac acc gaa ccc ggc caa  1008
Thr Leu Ala Val Thr Phe Val Asp Asn His Asp Thr Glu Pro Gly Gln
                325                 330                 335

gcg ctg cag tca tgg gtc gac cca tgg ttc aaa ccg ttg gct tac gcc  1056
Ala Leu Gln Ser Trp Val Asp Pro Trp Phe Lys Pro Leu Ala Tyr Ala
            340                 345                 350

ttt att cta act cgg cag gaa gga tac ccg tgc gtc ttt tat ggt gac  1104
Phe Ile Leu Thr Arg Gln Glu Gly Tyr Pro Cys Val Phe Tyr Gly Asp
        355                 360                 365

tat tat ggc att cca caa tat aac att cct tcg ctg aaa agc aaa atc  1152
Tyr Tyr Gly Ile Pro Gln Tyr Asn Ile Pro Ser Leu Lys Ser Lys Ile
    370                 375                 380

gat ccg ctc ctc atc gcg cgc agg gat tat gct tac gga acg caa cat  1200
Asp Pro Leu Leu Ile Ala Arg Arg Asp Tyr Ala Tyr Gly Thr Gln His
385                 390                 395                 400

gat tat ctt gat cac tcc gac atc atc ggg tgg aca agg gaa ggg ggc  1248
Asp Tyr Leu Asp His Ser Asp Ile Ile Gly Trp Thr Arg Glu Gly Gly
                405                 410                 415

act gaa aaa cca gga tcc gga ctg gcc gca ctg atc acc gat ggg ccg  1296
Thr Glu Lys Pro Gly Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
            420                 425                 430

gga gga agc aaa tgg atg tac gtt ggc aaa caa cac gct gga aaa gtg  1344
Gly Gly Ser Lys Trp Met Tyr Val Gly Lys Gln His Ala Gly Lys Val
        435                 440                 445

ttc tat gac ctt acc ggc aac cgg agt gac acc gtc acc atc aac agt  1392
Phe Tyr Asp Leu Thr Gly Asn Arg Ser Asp Thr Val Thr Ile Asn Ser
    450                 455                 460
```

SEQUENCE LISTING

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gat gga tgg ggg gaa ttc aaa gtc aat ggc ggt tcg gtt tcg gtt tgg   1440
Asp Gly Trp Gly Glu Phe Lys Val Asn Gly Gly Ser Val Ser Val Trp
465                 470                 475                 480

gtt cct aga aaa acg acc gtt tct acc atc gct cgg ccg atc aca acc   1488
Val Pro Arg Lys Thr Thr Val Ser Thr Ile Ala Arg Pro Ile Thr Thr
                485                 490                 495

cga ccg tgg act ggt gaa ttc gtc cgt tgg acc gaa cca cgg ttg gtg   1536
Arg Pro Trp Thr Gly Glu Phe Val Arg Trp Thr Glu Pro Arg Leu Val
            500                 505                 510

gca tgg cct tga                                                  1548
Ala Trp Pro
        515
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<210> 6
<211> 515
<212> PRT
<213> Bacillus stearothermophilus

<400> 6

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Ala Ala Pro Phe Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu
  1               5                  10                  15

Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn
            20                  25                  30

Leu Ser Ser Leu Gly Ile Thr Ala Leu Trp Leu Pro Pro Ala Tyr Lys
        35                  40                  45

Gly Thr Ser Arg Ser Asp Val Gly Tyr Gly Val Tyr Asp Leu Tyr Asp
    50                  55                  60

Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr
65                  70                  75                  80

Lys Ala Gln Tyr Leu Gln Ala Ile Gln Ala Ala His Ala Ala Gly Met
                85                  90                  95

Gln Val Tyr Ala Asp Val Val Phe Asp His Lys Gly Gly Ala Asp Gly
            100                 105                 110

Thr Glu Trp Val Asp Ala Val Glu Val Asn Pro Ser Asp Arg Asn Gln
        115                 120                 125

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

Pro Gly Arg Gly Asn Thr Tyr Ser Ser Phe Lys Trp Arg Trp Tyr His
145                 150                 155                 160

Phe Asp Gly Val Asp Trp Asp Glu Ser Arg Lys Leu Ser Arg Ile Tyr
                165                 170                 175

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

Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Leu Asp Met Asp His
        195                 200                 205

Pro Glu Val Val Thr Glu Leu Lys Asn Trp Gly Lys Trp Tyr Val Asn
    210                 215                 220

Thr Thr Asn Ile Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys
225                 230                 235                 240
```

SEQUENCE LISTING

Phe Ser Phe Phe Pro Asp Trp Leu Ser Tyr Val Arg Ser Gln Thr Gly
245 250 255

Lys Pro Leu Phe Thr Val Gly Glu Tyr Trp Ser Tyr Asp Ile Asn Lys
260 265 270

Leu His Asn Tyr Ile Thr Lys Thr Asp Gly Thr Met Ser Leu Phe Asp
275 280 285

Ala Pro Leu His Asn Lys Phe Tyr Thr Ala Ser Lys Ser Gly Gly Ala
290 295 300

Phe Asp Met Arg Thr Leu Met Thr Asn Thr Leu Met Lys Asp Gln Pro
305 310 315 320

Thr Leu Ala Val Thr Phe Val Asp Asn His Asp Thr Glu Pro Gly Gln
325 330 335

Ala Leu Gln Ser Trp Val Asp Pro Trp Phe Lys Pro Leu Ala Tyr Ala
340 345 350

Phe Ile Leu Thr Arg Gln Glu Gly Tyr Pro Cys Val Phe Tyr Gly Asp
355 360 365

Tyr Tyr Gly Ile Pro Gln Tyr Asn Ile Pro Ser Leu Lys Ser Lys Ile
370 375 380

Asp Pro Leu Leu Ile Ala Arg Arg Asp Tyr Ala Tyr Gly Thr Gln His
385 390 395 400

Asp Tyr Leu Asp His Ser Asp Ile Ile Gly Trp Thr Arg Glu Gly Gly
405 410 415

Thr Glu Lys Pro Gly Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
420 425 430

Gly Gly Ser Lys Trp Met Tyr Val Gly Lys Gln His Ala Gly Lys Val
435 440 445

Phe Tyr Asp Leu Thr Gly Asn Arg Ser Asp Thr Val Thr Ile Asn Ser
450 455 460

Asp Gly Trp Gly Glu Phe Lys Val Asn Gly Gly Ser Val Ser Val Trp
465 470 475 480

Val Pro Arg Lys Thr Thr Val Ser Thr Ile Ala Arg Pro Ile Thr Thr
485 490 495

Arg Pro Trp Thr Gly Glu Phe Val Arg Trp Thr Glu Pro Arg Leu Val
500 505 510

Ala Trp Pro
515

<210> 7
<211> 1920
<212> DNA
<213> Bacillus licheniformis

<220>
<221> CDS
<222> (421)..(1872)
<223> Termamyl

<400> 7

SEQUENCE LISTING

cggaagattg gaagtacaaa aataagcaaa agattgtcaa tcatgtcatg agccatgcgg 60

gagacggaaa aatcgtctta atgcacgata tttatgcaac gttcgcagat gctgctgaag 120

agattattaa aaagctgaaa gcaaaaggct atcaattggt aactgtatct cagcttgaag 180

aagtgaagaa gcagagaggc tattgaataa atgagtagaa gcgccatatc ggcgcttttc 240

ttttggaaga aaatataggg aaaatggtac ttgttaaaaa ttcggaatat ttatacaaca 300

tcatatgttt cacattgaaa ggggaggaga atcatgaaac aacaaaaacg gctttacgcc 360

cgattgctga cgctgttatt tgcgctcatc ttcttgctgc ctcattctgc agcagcggcg 420

gca aat ctt aat ggg acg ctg atg cag tat ttt gaa tgg tac atg ccc 468
Ala Asn Leu Asn Gly Thr Leu Met Gln Tyr Phe Glu Trp Tyr Met Pro
1 5 10 15

aat gac ggc caa cat tgg agg cgt ttg caa aac gac tcg gca tat ttg 516
Asn Asp Gly Gln His Trp Arg Arg Leu Gln Asn Asp Ser Ala Tyr Leu
20 25 30

gct gaa cac ggt att act gcc gtc tgg att ccc ccg gca tat aag gga 564
Ala Glu His Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly
35 40 45

acg agc caa gcg gat gtg ggc tac ggt gct tac gac ctt tat gat tta 612
Thr Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
50 55 60

ggg gag ttt cat caa aaa ggg acg gtt cgg aca aag tac ggc aca aaa 660
Gly Glu Phe His Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys
65 70 75 80

gga gag ctg caa tct gcg atc aaa agt ctt cat tcc cgc gac att aac 708
Gly Glu Leu Gln Ser Ala Ile Lys Ser Leu His Ser Arg Asp Ile Asn
85 90 95

gtt tac ggg gat gtg gtc atc aac cac aaa ggc ggc gct gat gcg acc 756
Val Tyr Gly Asp Val Val Ile Asn His Lys Gly Gly Ala Asp Ala Thr
100 105 110

gaa gat gta acc gcg gtt gaa gtc gat ccc gct gac cgc aac cgc gta 804
Glu Asp Val Thr Ala Val Glu Val Asp Pro Ala Asp Arg Asn Arg Val
115 120 125

att tca gga gaa cac cta att aaa gcc tgg aca cat ttt cat ttt ccg 852
Ile Ser Gly Glu His Leu Ile Lys Ala Trp Thr His Phe His Phe Pro
130 135 140

ggg cgc ggc agc aca tac agc gat ttt aaa tgg cat tgg tac cat ttt 900
Gly Arg Gly Ser Thr Tyr Ser Asp Phe Lys Trp His Trp Tyr His Phe
145 150 155 160

gac gga acc gat tgg gac gag tcc cga aag ctg aac cgc atc tat aag 948
Asp Gly Thr Asp Trp Asp Glu Ser Arg Lys Leu Asn Arg Ile Tyr Lys
165 170 175

ttt caa gga aag gct tgg gat tgg gaa gtt tcc aat gaa aac ggc aac 996
Phe Gln Gly Lys Ala Trp Asp Trp Glu Val Ser Asn Glu Asn Gly Asn
180 185 190

tat gat tat ttg atg tat gcc gac atc gat tat gac cat cct gat gtc 1044
Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Tyr Asp His Pro Asp Val
195 200 205

gca gca gaa att aag aga tgg ggc act tgg tat gcc aat gaa ctg caa 1092
Ala Ala Glu Ile Lys Arg Trp Gly Thr Trp Tyr Ala Asn Glu Leu Gln

SEQUENCE LISTING

210 215 220

ttg gac ggt ttc cgt ctt gat gct gtc aaa cac att aaa ttt tct ttt 1140
Leu Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys Phe Ser Phe
225 230 235 240

ttg cgg gat tgg gtt aat cat gtc agg gaa aaa acg ggg aag gaa atg 1188
Leu Arg Asp Trp Val Asn His Val Arg Glu Lys Thr Gly Lys Glu Met
245 250 255

ttt acg gta gct gaa tat tgg cag aat gac ttg ggc gcg ctg gaa aac 1236
Phe Thr Val Ala Glu Tyr Trp Gln Asn Asp Leu Gly Ala Leu Glu Asn
260 265 270

tat ttg aac aaa aca aat ttt aat cat tca gtg ttt gac gtg ccg ctt 1284
Tyr Leu Asn Lys Thr Asn Phe Asn His Ser Val Phe Asp Val Pro Leu
275 280 285

cat tat cag ttc cat gct gca tcg aca cag gga ggc ggc tat gat atg 1332
His Tyr Gln Phe His Ala Ala Ser Thr Gln Gly Gly Gly Tyr Asp Met
290 295 300

agg aaa ttg ctg aac ggt acg gtc gtt tcc aag cat ccg ttg aaa tcg 1380
Arg Lys Leu Leu Asn Gly Thr Val Val Ser Lys His Pro Leu Lys Ser
305 310 315 320

gtt aca ttt gtc gat aac cat gat aca cag ccg ggg caa tcg ctt gag 1428
Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu
325 330 335

tcg act gtc caa aca tgg ttt aag ccg ctt gct tac gct ttt att ctc 1476
Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu
340 345 350

aca agg gaa tct gga tac cct cag gtt ttc tac ggg gat atg tac ggg 1524
Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly
355 360 365

acg aaa gga gac tcc cag cgc gaa att cct gcc ttg aaa cac aaa att 1572
Thr Lys Gly Asp Ser Gln Arg Glu Ile Pro Ala Leu Lys His Lys Ile
370 375 380

gaa ccg atc tta aaa gcg aga aaa cag tat gcg tac gga gca cag cat 1620
Glu Pro Ile Leu Lys Ala Arg Lys Gln Tyr Ala Tyr Gly Ala Gln His
385 390 395 400

gat tat ttc gac cac cat gac att gtc ggc tgg aca agg gaa ggc gac 1668
Asp Tyr Phe Asp His His Asp Ile Val Gly Trp Thr Arg Glu Gly Asp
405 410 415

agc tcg gtt gca aat tca ggt ttg gcg gca tta ata aca gac gga ccc 1716
Ser Ser Val Ala Asn Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
420 425 430

ggt ggg gca aag cga atg tat gtc ggc cgg caa aac gcc ggt gag aca 1764
Gly Gly Ala Lys Arg Met Tyr Val Gly Arg Gln Asn Ala Gly Glu Thr
435 440 445

tgg cat gac att acc gga aac cgt tcg gag ccg gtt gtc atc aat tcg 1812
Trp His Asp Ile Thr Gly Asn Arg Ser Glu Pro Val Val Ile Asn Ser
450 455 460

gaa ggc tgg gga gag ttt cac gta aac ggc ggg tcg gtt tca att tat 1860
Glu Gly Trp Gly Glu Phe His Val Asn Gly Gly Ser Val Ser Ile Tyr
465 470 475 480

gtt caa aga tag aagagcagag aggacggatt tcctgaagga aatccgtttt 1912
Val Gln Arg

SEQUENCE LISTING

tttatttt 1920

<210> 8
<211> 483
<212> PRT
<213> Bacillus licheniformis

<400> 8
Ala Asn Leu Asn Gly Thr Leu Met Gln Tyr Phe Glu Trp Tyr Met Pro
1 5 10 15

Asn Asp Gly Gln His Trp Arg Arg Leu Gln Asn Asp Ser Ala Tyr Leu
20 25 30

Ala Glu His Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly
35 40 45

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

Gly Glu Phe His Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys
65 70 75 80

Gly Glu Leu Gln Ser Ala Ile Lys Ser Leu His Ser Arg Asp Ile Asn
85 90 95

Val Tyr Gly Asp Val Val Ile Asn His Lys Gly Gly Ala Asp Ala Thr
100 105 110

Glu Asp Val Thr Ala Val Glu Val Asp Pro Ala Asp Arg Asn Arg Val
115 120 125

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

Gly Arg Gly Ser Thr Tyr Ser Asp Phe Lys Trp His Trp Tyr His Phe
145 150 155 160

Asp Gly Thr Asp Trp Asp Glu Ser Arg Lys Leu Asn Arg Ile Tyr Lys
165 170 175

Phe Gln Gly Lys Ala Trp Asp Trp Glu Val Ser Asn Glu Asn Gly Asn
180 185 190

Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Tyr Asp His Pro Asp Val
195 200 205

Ala Ala Glu Ile Lys Arg Trp Gly Thr Trp Tyr Ala Asn Glu Leu Gln
210 215 220

Leu Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys Phe Ser Phe
225 230 235 240

Leu Arg Asp Trp Val Asn His Val Arg Glu Lys Thr Gly Lys Glu Met
245 250 255

Phe Thr Val Ala Glu Tyr Trp Gln Asn Asp Leu Gly Ala Leu Glu Asn
260 265 270

Tyr Leu Asn Lys Thr Asn Phe Asn His Ser Val Phe Asp Val Pro Leu
275 280 285

His Tyr Gln Phe His Ala Ala Ser Thr Gln Gly Gly Gly Tyr Asp Met
290 295 300

Arg Lys Leu Leu Asn Gly Thr Val Val Ser Lys His Pro Leu Lys Ser

SEQUENCE LISTING

305 310 315 320

Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu
325 330 335

Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu
340 345 350

Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly
355 360 365

Thr Lys Gly Asp Ser Gln Arg Glu Ile Pro Ala Leu Lys His Lys Ile
370 375 380

Glu Pro Ile Leu Lys Ala Arg Lys Gln Tyr Ala Tyr Gly Ala Gln His
385 390 395 400

Asp Tyr Phe Asp His His Asp Ile Val Gly Trp Thr Arg Glu Gly Asp
405 410 415

Ser Ser Val Ala Asn Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
420 425 430

Gly Gly Ala Lys Arg Met Tyr Val Gly Arg Gln Asn Ala Gly Glu Thr
435 440 445

Trp His Asp Ile Thr Gly Asn Arg Ser Glu Pro Val Val Ile Asn Ser
450 455 460

Glu Gly Trp Gly Glu Phe His Val Asn Gly Gly Ser Val Ser Ile Tyr
465 470 475 480

Val Gln Arg

<210> 9
<211> 2084
<212> DNA
<213> Bacillus amyloliquefaciens

<220>
<221> CDS
<222> (343)..(1794)
<223> BAN

<400> 9
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ctgaagaagt ggatcgattg tttgagaaaa gaagaagacc ataaaaatac cttgtctgtc 120

atcagacagg gtatttttta tgctgtccag actgtccgct gtgtaaaaat aaggaataaa 180

ggggggttgt tattatttta ctgatatgta aaatataatt tgtataagaa aatgagaggg 240

agaggaaaca tgattcaaaa acgaaagcgg acagtttcgt tcagacttgt gcttatgtgc 300

acgctgttat ttgtcagttt gccgattaca aaaacatcag cc gta aat ggc acg 354
Val Asn Gly Thr
1

ctg atg cag tat ttt gaa tgg tat acg ccg aac gac ggc cag cat tgg 402
Leu Met Gln Tyr Phe Glu Trp Tyr Thr Pro Asn Asp Gly Gln His Trp
5 10 15 20

aaa cga ttg cag aat gat gcg gaa cat tta tcg gat atc gga atc act 450
Lys Arg Leu Gln Asn Asp Ala Glu His Leu Ser Asp Ile Gly Ile Thr
25 30 35

SEQUENCE LISTING

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gcc gtc tgg att cct ccc gca tac aaa gga ttg agc caa tcc gat aac   498
Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly Leu Ser Gln Ser Asp Asn
            40                  45                  50

gga tac gga cct tat gat ttg tat gat tta gga gaa ttc cag caa aaa   546
Gly Tyr Gly Pro Tyr Asp Leu Tyr Asp Leu Gly Glu Phe Gln Gln Lys
        55                  60                  65

ggg acg gtc aga acg aaa tac ggc aca aaa tca gag ctt caa gat gcg   594
Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys Ser Glu Leu Gln Asp Ala
    70                  75                  80

atc ggc tca ctg cat tcc cgg aac gtc caa gta tac gga gat gtg gtt   642
Ile Gly Ser Leu His Ser Arg Asn Val Gln Val Tyr Gly Asp Val Val
85                  90                  95                  100

ttg aat cat aag gct ggt gct gat gca aca gaa gat gta act gcc gtc   690
Leu Asn His Lys Ala Gly Ala Asp Ala Thr Glu Asp Val Thr Ala Val
                105                 110                 115

gaa gtc aat ccg gcc aat aga aat cag gaa act tcg gag gaa tat caa   738
Glu Val Asn Pro Ala Asn Arg Asn Gln Glu Thr Ser Glu Glu Tyr Gln
            120                 125                 130

atc aaa gcg tgg acg gat ttt cgt ttt ccg ggc cgt gga aac acg tac   786
Ile Lys Ala Trp Thr Asp Phe Arg Phe Pro Gly Arg Gly Asn Thr Tyr
        135                 140                 145

agt gat ttt aaa tgg cat tgg tat cat ttc gac gga gcg gac tgg gat   834
Ser Asp Phe Lys Trp His Trp Tyr His Phe Asp Gly Ala Asp Trp Asp
    150                 155                 160

gaa tcc cgg aag atc agc cgc atc ttt aag ttt cgt ggg gaa gga aaa   882
Glu Ser Arg Lys Ile Ser Arg Ile Phe Lys Phe Arg Gly Glu Gly Lys
165                 170                 175                 180

gcg tgg gat tgg gaa gta tca agt gaa aac ggc aac tat gac tat tta   930
Ala Trp Asp Trp Glu Val Ser Ser Glu Asn Gly Asn Tyr Asp Tyr Leu
                185                 190                 195

atg tat gct gat gtt gac tac gac cac cct gat gtc gtg gca gag aca   978
Met Tyr Ala Asp Val Asp Tyr Asp His Pro Asp Val Val Ala Glu Thr
            200                 205                 210

aaa aaa tgg ggt atc tgg tat gcg aat gaa ctg tca tta gac ggc ttc   1026
Lys Lys Trp Gly Ile Trp Tyr Ala Asn Glu Leu Ser Leu Asp Gly Phe
        215                 220                 225

cgt att gat gcc gcc aaa cat att aaa ttt tca ttt ctg cgt gat tgg   1074
Arg Ile Asp Ala Ala Lys His Ile Lys Phe Ser Phe Leu Arg Asp Trp
    230                 235                 240

gtt cag gcg gtc aga cag gcg acg gga aaa gaa atg ttt acg gtt gcg   1122
Val Gln Ala Val Arg Gln Ala Thr Gly Lys Glu Met Phe Thr Val Ala
245                 250                 255                 260

gag tat tgg cag aat aat gcc ggg aaa ctc gaa aac tac ttg aat aaa   1170
Glu Tyr Trp Gln Asn Asn Ala Gly Lys Leu Glu Asn Tyr Leu Asn Lys
                265                 270                 275

aca agc ttt aat caa tcc gtg ttt gat gtt ccg ctt cat ttc aat tta   1218
Thr Ser Phe Asn Gln Ser Val Phe Asp Val Pro Leu His Phe Asn Leu
            280                 285                 290

cag gcg gct tcc tca caa gga ggc gga tat gat atg agg cgt ttg ctg   1266
Gln Ala Ala Ser Ser Gln Gly Gly Gly Tyr Asp Met Arg Arg Leu Leu
        295                 300                 305
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SEQUENCE LISTING

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gac ggt acc gtt gtg tcc agg cat ccg gaa aag gcg gtt aca ttt gtt   1314
Asp Gly Thr Val Val Ser Arg His Pro Glu Lys Ala Val Thr Phe Val
    310                 315                 320

gaa aat cat gac aca cag ccg gga cag tca ttg gaa tcg aca gtc caa   1362
Glu Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu Ser Thr Val Gln
325                 330                 335                 340

act tgg ttt aaa ccg ctt gca tac gcc ttt att ttg aca aga gaa tcc   1410
Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu Thr Arg Glu Ser
                345                 350                 355

ggt tat cct cag gtg ttc tat ggg gat atg tac ggg aca aaa ggg aca   1458
Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly Thr Lys Gly Thr
            360                 365                 370

tcg cca aag gaa att ccc tca ctg aaa gat aat ata gag ccg att tta   1506
Ser Pro Lys Glu Ile Pro Ser Leu Lys Asp Asn Ile Glu Pro Ile Leu
        375                 380                 385

aaa gcg cgt aag gag tac gca tac ggg ccc cag cac gat tat att gac   1554
Lys Ala Arg Lys Glu Tyr Ala Tyr Gly Pro Gln His Asp Tyr Ile Asp
    390                 395                 400

cac ccg gat gtg atc gga tgg acg agg gaa ggt gac agc tcc gcc gcc   1602
His Pro Asp Val Ile Gly Trp Thr Arg Glu Gly Asp Ser Ser Ala Ala
405                 410                 415                 420

aaa tca ggt ttg gcc gct tta atc acg gac gga ccc ggc gga tca aag   1650
Lys Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro Gly Gly Ser Lys
                425                 430                 435

cgg atg tat gcc ggc ctg aaa aat gcc ggc gag aca tgg tat gac ata   1698
Arg Met Tyr Ala Gly Leu Lys Asn Ala Gly Glu Thr Trp Tyr Asp Ile
            440                 445                 450

acg ggc aac cgt tca gat act gta aaa atc gga tct gac ggc tgg gga   1746
Thr Gly Asn Arg Ser Asp Thr Val Lys Ile Gly Ser Asp Gly Trp Gly
        455                 460                 465

gag ttt cat gta aac gat ggg tcc gtc tcc att tat gtt cag aaa taa   1794
Glu Phe His Val Asn Asp Gly Ser Val Ser Ile Tyr Val Gln Lys
    470                 475                 480

ggtaataaaa aaacacctcc aagctgagtg cgggtatcag cttggaggtg cgtttatttt 1854

ttcagccgta tgacaaggtc ggcatcaggt gtgacaaata cggtatgctg gctgtcatag 1914

gtgacaaatc cgggttttgc gccgtttggc tttttcacat gtctgatttt tgtataatca 1974

acaggcacgg agccggaatc tttcgccttg gaaaaataag cggcgatcgt agctgcttcc 2034

aatatggatt gttcatcggg atcgctgctt ttaatcacaa cgtgggatcc            2084
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<210> 10
<211> 483
<212> PRT
<213> Bacillus amyloliquefaciens

<400> 10

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Val Asn Gly Thr Leu Met Gln Tyr Phe Glu Trp Tyr Thr Pro Asn Asp
  1               5                  10                  15

Gly Gln His Trp Lys Arg Leu Gln Asn Asp Ala Glu His Leu Ser Asp
             20                  25                  30
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SEQUENCE LISTING

Ile Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly Leu Ser
35 40 45

Gln Ser Asp Asn Gly Tyr Gly Pro Tyr Asp Leu Tyr Asp Leu Gly Glu
50 55 60

Phe Gln Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys Ser Glu
65 70 75 80

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

Gly Asp Val Val Leu Asn His Lys Ala Gly Ala Asp Ala Thr Glu Asp
100 105 110

Val Thr Ala Val Glu Val Asn Pro Ala Asn Arg Asn Gln Glu Thr Ser
115 120 125

Glu Glu Tyr Gln Ile Lys Ala Trp Thr Asp Phe Arg Phe Pro Gly Arg
130 135 140

Gly Asn Thr Tyr Ser Asp Phe Lys Trp His Trp Tyr His Phe Asp Gly
145 150 155 160

Ala Asp Trp Asp Glu Ser Arg Lys Ile Ser Arg Ile Phe Lys Phe Arg
165 170 175

Gly Glu Gly Lys Ala Trp Asp Trp Glu Val Ser Ser Glu Asn Gly Asn
180 185 190

Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Tyr Asp His Pro Asp Val
195 200 205

Val Ala Glu Thr Lys Lys Trp Gly Ile Trp Tyr Ala Asn Glu Leu Ser
210 215 220

Leu Asp Gly Phe Arg Ile Asp Ala Ala Lys His Ile Lys Phe Ser Phe
225 230 235 240

Leu Arg Asp Trp Val Gln Ala Val Arg Gln Ala Thr Gly Lys Glu Met
245 250 255

Phe Thr Val Ala Glu Tyr Trp Gln Asn Asn Ala Gly Lys Leu Glu Asn
260 265 270

Tyr Leu Asn Lys Thr Ser Phe Asn Gln Ser Val Phe Asp Val Pro Leu
275 280 285

His Phe Asn Leu Gln Ala Ala Ser Ser Gln Gly Gly Gly Tyr Asp Met
290 295 300

Arg Arg Leu Leu Asp Gly Thr Val Val Ser Arg His Pro Glu Lys Ala
305 310 315 320

Val Thr Phe Val Glu Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu
325 330 335

Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu
340 345 350

Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly
355 360 365

Thr Lys Gly Thr Ser Pro Lys Glu Ile Pro Ser Leu Lys Asp Asn Ile
370 375 380

Glu Pro Ile Leu Lys Ala Arg Lys Glu Tyr Ala Tyr Gly Pro Gln His
385 390 395 400

SEQUENCE LISTING

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Asp Tyr Ile Asp His Pro Asp Val Ile Gly Trp Thr Arg Glu Gly Asp
                405                 410                 415

Ser Ser Ala Ala Lys Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
            420                 425                 430

Gly Gly Ser Lys Arg Met Tyr Ala Gly Leu Lys Asn Ala Gly Glu Thr
        435                 440                 445

Trp Tyr Asp Ile Thr Gly Asn Arg Ser Asp Thr Val Lys Ile Gly Ser
    450                 455                 460

Asp Gly Trp Gly Glu Phe His Val Asn Asp Gly Ser Val Ser Ile Tyr
465                 470                 475                 480

Val Gln Lys
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<210> 11
<211> 1458
<212> DNA
<213> Bacillus sp.

<220>
<221> CDS
<222> (1)..(1458)
<223> AA560

<400> 11

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cac cat aat ggt acg aac ggc aca atg atg cag tac ttt gaa tgg tat   48
His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
  1               5                  10                  15

cta cca aat gac gga aac cat tgg aat aga tta agg tct gat gca agt   96
Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Ser Asp Ala Ser
            20                  25                  30

aac cta aaa gat aaa ggg atc tca gcg gtt tgg att cct cct gca tgg   144
Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp
        35                  40                  45

aag ggt gcc tct caa aat gat gtg ggg tat ggt gct tat gat ctg tat   192
Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
    50                  55                  60

gat tta gga gaa ttc aat caa aaa gga acc att cgt aca aaa tat gga   240
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly
65                  70                  75                  80

acg cgc aat cag tta caa gct gca gtt aac gcc ttg aaa agt aat gga   288
Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly
                85                  90                  95

att caa gtg tat ggc gat gtt gta atg aat cat aaa ggg gga gca gac   336
Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
            100                 105                 110

gct acc gaa atg gtt agg gca gtt gaa gta aac ccg aat aat aga aat   384
Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
        115                 120                 125

caa gaa gtg tcc ggt gaa tat aca att gag gct tgg aca aag ttt gac   432
Gln Glu Val Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
    130                 135                 140

ttt cca gga cga ggt aat act cat tca aac ttc aaa tgg aga tgg tat   480
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SEQUENCE LISTING

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Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr
145                 150                 155                 160

cac ttt gat gga gta gat tgg gat cag tca cgt aag ctg aac aat cga   528
His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg
                165                 170                 175

att tat aaa ttt aga ggt gat gga aaa ggg tgg gat tgg gaa gtc gat   576
Ile Tyr Lys Phe Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp
            180                 185                 190

aca gaa aac ggt aac tat gat tac cta atg tat gca gat att gac atg   624
Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met
        195                 200                 205

gat cac cca gag gta gtg aat gag cta aga aat tgg ggt gtt tgg tat   672
Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr
    210                 215                 220

acg aat aca tta ggc ctt gat ggt ttt aga ata gat gca gta aaa cat   720
Thr Asn Thr Leu Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225                 230                 235                 240

ata aaa tac agc ttt act cgt gat tgg att aat cat gtt aga agt gca   768
Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala
                245                 250                 255

act ggc aaa aat atg ttt gcg gtt gcg gaa ttt tgg aaa aat gat tta   816
Thr Gly Lys Asn Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
            260                 265                 270

ggt gct att gaa aac tat tta aac aaa aca aac tgg aac cat tca gtc   864
Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
        275                 280                 285

ttt gat gtt ccg ctg cac tat aac ctc tat aat gct tca aaa agc gga   912
Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly
    290                 295                 300

ggg aat tat gat atg agg caa ata ttt aat ggt aca gtc gtg caa aga   960
Gly Asn Tyr Asp Met Arg Gln Ile Phe Asn Gly Thr Val Val Gln Arg
305                 310                 315                 320

cat cca atg cat gct gtt aca ttt gtt gat aat cat gat tcg caa cct  1008
His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
                325                 330                 335

gaa gaa gct tta gag tct ttt gtt gaa gaa tgg ttc aaa cca tta gcg  1056
Glu Glu Ala Leu Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala
            340                 345                 350

tat gct ttg aca tta aca cgt gaa caa ggc tac cct tct gta ttt tat  1104
Tyr Ala Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
        355                 360                 365

gga gat tat tat ggc att cca acg cat ggt gta cca gcg atg aaa tcg  1152
Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
    370                 375                 380

aaa att gac ccg att cta gaa gcg cgt caa aag tat gca tat gga aga  1200
Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Arg
385                 390                 395                 400

caa aat gac tac tta gac cat cat aat atc atc ggt tgg aca cgt gaa  1248
Gln Asn Asp Tyr Leu Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
                405                 410                 415

ggg aat aca gca cac ccc aac tcc ggt tta gct act atc atg tcc gat  1296
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SEQUENCE LISTING

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Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
          420                 425                 430

ggg gca gga gga aat aag tgg atg ttt gtt ggg cgt aat aaa gct ggt   1344
Gly Ala Gly Gly Asn Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
      435                 440                 445

caa gtt tgg acc gat atc act gga aat cgt gca ggt act gtt acg att   1392
Gln Val Trp Thr Asp Ile Thr Gly Asn Arg Ala Gly Thr Val Thr Ile
  450                 455                 460

aat gct gat gga tgg ggt aat ttt tct gta aat gga gga tca gtt tct   1440
Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465                 470                 475                 480

att tgg gta aac aaa taa                                          1458
Ile Trp Val Asn Lys
              485
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<210> 12
<211> 485
<212> PRT
<213> Bacillus sp.

<400> 12

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His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
  1               5                  10                  15

Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Ser Asp Ala Ser
            20                  25                  30

Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp
        35                  40                  45

Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
    50                  55                  60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly
65                  70                  75                  80

Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly
                85                  90                  95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
            100                 105                 110

Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
        115                 120                 125

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

Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr
145                 150                 155                 160

His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg
                165                 170                 175

Ile Tyr Lys Phe Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp
            180                 185                 190

Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met
        195                 200                 205

Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr
    210                 215                 220
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SEQUENCE LISTING

Thr Asn Thr Leu Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225 230 235 240

Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala
245 250 255

Thr Gly Lys Asn Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
260 265 270

Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
275 280 285

Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly
290 295 300

Gly Asn Tyr Asp Met Arg Gln Ile Phe Asn Gly Thr Val Val Gln Arg
305 310 315 320

His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
325 330 335

Glu Glu Ala Leu Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala
340 345 350

Tyr Ala Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
355 360 365

Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
370 375 380

Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Arg
385 390 395 400

Gln Asn Asp Tyr Leu Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
405 410 415

Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
420 425 430

Gly Ala Gly Gly Asn Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
435 440 445

Gln Val Trp Thr Asp Ile Thr Gly Asn Arg Ala Gly Thr Val Thr Ile
450 455 460

Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465 470 475 480

Ile Trp Val Asn Lys
485

<210> 13
<211> 485
<212> PRT
<213> Bacillus sp. 707

<400> 13
His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
1 5 10 15

Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Asn Ser Asp Ala Ser
20 25 30

Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp
35 40 45

Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr

SEQUENCE LISTING

50 55 60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65 70 75 80

Thr Arg Ser Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly
85 90 95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
100 105 110

Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
115 120 125

Gln Glu Val Thr Gly Glu Tyr Thr Ile Glu Ala Trp Thr Arg Phe Asp
130 135 140

Phe Pro Gly Arg Gly Asn Thr His Ser Ser Phe Lys Trp Arg Trp Tyr
145 150 155 160

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

Ile Tyr Lys Phe Arg Gly His Gly Lys Ala Trp Asp Trp Glu Val Asp
180 185 190

Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met
195 200 205

Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr
210 215 220

Thr Asn Thr Leu Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
225 230 235 240

Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala
245 250 255

Thr Gly Lys Asn Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
260 265 270

Gly Ala Ile Glu Asn Tyr Leu Gln Lys Thr Asn Trp Asn His Ser Val
275 280 285

Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly
290 295 300

Gly Asn Tyr Asp Met Arg Asn Ile Phe Asn Gly Thr Val Val Gln Arg
305 310 315 320

His Pro Ser His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
325 330 335

Glu Glu Ala Leu Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala
340 345 350

Tyr Ala Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
355 360 365

Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Arg Ser
370 375 380

Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Lys
385 390 395 400

Gln Asn Asp Tyr Leu Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
405 410 415

SEQUENCE LISTING

Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
420 425 430

Gly Ala Gly Gly Ser Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
435 440 445

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

Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
465 470 475 480

Ile Trp Val Asn Lys
485

<210> 14
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CGATTGCTGA CGCTGTTATT TGCG
24

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<223> Primer 24814
<400> 15
GATCACCCGC GATACCGTC
29

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<223> Primer #24
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31
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<212> DNA
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<400> 17
GCCGCCGCTG CTGCAGAATG AGGCAGCAAG
30

<210> 18
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<212> DNA
<213> Artificial Sequence
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<223> Primer #312
<400> 18
CCCGAAAGCT GAACCGCATC TATAGGTTTC AAGGGAAGAC TTGGGATT 48

<210> 19
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<212> DNA
<213> Artificial Sequence
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SEQUENCE LISTING

<223> Primer #290
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23

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CCGACTTTGA TTATGACCAT CCTGTTGTCG TAGCAGAGAT TAAGAGATGG GG 52

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<223> Primer #314
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CGACAATGTC ATGGTGGTCG AAAAAATCAT GCTGTGCTCC GTACG 45

<210> 22
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<212> DNA
<213> Artificial Sequence
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<223> Primer #296
<400> 22
TTTCGACCAC CATGACATTG TCG
23

<210> 23
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<212> DNA
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<223> Primer #305
<400> 23
TATAGATGCG GTTCAGCTTT CGGG
24

<210> 24
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tttg atg aaa aga tgg gta gta gca atg ctg gca gtg tta ttt tta ttt 109
Met Lys Arg Trp Val Val Ala Met Leu Ala Val Leu Phe Leu Phe
-20 -15 -10

cct tcg gta gta gtt gca gat ggc ttg aat gga acg atg atg cag tat 157
Pro Ser Val Val Val Ala Asp Gly Leu Asn Gly Thr Met Met Gln Tyr
-5 -1 1 5 10

tat gag tgg cat cta gag aat gat ggg caa cac tgg aat cgg ttg cat 205

SEQUENCE LISTING

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Tyr Glu Trp His Leu Glu Asn Asp Gly Gln His Trp Asn Arg Leu His
                15                  20                  25

gat gat gcc gaa gct tta agt aat gcg ggt att aca gct att tgg ata      253
Asp Asp Ala Glu Ala Leu Ser Asn Ala Gly Ile Thr Ala Ile Trp Ile
            30                  35                  40

ccc cca gcc tac aaa gga aat agt cag gct gat gtt ggg tat ggt gca      301
Pro Pro Ala Tyr Lys Gly Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala
        45                  50                  55

tac gac ctt tat gat tta ggg gag ttt aat caa aaa ggt acc gtt cga      349
Tyr Asp Leu Tyr Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg
    60                  65                  70

acg aaa tac ggg aca aag gct cag ctt gag cga gct ata ggg tcc cta      397
Thr Lys Tyr Gly Thr Lys Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu
75                  80                  85                  90

aag tcg aat gat atc aat gtt tat ggg gat gtc gta atg aat cat aaa      445
Lys Ser Asn Asp Ile Asn Val Tyr Gly Asp Val Val Met Asn His Lys
                95                  100                 105

tta gga gct gat ttc acg gag gca gtg caa gct gtt caa gta aat cct      493
Leu Gly Ala Asp Phe Thr Glu Ala Val Gln Ala Val Gln Val Asn Pro
            110                 115                 120

tcg aac cgt tgg cag gat att tca ggt gtc tac acg att gat gca tgg      541
Ser Asn Arg Trp Gln Asp Ile Ser Gly Val Tyr Thr Ile Asp Ala Trp
        125                 130                 135

acg gga ttt gac ttt cca ggg cgc aac aat gcc tat tcc gat ttt aaa      589
Thr Gly Phe Asp Phe Pro Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys
    140                 145                 150

tgg aga tgg ttc cat ttt aat ggc gtt gac tgg gat caa cgc tat caa      637
Trp Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln
155                 160                 165                 170

gaa aac cat ctt ttt cgc ttt gca aat acg aac tgg aac tgg cga gtg      685
Glu Asn His Leu Phe Arg Phe Ala Asn Thr Asn Trp Asn Trp Arg Val
                175                 180                 185

gat gaa gag aat ggt aat tat gac tat tta tta gga tcg aac att gac      733
Asp Glu Glu Asn Gly Asn Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp
            190                 195                 200

ttt agc cac cca gag gtt caa gag gaa tta aag gat tgg ggg agc tgg      781
Phe Ser His Pro Glu Val Gln Glu Glu Leu Lys Asp Trp Gly Ser Trp
        205                 210                 215

ttt acg gat gag cta gat tta gat ggg tat cga ttg gat gct att aag      829
Phe Thr Asp Glu Leu Asp Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys
    220                 225                 230

cat att cca ttc tgg tat acg tca gat tgg gtt agg cat cag cga agt      877
His Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg Ser
235                 240                 245                 250

gaa gca gac caa gat tta ttt gtc gta ggg gag tat tgg aag gat gac      925
Glu Ala Asp Gln Asp Leu Phe Val Val Gly Glu Tyr Trp Lys Asp Asp
                255                 260                 265

gta ggt gct ctc gaa ttt tat tta gat gaa atg aat tgg gag atg tct      973
Val Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met Ser
            270                 275                 280

cta ttc gat gtt ccg ctc aat tat aat ttt tac cgg gct tca aag caa     1021
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SEQUENCE LISTING

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Leu Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Lys Gln
        285                 290                 295

ggc gga agc tat gat atg cgt aat att tta cga gga tct tta gta gaa     1069
Gly Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val Glu
    300                 305                 310

gca cat ccg att cat gca gtt acg ttt gtt gat aat cat gat act cag     1117
Ala His Pro Ile His Ala Val Thr Phe Val Asp Asn His Asp Thr Gln
315                 320                 325                 330

cca gga gag tca tta gaa tca tgg gtc gct gat tgg ttt aag cca ctt     1165
Pro Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro Leu
                335                 340                 345

gct tat gcg aca atc ttg acg cgt gaa ggt ggt tat cca aat gta ttt     1213
Ala Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe
            350                 355                 360

tac ggt gac tac tat ggg att cct aac gat aac att tca gct aag aag     1261
Tyr Gly Asp Tyr Tyr Gly Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys
        365                 370                 375

gat atg att gat gag ttg ctt gat gca cgt caa aat tac gca tat ggc     1309
Asp Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly
    380                 385                 390

aca caa cat gac tat ttt gat cat tgg gat atc gtt gga tgg aca aga     1357
Thr Gln His Asp Tyr Phe Asp His Trp Asp Ile Val Gly Trp Thr Arg
395                 400                 405                 410

gaa ggt aca tcc tca cgt cct aat tcg ggt ctt gct act att atg tcc     1405
Glu Gly Thr Ser Ser Arg Pro Asn Ser Gly Leu Ala Thr Ile Met Ser
                415                 420                 425

aat ggt cct gga gga tca aaa tgg atg tac gta gga cag caa cat gca     1453
Asn Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Gln Gln His Ala
            430                 435                 440

gga caa acg tgg aca gat tta act ggc aat cac gcg gcg tcg gtt acg     1501
Gly Gln Thr Trp Thr Asp Leu Thr Gly Asn His Ala Ala Ser Val Thr
        445                 450                 455

att aat ggt gat ggc tgg ggc gaa ttc ttt aca aat gga gga tct gta     1549
Ile Asn Gly Asp Gly Trp Gly Glu Phe Phe Thr Asn Gly Gly Ser Val
    460                 465                 470

tcc gtg tat gtg aac caa taataaaaag ccttgagaag ggattcctcc           1597
Ser Val Tyr Val Asn Gln
475                 480

ctaactcaag gctttcttta tgtcgtttag ctcaacgctt ctacgaagct tta         1650
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<210> 25
<211> 501
<212> PRT
<213> Bacillus sp.
<400> 25

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Met Lys Arg Trp Val Val Ala Met Leu Ala Val Leu Phe Leu Phe Pro
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Ser Val Val Val Ala Asp Gly Leu Asn Gly Thr Met Met Gln Tyr Tyr
-5              -1  1               5                   10

Glu Trp His Leu Glu Asn Asp Gly Gln His Trp Asn Arg Leu His Asp
            15                  20                  25
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SEQUENCE LISTING

Asp Ala Glu Ala Leu Ser Asn Ala Gly Ile Thr Ala Ile Trp Ile Pro
30 35 40

Pro Ala Tyr Lys Gly Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr
45 50 55

Asp Leu Tyr Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr
60 65 70 75

Lys Tyr Gly Thr Lys Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys
80 85 90

Ser Asn Asp Ile Asn Val Tyr Gly Asp Val Val Met Asn His Lys Leu
95 100 105

Gly Ala Asp Phe Thr Glu Ala Val Gln Ala Val Gln Val Asn Pro Ser
110 115 120

Asn Arg Trp Gln Asp Ile Ser Gly Val Tyr Thr Ile Asp Ala Trp Thr
125 130 135

Gly Phe Asp Phe Pro Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp
140 145 150 155

Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu
160 165 170

Asn His Leu Phe Arg Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp
175 180 185

Glu Glu Asn Gly Asn Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe
190 195 200

Ser His Pro Glu Val Gln Glu Glu Leu Lys Asp Trp Gly Ser Trp Phe
205 210 215

Thr Asp Glu Leu Asp Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His
220 225 230 235

Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg Ser Glu
240 245 250

Ala Asp Gln Asp Leu Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val
255 260 265

Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu
270 275 280

Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Lys Gln Gly
285 290 295

SEQUENCE LISTING

Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala
300 305 310 315

His Pro Ile His Ala Val Thr Phe Val Asp Asn His Asp Thr Gln Pro
320 325 330

Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala
335 340 345

Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr
350 355 360

Gly Asp Tyr Tyr Gly Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp
365 370 375

Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr
380 385 390 395

Gln His Asp Tyr Phe Asp His Trp Asp Ile Val Gly Trp Thr Arg Glu
400 405 410

Gly Thr Ser Ser Arg Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn
415 420 425

Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Gln Gln His Ala Gly
430 435 440

Gln Thr Trp Thr Asp Leu Thr Gly Asn His Ala Ala Ser Val Thr Ile
445 450 455

Asn Gly Asp Gly Trp Gly Glu Phe Phe Thr Asn Gly Gly Ser Val Ser
460 465 470 475

Val Tyr Val Asn Gln
480

<210> 26
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<213> Bacillus sp.
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<222> (253)..()
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<222> (190)..(253)
<400> 26
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actagcactc ttcagggact aaaccacctt ttttccaaaa atgacatcat ataaacaaat 120

SEQUENCE LISTING

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ttgtctacca atcactattt aaagctgttt atgatatatg taagcgttat cattaaaagg    180

aggtatttg atg aga aga tgg gta gta gca atg ttg gca gtg tta ttt tta    231
          Met Arg Arg Trp Val Val Ala Met Leu Ala Val Leu Phe Leu
              -20                 -15                 -10

ttt cct tcg gta gta gtt gca gat gga ttg aac ggt acg atg atg cag      279
Phe Pro Ser Val Val Val Ala Asp Gly Leu Asn Gly Thr Met Met Gln
        -5              -1  1               5

tat tat gag tgg cat ttg gaa aac gac ggg cag cat tgg aat cgg ttg      327
Tyr Tyr Glu Trp His Leu Glu Asn Asp Gly Gln His Trp Asn Arg Leu
10                  15                  20                  25

cac gat gat gcc gca gct ttg agt gat gct ggt att aca gct att tgg      375
His Asp Asp Ala Ala Ala Leu Ser Asp Ala Gly Ile Thr Ala Ile Trp
                30                  35                  40

att ccg cca gcc tac aaa ggt aat agt cag gcg gat gtt ggg tac ggt      423
Ile Pro Pro Ala Tyr Lys Gly Asn Ser Gln Ala Asp Val Gly Tyr Gly
            45                  50                  55

gca tac gat ctt tat gat tta gga gag ttc aat caa aag ggt act gtt      471
Ala Tyr Asp Leu Tyr Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val
        60                  65                  70

cga acg aaa tac gga act aag gca cag ctt gaa cga gct att ggg tcc      519
Arg Thr Lys Tyr Gly Thr Lys Ala Gln Leu Glu Arg Ala Ile Gly Ser
    75                  80                  85

ctt aaa tct aat gat atc aat gta tac gga gat gtc gtg atg aat cat      567
Leu Lys Ser Asn Asp Ile Asn Val Tyr Gly Asp Val Val Met Asn His
90                  95                  100                 105

aaa atg gga gct gat ttt acg gag gca gtg caa gct gtt caa gta aat      615
Lys Met Gly Ala Asp Phe Thr Glu Ala Val Gln Ala Val Gln Val Asn
                110                 115                 120

cca acg aat cgt tgg cag gat att tca ggt gcc tac acg att gat gcg      663
Pro Thr Asn Arg Trp Gln Asp Ile Ser Gly Ala Tyr Thr Ile Asp Ala
            125                 130                 135

tgg acg ggt ttc gac ttt tca ggg cgt aac aac gcc tat tca gat ttt      711
Trp Thr Gly Phe Asp Phe Ser Gly Arg Asn Asn Ala Tyr Ser Asp Phe
        140                 145                 150

aag tgg aga tgg ttc cat ttt aat ggt gtt gac tgg gat cag cgc tat      759
Lys Trp Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr
    155                 160                 165

caa gaa aat cat att ttc cgc ttt gca aat acg aac tgg aac tgg cga      807
Gln Glu Asn His Ile Phe Arg Phe Ala Asn Thr Asn Trp Asn Trp Arg
170                 175                 180                 185

gtg gat gaa gag aac ggt aat tat gat tac ctg tta gga tcg aat atc      855
Val Asp Glu Glu Asn Gly Asn Tyr Asp Tyr Leu Leu Gly Ser Asn Ile
                190                 195                 200

gac ttt agt cat cca gaa gta caa gat gag ttg aag gat tgg ggt agc      903
Asp Phe Ser His Pro Glu Val Gln Asp Glu Leu Lys Asp Trp Gly Ser
            205                 210                 215

tgg ttt acc gat gag tta gat ttg gat ggt tat cgt tta gat gct att      951
Trp Phe Thr Asp Glu Leu Asp Leu Asp Gly Tyr Arg Leu Asp Ala Ile
        220                 225                 230

aaa cat att cca ttc tgg tat aca tct gat tgg gtt cgg cat cag cgc      999
Lys His Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg
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SEQUENCE LISTING

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    235                 240                 245

aac gaa gca gat caa gat tta ttt gtc gta ggg gaa tat tgg aag gat      1047
Asn Glu Ala Asp Gln Asp Leu Phe Val Val Gly Glu Tyr Trp Lys Asp
250                 255                 260                 265

gac gta ggt gct ctc gaa ttt tat tta gat gaa atg aat tgg gag atg      1095
Asp Val Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met
                270                 275                 280

tct cta ttc gat gtt cca ctt aat tat aat ttt tac cgg gct tca caa      1143
Ser Leu Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Gln
            285                 290                 295

caa ggt gga agc tat gat atg cgt aat att tta cga gga tct tta gta      1191
Gln Gly Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val
        300                 305                 310

gaa gcg cat ccg atg cat gca gtt acg ttt gtt gat aat cat gat act      1239
Glu Ala His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Thr
    315                 320                 325

cag cca ggg gag tca tta gag tca tgg gtt gct gat tgg ttt aag cca      1287
Gln Pro Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro
330                 335                 340                 345

ctt gct tat gcg aca att ttg acg cgt gaa ggt ggt tat cca aat gta      1335
Leu Ala Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val
                350                 355                 360

ttt tac ggt gat tac tat ggg att cct aac gat aac att tca gct aaa      1383
Phe Tyr Gly Asp Tyr Tyr Gly Ile Pro Asn Asp Asn Ile Ser Ala Lys
            365                 370                 375

aaa gat atg att gat gag ctg ctt gat gca cgt caa aat tac gca tat      1431
Lys Asp Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr
        380                 385                 390

ggc acg cag cat gac tat ttt gat cat tgg gat gtt gta gga tgg act      1479
Gly Thr Gln His Asp Tyr Phe Asp His Trp Asp Val Val Gly Trp Thr
    395                 400                 405

agg gaa gga tct tcc tcc aga cct aat tca ggc ctt gcg act att atg      1527
Arg Glu Gly Ser Ser Ser Arg Pro Asn Ser Gly Leu Ala Thr Ile Met
410                 415                 420                 425

tcg aat gga cct ggt ggt tcc aag tgg atg tat gta gga cgt cag aat      1575
Ser Asn Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Arg Gln Asn
                430                 435                 440

gca gga caa aca tgg aca gat tta act ggt aat aac gga gcg tcc gtt      1623
Ala Gly Gln Thr Trp Thr Asp Leu Thr Gly Asn Asn Gly Ala Ser Val
            445                 450                 455

aca att aat ggc gat gga tgg ggc gaa ttc ttt acg aat gga gga tct      1671
Thr Ile Asn Gly Asp Gly Trp Gly Glu Phe Phe Thr Asn Gly Gly Ser
        460                 465                 470

gta tcc gtg tac gtg aac caa taacaaaaag ccttgagaag ggattcctcc        1722
Val Ser Val Tyr Val Asn Gln
    475                 480

ctaactcaag gctttcttta tgt                                           1745
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<210> 27
<211> 501
<212> PRT

SEQUENCE LISTING

<213> Bacillus sp.
<400> 27

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Met Arg Arg Trp Val Val Ala Met Leu Ala Val Leu Phe Leu Phe Pro
 -20                 -15                 -10

Ser Val Val Val Ala Asp Gly Leu Asn Gly Thr Met Met Gln Tyr Tyr
-5              -1  1                   5                  10

Glu Trp His Leu Glu Asn Asp Gly Gln His Trp Asn Arg Leu His Asp
            15                  20                  25

Asp Ala Ala Ala Leu Ser Asp Ala Gly Ile Thr Ala Ile Trp Ile Pro
        30                  35                  40

Pro Ala Tyr Lys Gly Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr
    45                  50                  55

Asp Leu Tyr Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr
60                  65                  70                  75

Lys Tyr Gly Thr Lys Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys
                80                  85                  90

Ser Asn Asp Ile Asn Val Tyr Gly Asp Val Val Met Asn His Lys Met
            95                  100                 105

Gly Ala Asp Phe Thr Glu Ala Val Gln Ala Val Gln Val Asn Pro Thr
        110                 115                 120

Asn Arg Trp Gln Asp Ile Ser Gly Ala Tyr Thr Ile Asp Ala Trp Thr
    125                 130                 135

Gly Phe Asp Phe Ser Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp
140                 145                 150                 155

Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu
                160                 165                 170

Asn His Ile Phe Arg Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp
            175                 180                 185

Glu Glu Asn Gly Asn Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe
        190                 195                 200

Ser His Pro Glu Val Gln Asp Glu Leu Lys Asp Trp Gly Ser Trp Phe
    205                 210                 215

Thr Asp Glu Leu Asp Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His
220                 225                 230                 235

Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg Asn Glu
                240                 245                 250
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SEQUENCE LISTING

Ala Asp Gln Asp Leu Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val
255 260 265

Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu
270 275 280

Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Gln Gln Gly
285 290 295

Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala
300 305 310 315

His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Thr Gln Pro
320 325 330

Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala
335 340 345

Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr
350 355 360

Gly Asp Tyr Tyr Gly Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp
365 370 375

Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr
380 385 390 395

Gln His Asp Tyr Phe Asp His Trp Asp Val Val Gly Trp Thr Arg Glu
400 405 410

Gly Ser Ser Ser Arg Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn
415 420 425

Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Arg Gln Asn Ala Gly
430 435 440

Gln Thr Trp Thr Asp Leu Thr Gly Asn Asn Gly Ala Ser Val Thr Ile
445 450 455

Asn Gly Asp Gly Trp Gly Glu Phe Phe Thr Asn Gly Gly Ser Val Ser
460 465 470 475

Val Tyr Val Asn Gln
480

<210> 28
<211> 1920
<212> DNA
<213> Bacillus licheniformis
<220>
<221> CDS
<222> (421)..(1872)

SEQUENCE LISTING

<400> 28

cggaagattg gaagtacaaa aataagcaaa agattgtcaa tcatgtcatg agccatgcgg 60

gagacggaaa aatcgtctta atgcacgata tttatgcaac gttcgcagat gctgctgaag 120

agattattaa aaagctgaaa gcaaaaggct atcaattggt aactgtatct cagcttgaag 180

aagtgaagaa gcagagaggc tattgaataa atgagtagaa gcgccatatc ggcgcttttc 240

ttttggaaga aaatataggg aaaatggtac ttgttaaaaa ttcggaatat ttatacaaca 300

tcatatgttt cacattgaaa ggggaggaga atcatgaaac aacaaaaacg gctttacgcc 360

cgattgctga cgctgttatt tgcgctcatc ttcttgctgc ctcattctgc agcagcggcg 420

gca aat ctt aat ggg acg ctg atg cag tat ttt gaa tgg tac atg ccc 468
Ala Asn Leu Asn Gly Thr Leu Met Gln Tyr Phe Glu Trp Tyr Met Pro
1 5 10 15

aat gac ggc caa cat tgg agg cgt ttg caa aac gac tcg gca tat ttg 516
Asn Asp Gly Gln His Trp Arg Arg Leu Gln Asn Asp Ser Ala Tyr Leu
20 25 30

gct gaa cac ggt att act gcc gtc tgg att ccc ccg gca tat aag gga 564
Ala Glu His Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly
35 40 45

acg agc caa gcg gat gtg ggc tac ggt gct tac gac ctt tat gat tta 612
Thr Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
50 55 60

ggg gag ttt cat caa aaa ggg acg gtt cgg aca aag tac ggc aca aaa 660
Gly Glu Phe His Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys
65 70 75 80

gga gag ctg caa tct gcg atc aaa agt ctt cat tcc cgc gac att aac 708
Gly Glu Leu Gln Ser Ala Ile Lys Ser Leu His Ser Arg Asp Ile Asn
85 90 95

gtt tac ggg gat gtg gtc atc aac cac aaa ggc ggc gct gat gcg acc 756
Val Tyr Gly Asp Val Val Ile Asn His Lys Gly Gly Ala Asp Ala Thr
100 105 110

gaa gat gta acc gcg gtt gaa gtc gat ccc gct gac cgc aac cgc gta 804
Glu Asp Val Thr Ala Val Glu Val Asp Pro Ala Asp Arg Asn Arg Val
115 120 125

att tca gga gaa cac cta att aaa gcc tgg aca cat ttt cat ttt ccg 852
Ile Ser Gly Glu His Leu Ile Lys Ala Trp Thr His Phe His Phe Pro
130 135 140

ggg cgc ggc agc aca tac agc gat ttt aaa tgg cat tgg tac cat ttt 900
Gly Arg Gly Ser Thr Tyr Ser Asp Phe Lys Trp His Trp Tyr His Phe
145 150 155 160

gac gga acc gat tgg gac gag tcc cga aag ctg aac cgc atc tat aag 948
Asp Gly Thr Asp Trp Asp Glu Ser Arg Lys Leu Asn Arg Ile Tyr Lys
165 170 175

ttt caa gga aag gct tgg gat tgg gaa gtt tcc aat gaa aac ggc aac 996
Phe Gln Gly Lys Ala Trp Asp Trp Glu Val Ser Asn Glu Asn Gly Asn
180 185 190

tat gat tat ttg atg tat gcc gac atc gat tat gac cat cct gat gtc 1044
Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Tyr Asp His Pro Asp Val
195 200 205

gca gca gaa att aag aga tgg ggc act tgg tat gcc aat gaa ctg caa 1092

SEQUENCE LISTING

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Ala Ala Glu Ile Lys Arg Trp Gly Thr Trp Tyr Ala Asn Glu Leu Gln
    210                 215                 220

ttg gac ggt ttc cgt ctt gat gct gtc aaa cac att aaa ttt tct ttt     1140
Leu Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys Phe Ser Phe
225                 230                 235                 240

ttg cgg gat tgg gtt aat cat gtc agg gaa aaa acg ggg aag gaa atg     1188
Leu Arg Asp Trp Val Asn His Val Arg Glu Lys Thr Gly Lys Glu Met
                245                 250                 255

ttt acg gta gct gaa tat tgg cag aat gac ttg ggc gcg ctg gaa aac     1236
Phe Thr Val Ala Glu Tyr Trp Gln Asn Asp Leu Gly Ala Leu Glu Asn
            260                 265                 270

tat ttg aac aaa aca aat ttt aat cat tca gtg ttt gac gtg ccg ctt     1284
Tyr Leu Asn Lys Thr Asn Phe Asn His Ser Val Phe Asp Val Pro Leu
        275                 280                 285

cat tat cag ttc cat gct gca tcg aca cag gga ggc ggc tat gat atg     1332
His Tyr Gln Phe His Ala Ala Ser Thr Gln Gly Gly Gly Tyr Asp Met
    290                 295                 300

agg aaa ttg ctg aac ggt acg gtc gtt tcc aag cat ccg ttg aaa tcg     1380
Arg Lys Leu Leu Asn Gly Thr Val Val Ser Lys His Pro Leu Lys Ser
305                 310                 315                 320

gtt aca ttt gtc gat aac cat gat aca cag ccg ggg caa tcg ctt gag     1428
Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu
                325                 330                 335

tcg act gtc caa aca tgg ttt aag ccg ctt gct tac gct ttt att ctc     1476
Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu
            340                 345                 350

aca agg gaa tct gga tac cct cag gtt ttc tac ggg gat atg tac ggg     1524
Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly
        355                 360                 365

acg aaa gga gac tcc cag cgc gaa att cct gcc ttg aaa cac aaa att     1572
Thr Lys Gly Asp Ser Gln Arg Glu Ile Pro Ala Leu Lys His Lys Ile
    370                 375                 380

gaa ccg atc tta aaa gcg aga aaa cag tat gcg tac gga gca cag cat     1620
Glu Pro Ile Leu Lys Ala Arg Lys Gln Tyr Ala Tyr Gly Ala Gln His
385                 390                 395                 400

gat tat ttc gac cac cat gac att gtc ggc tgg aca agg gaa ggc gac     1668
Asp Tyr Phe Asp His His Asp Ile Val Gly Trp Thr Arg Glu Gly Asp
                405                 410                 415

agc tcg gtt gca aat tca ggt ttg gcg gca tta ata aca gac gga ccc     1716
Ser Ser Val Ala Asn Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
            420                 425                 430

ggt ggg gca aag cga atg tat gtc ggc cgg caa aac gcc ggt gag aca     1764
Gly Gly Ala Lys Arg Met Tyr Val Gly Arg Gln Asn Ala Gly Glu Thr
        435                 440                 445

tgg cat gac att acc gga aac cgt tcg gag ccg gtt gtc atc aat tcg     1812
Trp His Asp Ile Thr Gly Asn Arg Ser Glu Pro Val Val Ile Asn Ser
    450                 455                 460

gaa ggc tgg gga gag ttt cac gta aac ggc ggg tcg gtt tca att tat     1860
Glu Gly Trp Gly Glu Phe His Val Asn Gly Gly Ser Val Ser Ile Tyr
465                 470                 475                 480

gtt caa aga tag aagagcagag aggacggatt tcctgaagga aatccgtttt        1912
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SEQUENCE LISTING

Val Gln Arg

tttatttt 1920