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## DESCRIPTION

### REFERENCE TO A SEQUENCE LISTING

[0001] This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

### FIELD OF THE INVENTION

[0002] The present invention relates to an improved mashing process for production of a brewer's wort and for production of a beer.

### BACKGROUND OF THE INVENTION

[0003] In modern mashing processes, enzymes are often added as a supplement when mashing malt is low in enzymes or to allow use of all adjunct grists. Enzymes may also be applied in mashing of well modified malts with high enzyme content in order to increase the extract recovery as well as the amount of fermentable sugars. It is thus well known to apply debranching enzymes, e.g., isoamylase or pullulanase to increase the yield of fermentable sugars. Debranching enzymes may be applied in processes for production of low calorie beer. Such processes are the subject of Willox et al. (MBAA Technical Quarterly, 1977, 14: 105), U.S. Patent Nos. 4,528,198, 4,666,718, and 4,318,927, and GB 2056484 and GB 2069527, and US 4,355,047.

### SUMMARY OF THE INVENTION

[0004] The present inventors have now surprisingly discovered that by using a certain pullulanase, mashing can be achieved using a smaller amount of enzyme protein.

[0005] Accordingly, in a first aspect the invention provides a process for producing a brewers wort comprising forming a mash from a grist, and contacting said mash with a pullulanase (E.C. 3.2.1.41), wherein said pullulanase has an amino acid sequence which a) is at least 98% identical to the amino acid sequence shown in SEQ ID NO: 4.

[0006] In a further aspect the invention provides a composition suitable for use in the process of the first aspect, said composition comprising pullulanase (E.C. 3.2.1.41), glucoamylase and optionally alpha-amylase, wherein the pullulanase has an amino acid sequence which a) is at least 98% identical to the amino acid sequence shown in SEQ ID NO: 4.

### DETAILED DESCRIPTION OF THE INVENTION

[0007] Brewing processes are well-known in the art, and generally involve the steps of malting, mashing, and fermentation. Mashing is the process of converting starch from the milled barley malt and solid adjuncts into fermentable and unfermentable sugars to produce wort of the desired composition. Traditional mashing involves mixing milled barley malt and adjuncts with water at a set temperature and volume to continue the biochemical changes initiated during the malting process. The mashing process is conducted over a period of time at various temperatures in order to activate the endogenous enzymes responsible for the degradation of proteins and carbohydrates. By far the most important change brought about in mashing is the conversion of starch molecules into fermentable sugars. The principal enzymes responsible for starch conversion in a traditional mashing process are alpha- and beta-amylases. Alpha-amylase very rapidly reduces insoluble and soluble starch by splitting starch molecules into many shorter chains that can be attacked by beta-amylase. The disaccharide produced is maltose. In addition to the maltose formed during mashing short branched glucose oligomers are produced. The short branched glucose oligomers are non fermentable sugars and add to the taste as well as the amount of calories of the finished beer.

[0008] After mashing, when all the starch has been broken down, it is necessary to separate the liquid extract (the wort) from the solids (spent grains). Wort separation, lautering, is important because the solids contain large amounts of protein, poorly modified starch, fatty material, silicates, and polyphenols (tannins). Following the separation of the wort from the spent grains the wort may

be fermented with brewers yeast to produce a beer.

[0009] Further information on conventional brewing processes may be found in "Technology Brewing and Malting" by Wolfgang Kunze of the Research and Teaching Institute of Brewing, Berlin (VLB), 2nd revised Edition 1999, ISBN 3-921690-39-0.

[0010] The short branched glucose oligomers formed during mashing may be further hydrolyzed by addition of exogenous enzymes (enzymes added in addition to the malt). Debranching enzymes such as pullulanase and isoamylase hydrolyze the branching alpha-1-6 glucosidic bonds in these oligomers, thereby releasing glucose or maltose and straight-chained oligomers which are subject to the action of endogenous (malt derived) and/or exogenous enzymes, *e.g.*, alpha-amylases, beta-amylases and glucoamylases.

[0011] The present invention provides a new process suitable for producing a wort that is low in non-fermentable sugars. The process applies an expressly selected pullulanase activity.

## Definitions

[0012] Throughout this disclosure, various terms that are generally understood by those of ordinary skill in the arts, are used. Several terms are used with specific meaning, as defined below.

[0013] As used herein the term **"grist"** is understood as the starch or sugar containing material that is the basis for beer production, *e.g.*, the barley malt and the adjunct. Generally, the grist does not contain any added water.

[0014] The term **"malt"** is understood as any malted cereal grain, in particular barley.

[0015] The term **"adjunct"** is understood as the part of the grist which is not barley malt. The adjunct may comprise any starch rich plant material, *e.g.*, unmalted grain, such as barley, rice, corn, wheat, rye, sorghum and readily fermentable sugar and/or syrup.

[0016] The term **"mash"** is understood as a starch containing slurry comprising grist steeped in water.

[0017] The term **"wort"** is understood as the unfermented liquor run-off following extracting the grist during mashing.

[0018] The term **"spent grains"** is understood as the drained solids remaining when the grist has been extracted and the wort separated.

[0019] The term **"beer"** is understood as fermented wort, *i.e.*, an alcoholic beverage brewed from barley malt, optionally adjunct and hops.

[0020] The term **"homologous sequence"** is used to characterize a sequence having an amino acid sequence that is at least 70%, preferably at least 75%, or at least 80%, or at least 85%, or 90%, or at least 95%, at least 96%, at least 97%, at least 98% at least 99% or even at least 100% identical to a known sequence. The relevant part of the amino acid sequence for the homology determination is the mature polypeptide, *i.e.*, without the signal peptide. The term **"homologous sequence"** is also used to characterize DNA sequences which hybridize at low stringency, medium stringency, medium/high stringency, high stringency, or even very high stringency with a known sequence. Suitable experimental conditions for determining hybridization at low, medium, or high stringency between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC (Sodium chloride/Sodium citrate, Sambrook et al., 1989) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al., 1989), 0.5% SDS and 100 micrograms/ml of denatured sonicated salmon sperm DNA (Sambrook et al., 1989), followed by hybridization in the same solution containing a concentration of 10ng/ml of a random-primed (Feinberg and Vogelstein, 1983, Anal. Biochem. 132:6-13), 32P-dCTP-labeled (specific activity > 1 x 10<sup>9</sup> cpm/microgram) probe for 12 hours at about 45°C. The filter is then washed twice for 30 minutes in 2 x SSC, 0.5% SDS at about 55°C (low stringency), more preferably at about 60°C (medium stringency), still more preferably at about 65°C (medium/high stringency), even more preferably at about 70°C (high stringency), and even more preferably at about 75°C (very high stringency). Molecules to which the oligonucleotide probe hybridizes under these conditions are detected using an x-ray film.

[0021] The term **"identity"** when used about polypeptide or DNA sequences and referred to in this disclosure is understood as

the degree of identity between two sequences indicating a derivation of the first sequence from the second. The identity may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman and Wunsch, 1970, Journal of Molecular Biology 48: 443-453. The following settings for polypeptide sequence comparison are used: GAP creation penalty of 3.0 and GAP extension penalty of 0.1. The degree of identity between an amino acid sequence of the present invention and a different amino acid sequence ("foreign sequence") is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence" or the length of the "foreign sequence", whichever is the shortest. The result is expressed in percent identity.

#### **Wort production**

**[0022]** In accordance with the first aspect the invention provides a process for producing a brewer's wort comprising forming a mash from a grist, and contacting said mash with a pullulanase (E.C. 3.2.1.41), wherein said pullulanase has an amino acid sequence which is at least 98% or even at least 99% identical to the amino acid sequence shown in SEQ ID NO: 4.

**[0023]** The grist of the first aspect comprises starch containing malted grain and/or adjunct. The grist may preferably comprise from 0% to 100%, preferably from 20% to 100%, preferably from 30% to 100%, more preferably from 40% to 100%, even more preferably from 50% to 100%, yet more preferably from 60% to 100%, such as from 80% to 100% or even most preferably from 90% to 100% adjunct, unmalted grain and/or unmalted barley. In a particular embodiment the adjunct is composed of 100% unmalted barley. Furthermore, the grist preferably comprises from 0% to 100%, preferably from 20% to 100%, preferably from 30% to 100%, more preferably from 40% to 100%, even more preferably from 50% to 100%, yet more preferably from 60% to 100%, or most preferably from 70% to 100%, or even most preferably from 90% to 100% malted grain and/or malted barley. In a particular embodiment the grist comprises approximately 50% malted grain, *e.g.*, malted barley, and approximately 50% adjunct, *e.g.*, unmalted grain, such as unmalted barley.

**[0024]** Malted grain used in the process of the first aspect may comprise any malted grain, and preferably malted grain selected from malted barley, wheat, rye, sorghum, millet, corn, and rice, and most preferably malted barley.

**[0025]** The adjunct used in the process of the first aspect may be obtained from tubers, roots, stems, leaves, legumes, cereals and/or whole grain. The adjunct may comprise raw and/or refined starch and/or sugar containing material derived from plants like wheat, rye, oat, corn, rice, milo, millet, sorghum, potato, sweet potato, cassava, tapioca, sago, banana, sugar beet and/or sugar cane. Preferably, the adjunct comprises unmalted grain, *e.g.*, unmalted grain selected from the list consisting of barley, wheat, rye, sorghum, millet, corn, and rice, and most preferably unmalted barley. Adjunct comprising readily fermentable carbohydrates such as sugars or syrups may be added to the barley malt mash before, during or after mashing process of the invention but is preferably added after the mashing process.

**[0026]** According to the invention, a pullulanase (E.C. 3.2.1.41) enzyme having at least 98% identity to the amino acid sequence of SEQ ID NO. 4, is exogenously supplied and present in the mash. The pullulanase may be added to the mash ingredients, *e.g.*, the water and/or the grist before, during or after forming the mash. In a particularly preferred embodiment an alpha-amylase (E.C. 3.2.1.1) and/or a glucoamylase (E.C. 3.2.1.3), is added and present in the mash together with the pullulanase.

**[0027]** In another preferred embodiment, a further enzyme is added to the mash, said enzyme being selected from the group consisting of cellulose, isoamylase, protease, xylanase.

**[0028]** During the mashing process, starch extracted from the grist is gradually hydrolyzed into fermentable sugars and smaller dextrins. Preferably, the mash is starch negative to iodine testing, before extracting the wort.

**[0029]** The mashing process generally applies a controlled stepwise increase in temperature, where each step favors one enzymatic action over the other, eventually degrading proteins, cell walls and starch. Mashing temperature profiles are generally known in the art. In the present invention the saccharification (starch degradation) step in the mashing process is preferably performed between 60°C and 66°C, more preferably between 61°C and 65°C, even more preferably between 62°C and 64°C, and most preferably between 63°C and 64°C. In a particular embodiment of the present invention the saccharification temperature is 64°C.

**[0030]** Obtaining the wort from the mash typically includes straining the wort from the spent grains, *i.e.*, the insoluble grain and husk material forming part of grist. Hot water may be run through the spent grains to rinse out, or sparge, any remaining extract

from the grist. The application of a thermostable cellulase in the process of the present invention results in efficient reduction of beta-glucan level facilitating wort straining thus ensuring reduced cycle time and high extract recovery. Preferably the extract recovery is at least 80%, preferably at least 81%, more preferably at least 82%, even more preferably at least 83%, such as at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, and most preferably at least 91%.

**[0031]** Following the separation of the wort from the spent grains of the grist of any of the aforementioned embodiments of the first aspect, the wort may be used as it is or it may be dewatered to provide a concentrated and/or dried wort. The concentrated and/or dried wort may be used as brewing extract, as malt extract flavoring, for non-alcoholic malt beverages, malt vinegar, breakfast cereals, for confectionary etc.

**[0032]** In a preferred embodiment, the wort is fermented to produce an alcoholic beverage, preferably a beer, *e.g.*, ale, strong ale, bitter, stout, porter, lager, export beer, malt liquor, barley wine, happoushu, high-alcohol beer, low-alcohol beer, low-calorie beer or light beer. Fermentation of the wort may include pitching the wort with a yeast slurry comprising fresh yeast, *i.e.*, yeast not previously used for the invention or the yeast may be recycled yeast. The yeast applied may be any yeast suitable for beer brewing, especially yeasts selected from *Saccharomyces* spp. such as *S. cerevisiae* and *S. uvarum*, including natural or artificially produced variants of these organisms. The methods for fermentation of wort for production of beer are well known to the person skilled in the arts.

**[0033]** The process of the invention may include adding silica hydrogel to the fermented wort to increase the colloidal stability of the beer. The processes may further include adding kieselguhr to the fermented wort and filtering to render the beer bright.

**[0034]** Further disclosed is beer produced from the wort of the second or third aspect, such as a beer produced by fermenting the wort to produce a beer. The beer may be any type of beer, *e.g.*, ales, strong ales, stouts, porters, lagers, bitters, export beers, malt liquors, happoushu, high-alcohol beer, low-alcohol beer, low-calorie beer or light beer.

## Enzymes

**[0035]** The enzymes to be applied in the present invention should be selected for their ability to retain sufficient activity at the process temperature of the processes of the invention, as well as under the pH regime in the mash and should be added in effective amounts. The enzymes may be derived from any source, preferably from a plant or an alga, and more preferably from a microorganism, such as from a bacterium or a fungus.

### Pullulanase (E.C. 3.2.1.41)

**[0036]** A preferred pullulanase enzyme to be used in the processes and/or compositions of the invention is a pullulanase having an amino acid sequence which is at least 98% or even 100% identical to the sequence shown in SEQ ID NO: 4; in particular when aligned using the Program Needle using Matrix: BLOSUM62; Gap initiation penalty: 10.0; Gap extension penalty: 0.5; Gapless Identity Matrix.

**[0037]** Most preferably the pullulanase is derived from *Bacillus acidopullulyticus*. The pullulanase may have the amino acid sequence disclosed by Kelly et al., 1994 (FEMS Microbiol. Letters 115: 97-106) (SEQ ID NO: 6).

### Isoamylase (E.C. 3.2.1.68)

**[0038]** Another enzyme applied in the processes and/or compositions of the invention may be an alternative debranching enzyme, such as an isoamylase (E.C. 3.2.1.68). Isoamylase hydrolyzes alpha-1,6-D-glucosidic branch linkages in amylopectin and beta-limit dextrins and can be distinguished from pullulanases by the inability of isoamylase to attack pullulan, and by the limited action on alpha-limit dextrins. Isoamylase may be added in effective amounts well known to the person skilled in the art. Isoamylase may be added alone or together with a pullulanases.

### Alpha-amylase (EC 3.2.1.1)

[0039] A particular alpha-amylase enzyme to be used in the processes and/or compositions of the invention may be a *Bacillus* alpha-amylase. Well-known *Bacillus* alpha-amylases include alpha-amylase derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus*. In the context of the present invention, a contemplated *Bacillus* alpha-amylase is an alpha-amylase as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. A preferred alpha-amylase has an amino acid sequence having at least 90% identity to SEQ ID NO: 4 in WO 99/19467 (herein disclosed as SEQ ID NO: 7), such as at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99%. Most preferred maltogenic alpha-amylase is SEQ ID NO: 9 or comprise the variants thereof disclosed in WO 99/43794. Contemplated variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Specifically contemplated is an alpha-amylase (E.C. 3.2.1.1) from *B. stearothermophilus* having the amino acid sequence disclosed as SEQ ID NO: 3 in WO 99/19467 (herein disclosed as SEQ ID NO: 10) with the mutations: I181\* + G182\* + N193F.

[0040] *Bacillus* alpha-amylases may be added in the amounts of 1.0-1000 NU/kg DS, preferably from 2.0-500 NU/kg DS, preferably 10-200 NU/kg DS.

[0041] Another particular alpha-amylase to be used in the processes of the invention may be any fungal alpha-amylase, e.g., an alpha-amylase derived from a species within *Aspergillus*, and preferably from a strain of *Aspergillus niger*. Especially contemplated are fungal alpha-amylases which exhibit a high identity, i.e., at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85% or even at least 90% identity to the amino acid sequences shown SEQ ID NO: 1 in WO 2002/038787 (herein disclosed as SEQ ID NO: 11). Fungal alpha-amylases may be added in an amount of 1-1000 AFAU/kg DS, preferably from 2-500 AFAU/kg DS, preferably 20-100 AFAU/kg DS.

### **Glucoamylases (E.C.3.2.1.3)**

[0042] A further particular enzyme to be used in the processes and/or compositions of the invention may be a glucoamylase (E.C.3.2.1.3) derived from a microorganism or a plant. Preferred are glucoamylases of fungal or bacterial origin selected from the group consisting of *Aspergillus* glucoamylases, in particular *A. niger* G1 or G2 glucoamylase (Boel et al., 1984, EMBO J. 3(5): 1097-1102), or variants thereof, such as disclosed in WO 92/00381 and WO 00/04136; the *A. awamori* glucoamylase (WO 84/02921), *A. oryzae* (Agric. Biol. Chem., 1991, 55(4): 941-949), or variants or fragments thereof.

[0043] Other contemplated glucoamylases include *Talaromyces* glucoamylases, in particular derived from *Talaromyces emersonii* (WO 99/28448), *Talaromyces leycettanus* (U.S. Patent No. Re. 32,153), *Talaromyces duponti*, and *Talaromyces thermophilus* (U.S. Patent No. 4,587,215). Preferred glucoamylases include the glucoamylases derived from *Aspergillus oryzae*, such as a glucoamylase having at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% or even at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 in WO00/04136. Other preferred glucoamylases include the glucoamylases derived from *Talaromyces emersonii* such as a glucoamylase having at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% or even at least 90% identity to the amino acid sequence of *Talaromyces emersonii* (WO 99/28448).

[0044] Bacterial glucoamylases contemplated include glucoamylases from the genus *Clostridium*, in particular *C. thermoamylolyticum* (EP 135,138), and *C. thermohydrosulfuricum* (WO 86/01831).

[0045] Also contemplated are the commercial products AMG 200L; AMG 300 L; SAN™ SUPER and AMG™ E (from Novozymes); OPTIDEX™ 300 (from Genencor Int.); AMIGASE™ and AMIGASE™ PLUS (from DSM); G-ZYME™ G900 (from Enzyme Bio-Systems); G-ZYME™ G990 ZR (*A. niger* glucoamylase and low protease content). Glucoamylases may be added in effective amounts well known to the person skilled in the art.

### **Protease**

[0046] Suitable proteases include microbial proteases, such as fungal and bacterial proteases. Preferred proteases are acidic proteases, i.e., proteases characterized by the ability to hydrolyze proteins under acidic conditions below pH 7.

[0047] The proteases are responsible for reducing the overall length of high-molecular-weight proteins to low-molecular-weight proteins in the mash. The low-molecular-weight proteins are a necessity for yeast nutrition and the high-molecular-weight-proteins ensure foam stability. Thus it is well-known to the skilled person that protease should be added in a balanced amount which at the same time allows ample free amino acids for the yeast and leaves enough high-molecular-weight-proteins to stabilize the foam.

Proteases may be added in the amounts of 0.1-1000 AU/kg DS, preferably 1-100 AU/kg DS and most preferably 5-25 AU/kg DS.

## **Cellulase (E.C. 3.2.1.4)**

[0048] The cellulase may be of microbial origin, such as derivable from a strain of a filamentous fungus (e.g., *Aspergillus*, *Trichoderma*, *Humicola*, *Fusarium*). Specific examples of cellulases include the endoglucanase (endoglucanase I) obtainable from *H. insoles* and further defined by the amino acid sequence of fig. 14 in WO 91/17244 (herein disclosed as SEQ ID NO: 12) and the 43 kD *H. insolens* endoglucanase described in WO 91/17243.

[0049] A particular cellulase to be used in the processes of the invention may be an endo-glucanase, such as an endo-1,4-beta-glucanase. Especially contemplated is the beta-glucanase shown in SEQ.ID.NO: 2 in WO 2003/062409 (herein disclosed as SEQ ID NO: 14) and homologous sequences. Commercially available cellulase preparations which may be used include CELLUCLAST®, CELLUZYME®, CEREFLO® and ULTRAFLO® (available from Novozymes A/S), LAMINEX™ and SPEZYME® CP (available from Genencor Int.) and ROHAMENT® 7069 W (available from Röhm, Germany).

[0050] Beta-glucanases may be added in the amounts of 1.0-10000 BGU/kg DS, preferably from 10-5000 BGU/kg DS, preferably from 50-1000 BGU/kg DS and most preferably from 100-500 BGU/kg DS.

## **MATERIALS AND METHODS**

### **Enzymes**

[0051] Pullulanase 1 derived from *Bacillus acidopullulyticus* and having the sequence showed in SEQ ID NO: 1. Pullulanase 1 is available from Novozymes as Promozyme 400L.

[0052] Pullulanase 2 derived from *Bacillus deramificans* (U.S. Patent No. 5,736,375) and having the sequence showed in SEQ ID NO: 2. Pullulanase 2 is available from Novozymes as Promozyme D2.

[0053] Pullulanase 3 derived from *Bacillus acidopullulyticus* and having the sequence showed in SEQ ID NO: 4.

[0054] Acid fungal alpha-amylase derived from *Aspergillus niger* and having the sequence showed in SEQ ID NO: 11.

[0055] Glucoamylase G1 derived from *Aspergillus niger* (Boel et al., supra).

### **Methods**

#### **Alpha-amylase activity (NU)**

[0056] Alpha-amylase activity may be determined using potato starch as substrate. This method is based on the break-down of modified potato starch by the enzyme, and the reaction is followed by mixing samples of the starch/enzyme solution with an iodine solution. Initially, a blackish-blue color is formed, but during the break-down of the starch the blue color gets weaker and gradually turns into a reddish-brown, which is compared to a colored glass standard.

[0057] One Kilo Novo alpha amylase Unit (KNU) equals 1000 NU. One KNU is defined as the amount of enzyme which, under standard conditions (i.e., at 37°C +/- 0.05; 0.0003 M Ca<sup>2+</sup>; and pH 5.6) degrades 5.26 g starch dry matter (Merck Amylum soluble).

#### **Acid alpha-amylase activity (AFAU)**

**[0058]** Acid alpha-amylase activity may be measured in AFAU (Acid Fungal Alpha-amylase Units), which are determined relative to an enzyme standard. 1 FAU is defined as the amount of enzyme which degrades 5.260 mg starch dry matter per hour under the below mentioned standard conditions.

**[0059]** Acid alpha-amylase, an endo-alpha-amylase (1,4-alpha-D-glucan-glucanohydrolase, E.C. 3.2.1.1) hydrolyzes alpha-1,4-glucosidic bonds in the inner regions of the starch molecule to form dextrins and oligosaccharides with different chain lengths. The intensity of color formed with iodine is directly proportional to the concentration of starch. Amylase activity is determined using reverse colorimetry as a reduction in the concentration of starch under the specified analytical conditions.

**ALPHA - AMYLASE**



$\lambda = 590 \text{ nm}$

blue/violet  $t = 23 \text{ sec.}$  decoloration

**Standard conditions/reaction conditions:**

**[0060]**

|                           |                                  |
|---------------------------|----------------------------------|
| Substrate:                | Soluble starch, approx. 0.17 g/L |
| Buffer:                   | Citrate, approx. 0.03 M          |
| Iodine (I <sub>2</sub> ): | 0.03 g/L                         |
| CaCl <sub>2</sub> :       | 1.85 mM                          |
| pH:                       | 2.50 ± 0.05                      |
| Incubation temperature:   | 40°C                             |
| Reaction time:            | 23 seconds                       |
| Wavelength:               | 590 nm                           |
| Enzyme concentration:     | 0.025 AFAU/mL                    |
| Enzyme working range:     | 0.01-0.04 AFAU/mL                |

**Glucoamylase activity (AGU)**

**[0061]** The Novo Glucoamylase Unit (AGU) is defined as the amount of enzyme, which hydrolyzes 1 micromole maltose per minute at 37°C and pH 4.3.

**[0062]** The activity is determined as AGU/ml by a method modified after (AEL-SM-0131, available on request from Novozymes) using the Glucose GOD-Perid kit from Boehringer Mannheim, 124036. Standard: AMG-standard, batch 7-1195, 195 AGU/ml. 375 microL substrate (1% maltose in 50 mM Sodium acetate, pH 4.3) is incubated 5 minutes at 37°C. 25 microL enzyme diluted in sodium acetate is added. The reaction is stopped after 10 minutes by adding 100 microL 0.25 M NaOH. 20 microL is transferred to a 96 well microtitre plate and 200 microL GOD-Perid solution (124036, Boehringer Mannheim) is added. After 30 minutes at room temperature, the absorbance is measured at 650 nm and the activity calculated in AGU/ml from the AMG-standard. A detailed description of the analytical method (AEL-SM-0131) is available on request from Novozymes.

**Pullulanase activity (PUN):**

**[0063]** One pullulanase unit (PUN) is defined as the amount of enzyme, which is capable of forming 1 micromole glucose from pullulan substrate per minute at 50°C in a pH 5 citrate buffer.

**[0064]** Pullulanase samples are incubated with substrate (red pullulan). Endo-pullulanase hydrolyses the alpha-1,6-glycosidic bonds in red pullulan, releasing red substrate degradation products. Non-degraded substrate is precipitated using ethanol. The amount of color released is measured spectrophotometrically at 510 nm and is proportional to the endo-pullulanase activity in the sample. The color formation of samples is compared to the color formation produced by samples with known pullulanase activity.

[0065] Pullulanase is a pullulan 6-glucano-hydrolase with the enzyme classification number E.C.3.2.1.41.

**Reaction conditions**

[0066]

|                         |                     |
|-------------------------|---------------------|
| Temperature             | 50°C ± 2°C          |
| pH                      | 5.0                 |
| Substrate concentration | 0.67 % red pullulan |
| Enzyme concentration    | 0.04 - 0.13 PUN/ml  |
| Reaction time           | 30 min.             |
| Wavelength              | 510 nm              |

**Reagents/ Substrates**

[0067]

Potassium chloride solution 0.5 M

Red pullulan substrate 2%. Supplier Megazyme, Australia

Citrate buffer 0.05 M pH 5.0

Citrate buffer 0.05 M pH 5.0 with 25 mM cysteine

Ethanol 99.8%

Pullulanase Standard preparation of 904 PUN/g diluted into citrate buffer 0.05 M to a standard dilution series from 0.05 - 0.20 PUN/ml

Blank Citrate buffer 0.05 M

Enzyme samples are diluted in citrate buffer 0.05 M to an activity between 0.06 - 0.20 PUN/ml and compared to the standard dilution series.

**Example 1**

[0068] In this example, the ability of different pullulanases to reduce the amount of non-fermentable carbohydrates (dextrin/DP4/4+) in a wort was analyzed.

[0069] 100% well modified malt was mashed using a mashing temperature profile comprising 46°C for 26 minutes, followed by a 1°C/minute increase till 64°C after which the temperature was held constant. Samples were collected at 98, 128 and 158 minutes.

[0070] Enzymes were added at 0 minutes. Glucoamylase and alpha-amylase were added to all treatments in amounts of 1000 AGU/kg DS and 250 AFAU/kg DS respectively. Pullulanase was added according to table 1.

[0071] The samples were boiled 10 minutes and filtered (Pore size 0.20 micro-m). The samples were analyzed by HPLC and % non fermentable carbohydrate (DP4/4+) was calculated.

| Table 1: % non fermentable carbohydrate after mashing times of 98, 128 and 158 minutes. |                    |              |             |             |
|-----------------------------------------------------------------------------------------|--------------------|--------------|-------------|-------------|
| Type of pullulanase                                                                     | Amount in mg/kg DS | Mashing time |             |             |
|                                                                                         |                    | 98 minutes   | 128 minutes | 158 minutes |
| None                                                                                    | 0                  | 28.87        | 25.16       | 22.94       |
| Pullulanase 1                                                                           | 0.74               | 27.64        | 24.55       | 21.96       |
| Pullulanase 1                                                                           | 3.65               | 23.96        | 21.35       | 18.92       |
| Pullulanase 1                                                                           | 7.25               | 20.71        | 17.74       | 16.11       |
| Pullulanase 1                                                                           | 14.39              | 16.36        | 14.47       | 13.22       |
| Pullulanase 2                                                                           | 1.86               | 28.34        | 25.21       | 22.79       |
| Pullulanase 2                                                                           | 9.26               | 26.81        | 23.28       | 21.36       |
| Pullulanase 2                                                                           | 18.40              | 25.68        | 22.33       | 20.17       |
| Pullulanase 2                                                                           | 36.59              | 23.56        | 20.58       | 18.35       |
| Pullulanase 3                                                                           | 1.37               | 27.11        | 23.38       | 20.51       |
| Pullulanase 3                                                                           | 2.74               | 24.46        | 20.93       | 18.18       |

[0072] The data in table 1 was used to calculate, by regression, the enzyme dosages of pullulanase 1 and pullulanase 2 needed to get the same effect as 2.74 mg enzyme protein/kg of pullulanase 3 (see table 2).

| Table 2. Pullulanase enzyme protein mg/kg DS needed to achieve the same effect as with 2.74 mg Pullulanase 3 and mashing times of 98, 128 and 158 minutes. |               |               |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|---------------|
| Mashing time                                                                                                                                               | Pullulanase 1 | Pullulanase 2 |
| 98 minutes                                                                                                                                                 | 3.38          | 27.33         |
| 128 minutes                                                                                                                                                | 4.04          | 31.06         |
| 158 minutes                                                                                                                                                | 4.56          | 40.37         |

[0073] From these results it can be seen that Pullulanase 3 is the most efficient enzyme. Consequently, less Pullulanase 3 enzyme protein is needed to reduce the amount of non-fermentable carbohydrates (dextrin/DP4/4+) and thereby increase attenuation of the wort.

## Example 2

[0074] The pH profile and temperature profile of different pullulanases were analyzed in the present example.

[0075] The pH and temperature profile investigations were based on relative enzyme activity analysis with the conditions described below.

[0076] Principals of the analytical method:

The alpha-1,6-glycosidic bonds in pullulan were hydrolyzed by a pullulanase enzyme and the increased reducing sugar capacity was detected by a modified Somogyi-Nelson method.

[0077] In the present experiment the activity is assessed as relative activity, where the most active sample is given as 100%. The assay conditions are as follows:

Buffer: citrate 0.1 M + 0.2 M phosphate (adjusted in the pH profile, pH 5 in temperature profile)

Substrate: 0.2% pullulan Sigma (p-4516)

Temperature: 60°C in pH profile, adjusted in temperature profile

Reaction time: 30 minutes

**[0078]** The reducing sugars released by pullulanases were detected according to the principle described in Nelson, 1944, J. Biol. Chem. 153: 375-380 and Somogyi, 1945, J. Biol. Chem. 160: 61-68. In brief, the hydrolysis reaction is stopped by adding Somogyi's cobber reagent in a volume corresponding to the sample volume (e.g., 2 ml to a sample of 2 ml). The samples are boiled for 20 minutes and cooled down prior to the color reaction. This reaction is performed by adding Nelson's reagent corresponding to ½ the volume of the sample (e.g., 2 ml to 4 ml sample+ Somogyi's cobber reagent). The samples are mixed for 2 minutes followed by addition of water in the same amount as Nelson's reagent. The samples are incubated 30 minutes in the dark and measured in a spectrophotometer at 540 nm.

**[0079]** Reagents can be prepared as follows:

Somogyi's cobber reagent:

Dissolve 70.2 g  $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$  and 80.0 g  $\text{KNaC}_4\text{H}_4\text{O}_6 \times 4\text{H}_2\text{O}$  (kaliumsodiumtartrat) in 1000 ml  $\text{H}_2\text{O}$  (heat slightly). Furthermore add 60 g NaOH; 16.0 g  $\text{CuSO}_4 \times 5\text{H}_2\text{O}$  and 360.0 g  $\text{Na}_2\text{SO}_4$  and fill to 2000 ml. Adjust pH to 10.8 with NaOH

Nelson's reagent:

Dissolve 100.0 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \times 7\text{H}_2\text{O}$  in 1200 ml  $\text{H}_2\text{O}$ . Add 84.0 ml  $\text{H}_2\text{SO}_4$  carefully. Additionally, dissolve 12.00 g  $\text{Na}_2\text{HAsO}_4 \times 7\text{H}_2\text{O}$  (disodiumhydrogenarsenate) in 100 ml  $\text{H}_2\text{O}$ , and add this solution slowly to the first solution and fill to 2000 ml.

**[0080]** The pH and temperature profiles for the three pullulanases are given in table 3 and 4 below.

| Table 3: pH profile given in relative (%) enzyme activity at 60°C            |               |               |               |
|------------------------------------------------------------------------------|---------------|---------------|---------------|
| pH                                                                           | Pullulanase 1 | Pullulanase 2 | Pullulanase 3 |
| 2.4                                                                          | 2.2           | 1             | 0.2           |
| 2.8                                                                          | 6             | 2.4           | 30.7          |
| 3.7                                                                          | 10.8          | 68.5          | 90            |
| 4.2                                                                          | 23.1          | 100           | 100           |
| 5                                                                            | 94            | 77.4          | 92.4          |
| 5.8                                                                          | 92.8          | 31.7          | 74.2          |
| 6.3                                                                          | 43.1          | 4.4           | 45.3          |
| 6.7                                                                          | 3.7           | 0             | 1.8           |
| 7.3                                                                          | 0             | 0             | 0             |
| Table 4: Temperature profile given in relative (%) enzyme activity at pH 5.0 |               |               |               |
| Temp. °C                                                                     | Pullulanase 1 | Pullulanase 2 | Pullulanase 3 |
| 30                                                                           | 22.8          | 36.7          | 19.7          |
| 45                                                                           | 64.4          | 72.5          | 47.3          |
| 55                                                                           | 100           | 100           | 76.8          |
| 60                                                                           | 99.6          | 85.2          | 92.6          |
| 62.5                                                                         | 87.1          | 70            | 101.2         |
| 65                                                                           | 42.1          | 54.4          | 100           |
| 70                                                                           | 9.4           | 12.7          | 75.6          |

**[0081]** These results show that pullulanase 3 has a broad pH profile and activity at high temperatures when compared to the other two pullulanases. These properties make pullulanase 3 a very robust enzyme in brewing (mashing conditions), in particular for saccharification temperatures between 62°C and 65°C.

**Example 3**

[0082] Infusion mashing test were made with Pullulanase 1 and 3 (SEQ ID NOS: 1 and 4). 6 mashing samples were prepared with 100% barley as substrate (grist).

[0083] Barley (DS%: 86.73) was milled and for each sample 50.0 g (total DS 43.34 g) were mixed with 200 g tap water at 50°C and 3.0 ml 1 M H<sub>3</sub>PO<sub>4</sub>.

[0084] All samples were added an identical enzyme mix with no pullulanase.

[0085] Samples 1-6 were then added pullulanase according to the following table:

Enzyme dose Activity/g

| Sample no. | Pullulanase 1 PUN/g | Pullulanase 3 PUN/g |
|------------|---------------------|---------------------|
| 1          | 0.1                 | -                   |
| 2          | 0.2                 | -                   |
| 3          | 0.5                 | -                   |
| 4          | -                   | 0.1                 |
| 5          | -                   | 0.2                 |
| 6          | -                   | 0.5                 |

[0086] The samples were then tested in automated mashing equipment running the following program.

| Time in minutes | Temp. °C      |
|-----------------|---------------|
| 0-30            | 50            |
| 30-44           | rising to 64  |
| 44-104          | 64            |
| 104-120         | rising to 80  |
| 120-140         | 80            |
| 140-155         | falling to 20 |

[0087] After mashing all samples were added tap water to a total of 300 g and filtered. The filtered samples were then boiled for 10 minutes and diluted 1:1 with deionised water. Subsamples of 50 ml were centrifuged and subjected to standard density analysis for calculation of RDF % (real degree of fermentation). The results are given as RDF % and wort sugar (DP2 and DP4+) in %.

| PUN/g | Pullulanase 1 |      | Pullulanase 3 |      |
|-------|---------------|------|---------------|------|
| 0     | 0             |      | 0             |      |
| 0.1   | 64            |      | 64.6          |      |
| 0.2   | 65            |      | 66.1          |      |
| 0.5   | 67.3          |      | 69.5          |      |
|       | Pullulanase 1 |      | Pullulanase 3 |      |
| PUN/g | DP2           | DP4+ | DP2           | DP4+ |
| 0     | 48.5          | 31.5 | 48.5          | 31.5 |
| 0.1   | 48.5          | 30   | 49            | 29   |
| 0.2   | 49.5          | 29   | 50            | 27.5 |
| 0.5   | 50.5          | 36.5 | n.d.          | n.d. |

[0088] The results show that pullulanase 3 is clearly better than pullulanase 1 for making a high % RDF, and still pullulanase 3 is

capable of producing maltose levels (DP2 levels) even better than pullulanase 1.

#### Example 4

[0089] Infusion mashing test were made with Pullulanase 3 (SEQ ID NO: 4) to evaluate the maltose generating properties of pullulanase 3. 6 mashing samples were prepared with 100% barley as substrate (grist).

[0090] Barley (DS%: 86.73) was milled and for each sample 50.0 g (total DS 43.34 g) were mixed with 200 g tap water at 50°C and 5% Na<sub>2</sub>SO<sub>3</sub> and 1 M H<sub>3</sub>PO<sub>4</sub>

[0091] All samples were added an identical enzyme mix with no pullulanase.

[0092] Samples 1-6 were then added pullulanase 3 according to the following table:

| Sample no. | Pullulanase 3 PUN/g |
|------------|---------------------|
| 1          | -                   |
| 2          | 0.1                 |
| 3          | 0.3                 |
| 4          | 0.5                 |
| 5          | 1.0                 |
| 6          | 2.0                 |

[0093] The samples were then tested in automated mashing equipment running the following program.

| Time in minutes | Temp. °C      |
|-----------------|---------------|
| 0-30            | 50            |
| 30-44           | rising to 64  |
| 44-104          | 64            |
| 104-120         | rising to 80  |
| 120-140         | 80            |
| 140-155         | falling to 20 |

[0094] After mashing all samples were added tap water to a total of 300 g and filtered. The filtered samples were then boiled for 10 minutes and diluted 1:1 with deionized water. Subsamples of 50 ml were centrifuged and subjected to analysis. The results were as follows:

| PUN/g | % glucose | % maltose | % dextrin | RDF % |
|-------|-----------|-----------|-----------|-------|
| 0     | 3.8       | 47.5      | 34        | 61.2  |
| 0.1   | 3.8       | 48.2      | 32        | 63.2  |
| 0.3   | 3.8       | 49.8      | 28.8      | 65.7  |
| 0.5   | 3.7       | 51.2      | 26.4      | 68.6  |
| 1     | 3.7       | 52.6      | 23.9      | 71    |
| 2     | 3.6       | 55.6      | 20.1      | 74.3  |

[0095] The results show that maltose concentration is increasing with increasing dosage of pullulanase 3, and the increase in maltose % is followed by the increase in attenuation (RDF%). The dextrin fraction (HPLC analysis DP4/4+) is at the same time decreasing.

[0096] Only barley beta-amylase can produce maltose in this reaction, and pullulanase 3 is facilitating the action of barley beta-amylase.

[0097] Glucose concentration is low and not effected by the action of pullulanase 3 which is an advantage when fermenting the produced wort. Pullulanase 3 help degrading dextrin and facilitate formation of maltose, by the barley beta-amylase, and by this increase attenuation (RDF%) of the wort.

## SEQUENCE LISTING

### [0098]

<110> Novozymes A/S

Novozymes NA, Inc.

Elvig, Niels

Joergensen, Per L.

Thomas, Michael

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 Ala Asn Glu Ile Asn Ser His Leu Gln Phe Leu Asn Ser Pro Glu Asn  
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 Thr Val Ala Tyr Glu Leu Thr Asp His Val Asn Lys Asp Lys Trp Gly  
 850 855 860  
 Asn Ile Ile Val Val Tyr Asn Pro Asn Lys Thr Val Ala Thr Ile Asn  
 865 870 875 880  
 Leu Pro Ser Gly Lys Trp Ala Ile Asn Ala Thr Ser Gly Lys Val Gly  
 885 890 895  
 Glu Ser Thr Leu Gly Gln Ala Glu Gly Ser Val Gln Val Pro Gly Ile  
 900 905 910  
 Ser Met Met Ile Leu His Gln Glu Val Ser Pro Asp His Gly Lys Lys  
 915 920 925

&lt;210&gt; 3

&lt;211&gt; 2589

&lt;212&gt; DNA

&lt;213&gt; Bacillus acidopullulyticus

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; (1)..(99)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(2586)

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (100)..(2589)

&lt;400&gt; 3

|                                                                 |      |
|-----------------------------------------------------------------|------|
| atg tcc cta ata cgt tct agg tat aat cat ttt gtc att ctt ttt act | 48   |
| Met Ser Leu Ile Arg Ser Arg Tyr Asn His Phe Val Ile Leu Phe Thr |      |
| -30 -25 -20                                                     |      |
| gtc gcc ata atg ttt cta aca gtt tgt ttc ccc gct tat aaa gct tta | 96   |
| Val Ala Ile Met Phe Leu Thr Val Cys Phe Pro Ala Tyr Lys Ala Leu |      |
| -15 -10 -5                                                      |      |
| gca gat tct acc tcg aca gaa gtc att gtg cat tat cat cgt ttt gat | 144  |
| Ala Asp Ser Thr Ser Thr Glu Val Ile Val His Tyr His Arg Phe Asp |      |
| -1 1 5 10 15                                                    |      |
| tct aac tat gca aat tgg gat cta tgg atg tgg cca tat caa cca gtt | 192  |
| Ser Asn Tyr Ala Asn Trp Asp Leu Trp Met Trp Pro Tyr Gln Pro Val |      |
| 20 25 30                                                        |      |
| aat ggt aat gga gca gca tac gag ttt tct gga aag gat gat ttt ggc | 240  |
| Asn Gly Asn Gly Ala Ala Tyr Glu Phe Ser Gly Lys Asp Asp Phe Gly |      |
| 35 40 45                                                        |      |
| gtt aaa gca gat gtt caa gtg cct ggg gat gat aca cag gta ggt ctg | 288  |
| Val Lys Ala Asp Val Gln Val Pro Gly Asp Asp Thr Gln Val Gly Leu |      |
| 50 55 60                                                        |      |
| att gtc cgt aca aat gat tgg agc caa aaa aat aca tca gac gat ctc | 336  |
| Ile Val Arg Thr Asn Asp Trp Ser Gln Lys Asn Thr Ser Asp Asp Leu |      |
| 65 70 75                                                        |      |
| cat att gat ctg aca aag ggg cat gaa ata tgg att gtt cag ggg gat | 384  |
| His Ile Asp Leu Thr Lys Gly His Glu Ile Trp Ile Val Gln Gly Asp |      |
| 80 85 90 95                                                     |      |
| ccc aat att tat tac aat ctg agt gat gcg cag gct gca gcg act cca | 432  |
| Pro Asn Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Thr Pro     |      |
| 100 105 110                                                     |      |
| aag gtt tcg aat gcg tat ttg gat aat gaa aaa aca gta ttg gca aag | 480  |
| Lys Val Ser Asn Ala Tyr Leu Asp Asn Glu Lys Thr Val Leu Ala Lys |      |
| 115 120 125                                                     |      |
| cta act aat cca atg aca tta tca gat gga tca agc ggc ttt acg gtt | 528  |
| Leu Thr Asn Pro Met Thr Leu Ser Asp Gly Ser Ser Gly Phe Thr Val |      |
| 130 135 140                                                     |      |
| aca gat aaa aca aca ggg gaa caa att cca gtt acc gct gca aca aat | 576  |
| Thr Asp Lys Thr Thr Gly Glu Gln Ile Pro Val Thr Ala Ala Thr Asn |      |
| 145 150 155                                                     |      |
| gcg aac tca gcc tcc tcg tct gag cag aca gac ttg gtt caa ttg acg | 624  |
| Ala Asn Ser Ala Ser Ser Ser Glu Gln Thr Asp Leu Val Gln Leu Thr |      |
| 160 165 170 175                                                 |      |
| tta gcc agt gca ccg gat gtt tcc cat aca ata caa gta gga gca gcc | 672  |
| Leu Ala Ser Ala Pro Asp Val Ser His Thr Ile Gln Val Gly Ala Ala |      |
| 180 185 190                                                     |      |
| ggt tat gaa gca gtc aat ctc ata cca cga aat gta tta aat ttg cct | 720  |
| Gly Tyr Glu Ala Val Asn Leu Ile Pro Arg Asn Val Leu Asn Leu Pro |      |
| 195 200 205                                                     |      |
| cgt tat tat tac agc gga aat gat tta ggt aac gtt tat tca aat aag | 768  |
| Arg Tyr Tyr Tyr Ser Gly Asn Asp Leu Gly Asn Val Tyr Ser Asn Lys |      |
| 210 215 220                                                     |      |
| gca acg gcc ttc cgt gta tgg gct cca act gct tcg gat gtc caa tta | 816  |
| Ala Thr Ala Phe Arg Val Trp Ala Pro Thr Ala Ser Asp Val Gln Leu |      |
| 225 230 235                                                     |      |
| ctt tta tac aat agt gaa aca gga cct gta acc aaa cag ctt gaa atg | 864  |
| Leu Leu Tyr Asn Ser Glu Thr Gly Pro Val Thr Lys Gln Leu Glu Met |      |
| 240 245 250 255                                                 |      |
| caa aag agt gat aac ggt aca tgg aaa ctg aag gtc cct ggt aat ctg | 912  |
| Gln Lys Ser Asp Asn Gly Thr Trp Lys Leu Lys Val Pro Gly Asn Leu |      |
| 260 265 270                                                     |      |
| aaa aat tgg tat tat ctc tat cag gta acg gtg aat ggg aag aca caa | 960  |
| Lys Asn Trp Tyr Tyr Leu Tyr Gln Val Thr Val Asn Gly Lys Thr Gln |      |
| 275 280 285                                                     |      |
| aca gcc gtt gac cct tat gtg cgt gct att tca gtc aat gca aca cgt | 1008 |
| Thr Ala Val Asp Pro Tyr Val Arg Ala Ile Ser Val Asn Ala Thr Arg |      |
| 290 295 300                                                     |      |
| ggt atg ata gtc gat tta gaa gat acg aat cct cct gga tgg aaa gaa | 1056 |
| Gly Met Ile Val Asp Leu Glu Asp Thr Asn Pro Pro Gly Trp Lys Glu |      |
| 305 310 315                                                     |      |

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| gat cat caa cag aca cct gcg aac cca gtg gat gaa gta atc tac gaa<br>Asp His Gln Gln Thr Pro Ala Asn Pro Val Asp Glu Val Ile Tyr Glu<br>320 325 330 335 | 1104 |
| gtg cat gtg cgt gat ttt tcg att gat gct aat tca ggc atg aaa aat<br>Val His Val Arg Asp Phe Ser Ile Asp Ala Asn Ser Gly Met Lys Asn<br>340 345 350     | 1152 |
| aaa ggg aaa tat ctt gcc ttt aca gaa cat ggc aca aaa ggc cct gat<br>Lys Gly Lys Tyr Leu Ala Phe Thr Glu His Gly Thr Lys Gly Pro Asp<br>355 360 365     | 1200 |
| aac gtg aaa acg ggt att gat agt ttg aag gaa tta gga atc aat gct<br>Asn Val Lys Thr Gly Ile Asp Ser Leu Lys Glu Leu Gly Ile Asn Ala<br>370 375 380     | 1248 |
| gtt caa tta cag ccg att gaa gaa ttt aac agc att gat gaa acc caa<br>Val Gln Leu Gln Pro Ile Glu Glu Phe Asn Ser Ile Asp Glu Thr Gln<br>385 390 395     | 1296 |
| cca aat atg tat aac tgg ggc tat gac cca aga aac tac aac gtc cct<br>Pro Asn Met Tyr Asn Trp Gly Tyr Asp Pro Arg Asn Tyr Asn Val Pro<br>400 405 410 415 | 1344 |
| gaa gga gcg tat gca act aca cca gaa gga acg gct cgc att acc cag<br>Glu Gly Ala Tyr Leu Ala Thr Thr Pro Glu Gly Thr Ala Arg Ile Thr Gln<br>420 425 430 | 1392 |
| tta aag caa ctg att caa agc att cat aaa gat cgg att gct atc aat<br>Leu Lys Gln Leu Ile Gln Ser Ile His Lys Asp Arg Ile Ala Ile Asn<br>435 440 445     | 1440 |
| atg gat gtg gtc tat aac cat acc ttt aac gta gga gtg tct gat ttt<br>Met Asp Val Val Tyr Asn His Thr Phe Asn Val Gly Val Ser Asp Phe<br>450 455 460     | 1488 |
| gat aag att gtt ccg caa tac tat tat cgg aca gac agc gca ggt aat<br>Asp Lys Ile Val Pro Gln Tyr Tyr Tyr Arg Thr Asp Ser Ala Gly Asn<br>465 470 475     | 1536 |
| tat acg aac ggc tca ggt gta ggt aat gaa att gcg acc gag cgt ccg<br>Tyr Thr Asn Gly Ser Gly Val Gly Asn Glu Ile Ala Thr Glu Arg Pro<br>480 485 490 495 | 1584 |
| atg gtc caa aag ttc gtt ctg gat tct gtt aaa tat tgg gta aag gaa<br>Met Val Gln Lys Phe Val Leu Asp Ser Val Lys Tyr Trp Val Lys Glu<br>500 505 510     | 1632 |
| tac cat atc gac ggc ttc cgt ttc gat ctt atg gct ctt tta gga aaa<br>Tyr His Ile Asp Gly Phe Arg Phe Asp Leu Met Ala Leu Leu Gly Lys<br>515 520 525     | 1680 |
| gac acc atg gcc aaa ata tca aaa gag ctt cat gct att aat cct ggc<br>Asp Thr Met Ala Lys Ile Ser Lys Glu Leu His Ala Ile Asn Pro Gly<br>530 535 540     | 1728 |
| att gtc ctg tat gga gaa cca tgg act ggc ggt acc tct gga tta tca<br>Ile Val Leu Tyr Gly Glu Pro Trp Thr Gly Gly Thr Ser Gly Leu Ser<br>545 550 555     | 1776 |

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agc gac caa ctc gtt acg aaa ggt cag caa aag ggc ttg gga att ggc      1824
Ser Asp Gln Leu Val Thr Lys Gly Gln Gln Lys Gly Leu Gly Ile Gly
560                      565                      570                      575

gta ttc aac gat aat att cgg aac gga ctc gat ggt aac gtt ttt gat      1872
Val Phe Asn Asp Asn Ile Arg Asn Gly Leu Asp Gly Asn Val Phe Asp
580                      585                      590

aaa tcg gca caa gga ttt gca aca gga gat cca aac caa gtt aat gtc      1920
Lys Ser Ala Gln Gly Phe Ala Thr Gly Asp Pro Asn Gln Val Asn Val
595                      600                      605

att aaa aat gga gtt atg gga agt att tca gat ttc act tcg gca cct      1968
Ile Lys Asn Gly Val Met Gly Ser Ile Ser Asp Phe Thr Ser Ala Pro
610                      615                      620

agc gaa acc att aac tat gta aca agc cat gat aat atg aca ttg tgg      2016
Ser Glu Thr Ile Asn Tyr Val Thr Ser His Asp Asn Met Thr Leu Trp
625                      630                      635

gat aaa att agc gca agt aat ccg aac gat aca caa gca gat cga att      2064
Asp Lys Ile Ser Ala Ser Asn Pro Asn Asp Thr Gln Ala Asp Arg Ile
640                      645                      650                      655

aag atg gat gaa ttg gct caa gct gtg gta ttt act tca caa ggg gta      2112
Lys Met Asp Glu Leu Ala Gln Ala Val Val Phe Thr Ser Gln Gly Val
660                      665                      670

cca ttt atg caa ggt gga gaa gaa atg ctg cgg aca aaa ggc ggt aat      2160
Pro Phe Met Gln Gly Gly Glu Glu Met Leu Arg Thr Lys Gly Gly Asn
675                      680                      685

gat aat agt tac aat gcc ggg gat agc gtg aat cag ttc gat tgg tca      2208
Asp Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn Gln Phe Asp Trp Ser
690                      695                      700

aga aaa gca caa ttt gaa aat gta ttc gac tac tat tct tgg ttg att      2256
Arg Lys Ala Gln Phe Glu Asn Val Phe Asp Tyr Thr Ser Trp Leu Ile
705                      710                      715

cat cta cgt gat aat cac cca gca ttc cgt atg acg aca gcg gat caa      2304
His Leu Arg Asp Asn His Pro Ala Phe Arg Met Thr Thr Ala Asp Gln
720                      725                      730                      735

atc aaa caa aat ctc act ttc ttg gat agc cca acg aac act gta gca      2352
Ile Lys Gln Asn Leu Thr Phe Leu Asp Ser Pro Thr Asn Thr Val Ala
740                      745                      750

ttt gaa tta aaa aat cat gcc aat cat gat aaa tgg aaa aac att ata      2400
Phe Glu Leu Lys Asn His Ala Asn His Asp Lys Trp Lys Asn Ile Ile
755                      760                      765

gtt atg tat aat cca aat aaa act gca caa act ctc act cta cca agt      2448
Val Met Tyr Asn Pro Asn Lys Thr Ala Gln Thr Leu Thr Leu Pro Ser
770                      775                      780

gga aat tgg aca att gta gga tta ggc aat caa gta ggt gag aaa tca      2496
Gly Asn Trp Thr Ile Val Gly Leu Gly Asn Gln Val Gly Glu Lys Ser
785                      790                      795

cta ggc cat gta aat ggc acg gtt gag gtg cca gct ctt agt acg atc      2544

Leu Gly His Val Asn Gly Thr Val Glu Val Pro Ala Leu Ser Thr Ile
800                      805                      810                      815

att ctt cat cag ggt aca tct gaa gat gtc att gat caa aat taa      2589
Ile Leu His Gln Gly Thr Ser Glu Asp Val Ile Asp Gln Asn
820                      825

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&lt;210&gt; 4

&lt;211&gt; 862

&lt;212&gt; PRT

&lt;213&gt; Bacillus acidopullulyticus

&lt;400&gt; 4

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

Ala Asn Ser Ala Ser Ser Ser Glu Gln Thr Asp Leu Val Gln Leu Thr  
 160 165 170 175  
 Leu Ala Ser Ala Pro Asp Val Ser His Thr Ile Gln Val Gly Ala Ala  
 180 185 190  
 Gly Tyr Glu Ala Val Asn Leu Ile Pro Arg Asn Val Leu Asn Leu Pro  
 195 200 205  
 Arg Tyr Tyr Tyr Ser Gly Asn Asp Leu Gly Asn Val Tyr Ser Asn Lys  
 210 215 220  
 Ala Thr Ala Phe Arg Val Trp Ala Pro Thr Ala Ser Asp Val Gln Leu  
 225 230 235  
 Leu Leu Tyr Asn Ser Glu Thr Gly Pro Val Thr Lys Gln Leu Glu Met  
 240 245 250 255  
 Gln Lys Ser Asp Asn Gly Thr Trp Lys Leu Lys Val Pro Gly Asn Leu  
 260 265 270  
 Lys Asn Trp Tyr Tyr Leu Tyr Gln Val Thr Val Asn Gly Lys Thr Gln  
 275 280 285  
 Thr Ala Val Asp Pro Tyr Val Arg Ala Ile Ser Val Asn Ala Thr Arg  
 290 295 300  
 Gly Met Ile Val Asp Leu Glu Asp Thr Asn Pro Pro Gly Trp Lys Glu  
 305 310 315  
 Asp His Gln Gln Thr Pro Ala Asn Pro Val Asp Glu Val Ile Tyr Glu  
 320 325 330 335  
 Val His Val Arg Asp Phe Ser Ile Asp Ala Asn Ser Gly Met Lys Asn  
 340 345 350  
 Lys Gly Lys Tyr Leu Ala Phe Thr Glu His Gly Thr Lys Gly Pro Asp  
 355 360 365  
 Asn Val Lys Thr Gly Ile Asp Ser Leu Lys Glu Leu Gly Ile Asn Ala  
 370 375 380  
 Val Gln Leu Gln Pro Ile Glu Glu Phe Asn Ser Ile Asp Glu Thr Gln  
 385 390 395

Pro Asn Met Tyr Asn Trp Gly Tyr Asp Pro Arg Asn Tyr Asn Val Pro  
 400 405 410 415  
 Glu Gly Ala Tyr Ala Thr Thr Pro Glu Gly Thr Ala Arg Ile Thr Gln  
 420 425 430  
 Leu Lys Gln Leu Ile Gln Ser Ile His Lys Asp Arg Ile Ala Ile Asn  
 435 440 445  
 Met Asp Val Val Tyr Asn His Thr Phe Asn Val Gly Val Ser Asp Phe  
 450 455 460  
 Asp Lys Ile Val Pro Gln Tyr Tyr Tyr Arg Thr Asp Ser Ala Gly Asn  
 465 470 475  
 Tyr Thr Asn Gly Ser Gly Val Gly Asn Glu Ile Ala Thr Glu Arg Pro  
 480 485 490 495  
 Met Val Gln Lys Phe Val Leu Asp Ser Val Lys Tyr Trp Val Lys Glu  
 500 505 510  
 Tyr His Ile Asp Gly Phe Arg Phe Asp Leu Met Ala Leu Leu Gly Lys  
 515 520 525  
 Asp Thr Met Ala Lys Ile Ser Lys Glu Leu His Ala Ile Asn Pro Gly  
 530 535 540  
 Ile Val Leu Tyr Gly Glu Pro Trp Thr Gly Gly Thr Ser Gly Leu Ser  
 545 550 555  
 Ser Asp Gln Leu Val Thr Lys Gly Gln Gln Lys Gly Leu Gly Ile Gly  
 560 565 570 575  
 Val Phe Asn Asp Asn Ile Arg Asn Gly Leu Asp Gly Asn Val Phe Asp  
 580 585 590  
 Lys Ser Ala Gln Gly Phe Ala Thr Gly Asp Pro Asn Gln Val Asn Val  
 595 600 605  
 Ile Lys Asn Gly Val Met Gly Ser Ile Ser Asp Phe Thr Ser Ala Pro  
 610 615 620  
 Ser Glu Thr Ile Asn Tyr Val Thr Ser His Asp Asn Met Thr Leu Trp  
 625 630 635

Asp Lys Ile Ser Ala Ser Asn Pro Asn Asp Thr Gln Ala Asp Arg Ile  
 640 645 650 655  
 Lys Met Asp Glu Leu Ala Gln Ala Val Val Phe Thr Ser Gln Gly Val  
 660 665  
 Pro Phe Met Gln Gly Gly Glu Glu Met Leu Arg Thr Lys Gly Gly Asn  
 675 680 685  
 Asp Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn Gln Phe Asp Trp Ser  
 690 695 700  
 Arg Lys Ala Gln Phe Glu Asn Val Phe Asp Tyr Tyr Ser Trp Leu Ile  
 705 710 715  
 His Leu Arg Asp Asn His Pro Ala Phe Arg Met Thr Thr Ala Asp Gln  
 720 725 730 735  
 Ile Lys Gln Asn Leu Thr Phe Leu Asp Ser Pro Thr Asn Thr Val Ala  
 740 745 750  
 Phe Glu Leu Lys Asn His Ala Asn His Asp Lys Trp Lys Asn Ile Ile  
 755 760 765  
 Val Met Tyr Asn Pro Asn Lys Thr Ala Gln Thr Leu Thr Leu Pro Ser  
 770 775 780  
 Gly Asn Trp Thr Ile Val Gly Leu Gly Asn Gln Val Gly Glu Lys Ser  
 785 790 795  
 Leu Gly His Val Asn Gly Thr Val Glu Val Pro Ala Leu Ser Thr Ile  
 800 805 810 815  
 Ile Leu His Gln Gly Thr Ser Glu Asp Val Ile Asp Gln Asn  
 820 825

<210> 5

<211> 2589

<212> DNA

<213> *Bacillus acidopullulyticus*

<220>

<221> CDS

<222> (1)..(2586)

<220>

<221> sig\_peptide

<222> (1)..(99)

<220>

<221> mat\_peptide

<222> (100)..(2586)

<400> 5

|                                                                                                                                                       |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| gtg tcc cta ata cgt tct agg tat aat cat ttt gtc att ctt ttt act<br>Val Ser Leu Ile Arg Ser Arg Tyr Asn His Phe Val Ile Leu Phe Thr<br>-30 -25 -20     | 48  |
| gtc gcc ata atg ttt cta aca gtt tgt ttc ccc gct tat aaa gct tta<br>Val Ala Ile Met Phe Leu Thr Val Cys Phe Pro Ala Tyr Lys Ala Leu<br>-15 -10 -5      | 96  |
| gca gat tct acc tcg aca gaa gtc att gtg cat tat cat cgt ttt gat<br>Ala Asp Ser Thr Ser Thr Glu Val Ile Val His Tyr His Arg Phe Asp<br>-1 1 5 10 15    | 144 |
| tct aac tat gca aat tgg gat cta tgg atg tgg cca tat caa cca gtt<br>Ser Asn Tyr Ala Asn Trp Asp Leu Trp Met Trp Pro Tyr Gln Pro Val<br>20 25 30        | 192 |
| aat ggt aat gga gca gca tac gag ttt tct gga aag gat gat ttt ggc<br>Asn Gly Asn Gly Ala Ala Tyr Glu Phe Ser Gly Lys Asp Asp Phe Gly<br>35 40 45        | 240 |
| gtt aaa gca gat gtt caa gtg cct ggg gat gat aca cag gta ggt ctg<br>Val Lys Ala Asp Val Gln Val Pro Gly Asp Asp Thr Gln Val Gly Leu<br>50 55 60        | 288 |
| att gtc cgt aca aat gat tgg agc caa aaa aat aca tca gac gat ctc<br>Ile Val Arg Thr Asn Asp Trp Ser Gln Lys Asn Thr Ser Asp Asp Leu<br>65 70 75        | 336 |
| cat att gat ctg aca aag ggg cat gaa ata tgg att gtt cag ggg gat<br>His Ile Asp Leu Thr Lys Gly His Glu Ile Trp Ile Val Gln Gly Asp<br>80 85 90 95     | 384 |
| ccc aat att tat tac aat ctg agt gat gcg cag gct gca gcg act cca<br>Pro Asn Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Thr Pro<br>100 105 110     | 432 |
| aag gtt tcg aat gcg tat ttg gat aat gaa aaa aca gta ttg gca aag<br>Lys Val Ser Asn Ala Tyr Leu Asp Asn Glu Lys Thr Val Leu Ala Lys<br>115 120 125     | 480 |
| cta act aat cca atg aca tta tca gat gga tca agc ggc ttt acg gtt<br>Leu Thr Asn Pro Met Thr Leu Ser Asp Gly Ser Ser Gly Phe Thr Val<br>130 135 140     | 528 |
| aca gat aaa aca aca ggg gaa caa att cca gtt acc gct gca aca aat<br>Thr Asp Lys Thr Thr Gly Glu Gln Ile Pro Val Thr Ala Ala Thr Asn<br>145 150 155     | 576 |
| gcg aac tca gcc tcc tcg tct gag cag aca gac ttg gtt caa ttg acg<br>Ala Asn Ser Ala Ser Ser Ser Glu Gln Thr Asp Leu Val Gln Leu Thr<br>160 165 170 175 | 624 |
| tta gcc agt gca ccg gat gtt tcc cat aca ata caa gta gga gca gcc<br>Leu Ala Ser Ala Pro Asp Val Ser His Thr Ile Gln Val Gly Ala Ala<br>180 185 190     | 672 |

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| ggt tat gaa gca gtc aat ctc ata cca cga aat gta tta aat ttg cct<br>Gly Tyr Glu Ala Val Asn Leu Ile Pro Arg Asn Val Leu Asn Leu Pro<br>195 200 205     | 720  |
| cgt tat tat tac agc gga aat gat tta ggt aac gtt tat tca aat aag<br>Arg Tyr Tyr Tyr Ser Gly Asn Asp Leu Gly Asn Val Tyr Ser Asn Lys<br>210 215 220     | 768  |
| gca acg gcc ttc cgt gta tgg gct cca act gct tcg gat gtc caa tta<br>Ala Thr Ala Phe Arg Val Trp Ala Pro Thr Ala Ser Asp Val Gln Leu<br>225 230 235     | 816  |
| ctt tta tac aat agt gaa aca gga cct gta acc aaa cag ctt gaa atg<br>Leu Leu Tyr Asn Ser Glu Thr Gly Pro Val Thr Lys Gln Leu Glu Met<br>240 245 250 255 | 864  |
| caa aag agt gat aac ggt aca tgg aaa ctg aag gtc cct ggt aat ctg<br>Gln Lys Ser Asp Asn Gly Thr Trp Lys Leu Lys Val Pro Gly Asn Leu<br>260 265 270     | 912  |
| aaa aat tgg tat tat ctc tat cag gta acg gtg aat ggg aag aca caa<br>Lys Asn Trp Tyr Tyr Leu Tyr Gln Val Thr Val Asn Gly Lys Thr Gln<br>275 280 285     | 960  |
| aca gcc gtt gac cct tat gtg cgt gct att tca gtc aat gca aca cgt<br>Thr Ala Val Asp Pro Tyr Val Arg Ala Ile Ser Val Asn Ala Thr Arg<br>290 295 300     | 1008 |
| ggt atg ata gtc gat tta gaa gat acg aat cct cct gga tgg aaa gaa<br>Gly Met Ile Val Asp Leu Glu Asp Thr Asn Pro Pro Gly Trp Lys Glu<br>305 310 315     | 1056 |
| gat cat caa cag aca cct gcg aac cca gtg gat gaa gta atc tac gaa<br>Asp His Gln Gln Thr Pro Ala Asn Pro Val Asp Glu Val Ile Tyr Glu<br>320 325 330 335 | 1104 |
| gtg cat gtg cgt gat ttt tcg att gat gct aat tca ggc atg aaa aat<br>Val His Val Arg Asp Phe Ser Ile Asp Ala Asn Ser Gly Met Lys Asn<br>340 345 350     | 1152 |
| aaa ggg aaa tat ctt gcc ttt aca gaa cat ggc aca aaa ggc cct gat<br>Lys Gly Lys Tyr Leu Ala Phe Thr Glu His Gly Thr Lys Gly Pro Asp<br>355 360 365     | 1200 |
| aac gtg aaa acg ggt att gat agt ttg aag gaa tta gga atc aat gct<br>Asn Val Lys Thr Gly Ile Asp Ser Leu Lys Glu Leu Gly Ile Asn Ala<br>370 375 380     | 1248 |
| gtt caa tta cag ccg att gaa gaa ttt aac agc att gat gaa acc caa<br>Val Gln Leu Gln Pro Ile Glu Glu Phe Asn Ser Ile Asp Glu Thr Gln<br>385 390 395     | 1296 |
| cca aat atg tat aac tgg ggc tat gac cca aga aac tac aac gtc cct<br>Pro Asn Met Tyr Asn Trp Gly Tyr Asp Pro Arg Asn Tyr Asn Val Pro<br>400 405 410 415 | 1344 |
| gaa gga gcg tat gca act aca cca gaa gga acg gct cgc att acc cag<br>Glu Gly Ala Tyr Ala Thr Thr Pro Glu Gly Thr Ala Arg Ile Thr Gln<br>420 425 430     | 1392 |

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| tta aag caa ctg att caa agc att cat aaa gat cgg att gct atc aat<br>Leu Lys Gln Leu Ile Gln Ser Ile His Lys Asp Arg Ile Ala Ile Asn<br>435 440 445     | 1440 |
| atg gat gtg gtc tat aac cat acc ttt aac gta gga gtg tct gat ttt<br>Met Asp Val Val Tyr Asn His Thr Phe Asn Val Gly Val Ser Asp Phe<br>450 455 460     | 1488 |
| gat aag att gtt ccg caa tac tat tat cgg aca gac agc gca ggt aat<br>Asp Lys Ile Val Pro Gln Tyr Tyr Arg Thr Asp Ser Ala Gly Asn<br>465 470 475         | 1536 |
| tat acg aac ggc tca ggt gta ggt aat gaa att gcg acc gag cgt ccg<br>Tyr Thr Asn Gly Ser Gly Val Gly Asn Glu Ile Ala Thr Glu Arg Pro<br>480 485 490 495 | 1584 |
| atg gtc caa aag ttc gtt ctg gat tct gtt aaa tat tgg gta aag gaa<br>Met Val Gln Lys Phe Val Leu Asp Ser Val Lys Tyr Trp Val Lys Glu<br>500 505 510     | 1632 |
| tac cat atc gac ggc ttc cgt ttc gat ctt atg gct ctt tta gga aaa<br>Tyr His Ile Asp Gly Phe Arg Phe Asp Leu Met Ala Leu Leu Gly Lys<br>515 520 525     | 1680 |
| gac acc atg gcc aaa ata tca aaa gag ctt cat gct att aat cct ggc<br>Asp Thr Met Ala Lys Ile Ser Lys Glu Leu His Ala Ile Asn Pro Gly<br>530 535 540     | 1728 |
| att gtc ctg tat gga gaa cca tgg act ggc ggt acc tct gga tta tca<br>Ile Val Leu Tyr Gly Glu Pro Trp Thr Gly Gly Thr Ser Gly Leu Ser<br>545 550 555     | 1776 |
| agc gac caa ctc gtt acg aaa ggt cag caa aag ggc ttg gga att ggc<br>Ser Asp Gln Leu Val Thr Lys Gly Gln Gln Lys Gly Leu Gly Ile Gly<br>560 565 570 575 | 1824 |
| gta ttc aac gat aat att cgg aac gga ctc gat ggt aac gtt ttt gat<br>Val Phe Asn Asp Asn Ile Arg Asn Gly Leu Asp Gly Asn Val Phe Asp<br>580 585 590     | 1872 |
| aaa tcg gca caa gga ttt gca aca gga gat cca aac caa gtt aat gtc<br>Lys Ser Ala Gln Gly Phe Ala Thr Gly Asp Pro Asn Gln Val Asn Val<br>595 600 605     | 1920 |
| att aaa aat aga gtt atg gga agt att tca gat ttc act tcg gca cct<br>Ile Lys Asn Arg Val Met Gly Ser Ile Ser Asp Phe Thr Ser Ala Pro<br>610 615 620     | 1968 |
| agc gaa acc att aac tat gta aca agc cat gat aat atg aca ttg tgg<br>Ser Glu Thr Ile Asn Tyr Val Thr Ser His Asp Asn Met Thr Leu Trp<br>625 630 635     | 2016 |
| gat aaa att agc gca agt aat ccg aac gat aca caa gca gat cga att<br>Asp Lys Ile Ser Ala Ser Asn Pro Asn Asp Thr Gln Ala Asp Arg Ile<br>640 645 650 655 | 2064 |
| aag atg gat gaa ttg gct caa gct gtg gta ttt act tca caa ggg gta<br>Lys Met Asp Glu Leu Ala Gln Ala Val Val Phe Thr Ser Gln Gly Val<br>660 665 670     | 2112 |
| cca ttt atg caa ggt gga gaa gaa atg ctg cgg aca aaa ggc ggt aat                                                                                       | 2160 |

Pro Phe Met Gln Gly Gly Glu Glu Met Leu Arg Thr Lys Gly Gly Asn  
675 680 685

gat aat agt tac aat gcc ggg gat agc gtg aat cag ttc gat tgg tca 2208  
Asp Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn Gln Phe Asp Trp Ser  
690 695 700

aga aaa gca caa ttt gaa aat gta ttc gac tac tat tct tgg ttg att 2256  
Arg Lys Ala Gln Phe Glu Asn Val Phe Asp Tyr Tyr Ser Trp Leu Ile  
705 710 715

cat cta cgt gat aat cac cca gca ttc cgt atg acg aca gcg gat caa 2304  
His Leu Arg Asp Asn His Pro Ala Phe Arg Met Thr Thr Ala Asp Gln  
720 725 730 735

atc aaa caa aat ctc act ttc ttg gat agc cca acg aac act gta gca 2352  
Ile Lys Gln Asn Leu Thr Phe Leu Asp Ser Pro Thr Asn Thr Val Ala  
740 745 750

ttt gaa tta aaa aat cat gcc aat cat gat aaa tgg aaa aac att ata 2400  
Phe Glu Leu Lys Asn His Ala Asn His Asp Lys Trp Lys Asn Ile Ile  
755 760 765

gtt atg tat aat cca aat aaa act gca caa act ctc act cta cca agt 2448  
Val Met Tyr Asn Pro Asn Lys Thr Ala Gln Thr Leu Thr Leu Pro Ser  
770 775 780

gga aat tgg aca att gta gga tta ggc aat caa gta ggt gag aaa tca 2496  
Gly Asn Trp Thr Ile Val Gly Leu Gly Asn Gln Val Gly Glu Lys Ser  
785 790 795

cta ggc cat gta aat ggc acg gtt gag gtg cca gct ctt agt acg atc 2544  
Leu Gly His Val Asn Gly Thr Val Glu Val Pro Ala Leu Ser Thr Ile  
800 805 810 815

att ctt cat cag ggt aca tct gaa gat gtc att gat caa aat taa 2589  
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820 825

&lt;210&gt; 6

&lt;211&gt; 862

&lt;212&gt; PRT

&lt;213&gt; Bacillus acidopullulyticus

&lt;400&gt; 6

Val Ser Leu Ile Arg Ser Arg Tyr Asn His Phe Val Ile Leu Phe Thr  
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-15 -10 -5

Ala Asp Ser Thr Ser Thr Glu Val Ile Val His Tyr His Arg Phe Asp  
-1 1 5 10 15

Ser Asn Tyr Ala Asn Trp Asp Leu Trp Met Trp Pro Tyr Gln Pro Val  
20 25 30

Asn Gly Asn Gly Ala Ala Tyr Glu Phe Ser Gly Lys Asp Asp Phe Gly  
 35 40 45  
 Val Lys Ala Asp Val Gln Val Pro Gly Asp Asp Thr Gln Val Gly Leu  
 50 55 60  
 Ile Val Arg Thr Asn Asp Trp Ser Gln Lys Asn Thr Ser Asp Asp Leu  
 65 70 75  
 His Ile Asp Leu Thr Lys Gly His Glu Ile Trp Ile Val Gln Gly Asp  
 80 85 90 95  
 Pro Asn Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Thr Pro  
 100 105 110  
 Lys Val Ser Asn Ala Tyr Leu Asp Asn Glu Lys Thr Val Leu Ala Lys  
 115 120 125  
 Leu Thr Asn Pro Met Thr Leu Ser Asp Gly Ser Ser Gly Phe Thr Val  
 130 135 140  
 Thr Asp Lys Thr Thr Gly Glu Gln Ile Pro Val Thr Ala Ala Thr Asn  
 145 150 155  
 Ala Asn Ser Ala Ser Ser Ser Glu Gln Thr Asp Leu Val Gln Leu Thr  
 160 165 170 175  
 Leu Ala Ser Ala Pro Asp Val Ser His Thr Ile Gln Val Gly Ala Ala  
 180 185 190  
 Gly Tyr Glu Ala Val Asn Leu Ile Pro Arg Asn Val Leu Asn Leu Pro  
 195 200 205  
 Arg Tyr Tyr Tyr Ser Gly Asn Asp Leu Gly Asn Val Tyr Ser Asn Lys  
 210 215 220  
 Ala Thr Ala Phe Arg Val Trp Ala Pro Thr Ala Ser Asp Val Gln Leu  
 225 230 235  
 Leu Leu Tyr Asn Ser Glu Thr Gly Pro Val Thr Lys Gln Leu Glu Met  
 240 245 250 255  
 Gln Lys Ser Asp Asn Gly Thr Trp Lys Leu Lys Val Pro Gly Asn Leu  
 260 265 270

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

Tyr His Ile Asp Gly Phe Arg Phe Asp Leu Met Ala Leu Leu Gly Lys  
 515 520 525  
 Asp Thr Met Ala Lys Ile Ser Lys Glu Leu His Ala Ile Asn Pro Gly  
 530 535 540  
 Ile Val Leu Tyr Gly Glu Pro Trp Thr Gly Gly Thr Ser Gly Leu Ser  
 545 550 555  
 Ser Asp Gln Leu Val Thr Lys Gly Gln Gln Lys Gly Leu Gly Ile Gly  
 560 565 570 575  
 Val Phe Asn Asp Asn Ile Arg Asn Gly Leu Asp Gly Asn Val Phe Asp  
 580 585 590  
 Lys Ser Ala Gln Gly Phe Ala Thr Gly Asp Pro Asn Gln Val Asn Val  
 595 600 605  
 Ile Lys Asn Arg Val Met Gly Ser Ile Ser Asp Phe Thr Ser Ala Pro  
 610 615 620  
 Ser Glu Thr Ile Asn Tyr Val Thr Ser His Asp Asn Met Thr Leu Trp  
 625 630 635  
 Asp Lys Ile Ser Ala Ser Asn Pro Asn Asp Thr Gln Ala Asp Arg Ile  
 640 645 650 655  
 Lys Met Asp Glu Leu Ala Gln Ala Val Val Phe Thr Ser Gln Gly Val  
 660 665 670  
 Pro Phe Met Gln Gly Gly Glu Glu Met Leu Arg Thr Lys Gly Gly Asn  
 675 680 685  
 Asp Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn Gln Phe Asp Trp Ser  
 690 695 700  
 Arg Lys Ala Gln Phe Glu Asn Val Phe Asp Tyr Tyr Ser Trp Leu Ile  
 705 710 715  
 His Leu Arg Asp Asn His Pro Ala Phe Arg Met Thr Thr Ala Asp Gln  
 720 725 730 735  
 Ile Lys Gln Asn Leu Thr Phe Leu Asp Ser Pro Thr Asn Thr Val Ala  
 740 745 750  
 Phe Glu Leu Lys Asn His Ala Asn His Asp Lys Trp Lys Asn Ile Ile  
 755 760 765  
 Val Met Tyr Asn Pro Asn Lys Thr Ala Gln Thr Leu Thr Leu Pro Ser  
 770 775 780  
 Gly Asn Trp Thr Ile Val Gly Leu Gly Asn Gln Val Gly Glu Lys Ser  
 785 790 795  
 Leu Gly His Val Asn Gly Thr Val Glu Val Pro Ala Leu Ser Thr Ile  
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 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  
 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  
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Glu Gly Trp Gly Glu Phe His Val Asn Gly Gly Ser Val Ser Ile Tyr  
 465 470 475 480

Val Gln Arg

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<211> 2160

<212> DNA

<213> Bacillus sp.

<220>

<221> CDS

<222> (1)..(2157)

<220>

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<222> (100)..(2157)

<400> 8

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| Met Lys Lys Lys Thr Leu Ser Leu Phe Val Gly Leu Met Leu Leu Ile |     |
| -30 -25 -20                                                     |     |
| ggt ctt ctg ttc agc ggt tct ctt ccg tac aat cca aac gcc gct gaa | 96  |
| Gly Leu Leu Phe Ser Gly Ser Leu Pro Tyr Asn Pro Asn Ala Ala Glu |     |
| -15 -10 -5                                                      |     |
| gcc agc agt tcc gca agc gtc aaa ggg gac gtg att tac cag att atc | 144 |
| Ala Ser Ser Ser Ala Ser Val Lys Gly Asp Val Ile Tyr Gln Ile Ile |     |
| -1 1 5 10 15                                                    |     |
| att gac cgg ttt tac gat ggg gac acg acg aac aac aat cct gcc aaa | 192 |

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| Ile Asp Arg Phe Tyr Asp Gly Asp Thr Thr Asn Asn Asn Pro Ala Lys |     |
| 20 25 30                                                        |     |
| agt tat gga ctt tac gat ccg acc aaa tcg aag tgg aaa atg tat tgg | 240 |
| Ser Tyr Gly Leu Tyr Asp Pro Thr Lys Ser Lys Trp Lys Met Tyr Trp |     |
| 35 40 45                                                        |     |
| ggc ggg gat ctg gag ggg gtt cgt caa aaa ctt cct tat ctt aaa cag | 288 |
| Gly Gly Asp Leu Glu Gly Val Arg Gln Lys Leu Pro Tyr Leu Lys Gln |     |
| 50 55 60                                                        |     |
| ctg ggc gta acg aca atc tgg ttg tcc ccg gtt ttg gac aat ctg gat | 336 |
| Leu Gly Val Thr Thr Ile Trp Leu Ser Pro Val Leu Asp Asn Leu Asp |     |
| 65 70 75                                                        |     |
| aca ctg gcg ggc acc gat aac acg ggc tat cac gga tac tgg acg cgc | 384 |
| Thr Leu Ala Gly Thr Asp Asn Thr Gly Tyr His Gly Tyr Trp Thr Arg |     |
| 80 85 90 95                                                     |     |
| gat ttt aaa cag att gag gaa cat ttc ggg aat tgg acc aca ttt gac | 432 |
| Asp Phe Lys Gln Ile Glu Glu His Phe Gly Asn Trp Thr Thr Phe Asp |     |
| 100 105 110                                                     |     |
| acg ttg gtc aat gat gct cac caa aac gga atc aag gtg att gtc gac | 480 |
| Thr Leu Val Asn Asp Ala His Gln Asn Gly Ile Lys Val Ile Val Asp |     |
| 115 120 125                                                     |     |
| ttt gtg ccc aat cat tcg act cct ttt aag gca aac gat tcc acc ttt | 528 |
| Phe Val Pro Asn His Ser Thr Pro Phe Lys Ala Asn Asp Ser Thr Phe |     |
| 130 135 140                                                     |     |
| gcg gaa ggc ggc gcc ctc tac aac aat gga acc tat atg ggc aat tat | 576 |
| Ala Glu Gly Gly Ala Leu Tyr Asn Asn Gly Thr Tyr Met Gly Asn Tyr |     |
| 145 150 155                                                     |     |
| ttt gat gac gca aca aaa ggg tac ttc cac cat aat ggg gac atc agc | 624 |
| Phe Asp Asp Ala Thr Lys Gly Tyr Phe His His Asn Gly Asp Ile Ser |     |
| 160 165 170 175                                                 |     |
| aac tgg gac gac ccg tac gag gcg caa tgg aaa aac ttc acg gat cca | 672 |
| Asn Trp Asp Asp Arg Tyr Glu Ala Gln Trp Lys Asn Phe Thr Asp Pro |     |
| 180 185 190                                                     |     |
| gcc ggt ttc tcg ctt gcc gat ttg tcg cag gaa aat ggc acg att gct | 720 |
| Ala Gly Phe Ser Leu Ala Asp Leu Ser Gln Glu Asn Gly Thr Ile Ala |     |
| 195 200 205                                                     |     |
| caa tac ctg acc gat gcg gcg gtt caa ttg gta gca cat gga gcg gat | 768 |
| Gln Tyr Leu Thr Asp Ala Ala Val Gln Leu Val Ala His Gly Ala Asp |     |
| 210 215 220                                                     |     |
| ggc ttg ccg att gat gcg gtg aag cat ttt aat tcg ggg ttc tcc aaa | 816 |
| Gly Leu Arg Ile Asp Ala Val Lys His Phe Asn Ser Gly Phe Ser Lys |     |
| 225 230 235                                                     |     |
| tcg ttg gcc gat aaa ctg tac caa aag aaa gac att ttc ctg gtg ggg | 864 |
| Ser Leu Ala Asp Lys Leu Tyr Gln Lys Lys Asp Ile Phe Leu Val Gly |     |
| 240 245 250 255                                                 |     |
| gaa tgg tac gga gat gac ccc gga aca gcc aat cat ctg gaa aag gtc | 912 |
| Glu Trp Tyr Gly Asp Asp Pro Gly Thr Ala Asn His Leu Glu Lys Val |     |

| 260                                                                                                                                                   | 265 | 270 |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|-----|------|
| cgg tac gcc aac aac agc ggt gtc aat gtg ctg gat ttt gat ctc aac<br>Arg Tyr Ala Asn Asn Ser Gly Val Asn Val Leu Asp Phe Asp Leu Asn<br>275 280 285     |     |     | 960  |
| acg gtg att cga aat gtg ttc ggc aca ttt acg caa acg atg tac gat<br>Thr Val Ile Arg Asn Val Phe Gly Thr Phe Thr Gln Thr Met Tyr Asp<br>290 295 300     |     |     | 1008 |
| ctt aac aat atg gtg aac caa acg ggg aac gag tac aaa tac aaa gaa<br>Leu Asn Asn Met Val Asn Gln Thr Gly Asn Glu Tyr Lys Tyr Lys Glu<br>305 310 315     |     |     | 1056 |
| aat cta atc aca ttt atc gat aac cat gat atg tca aga ttt ctt tcg<br>Asn Leu Ile Thr Phe Ile Asp Asn His Asp Met Ser Arg Phe Leu Ser<br>320 325 330 335 |     |     | 1104 |
| gta aat tcg aac aag gcg aat ttg cac cag gcg ctt gct ttc att ctc<br>Val Asn Ser Asn Lys Ala Asn Leu His Gln Ala Leu Ala Phe Ile Leu<br>340 345 350     |     |     | 1152 |
| act tcg cgc ggt acg ccc tcc atc tat tat gga acc gaa caa tac atg<br>Thr Ser Arg Gly Thr Pro Ser Ile Tyr Tyr Gly Thr Glu Gln Tyr Met<br>355 360 365     |     |     | 1200 |
| gca ggc gcc aat gac ccg tac aac cgg ggg atg atg ccg gcg ttt gat<br>Ala Gly Gly Asn Asp Pro Tyr Asn Arg Gly Met Met Pro Ala Phe Asp<br>370 375 380     |     |     | 1248 |
| acg aca acc acc gcc ttt aaa gag gtg tca act ctg gcg ggg ttg cgc<br>Thr Thr Thr Thr Ala Phe Lys Glu Val Ser Thr Leu Ala Gly Leu Arg<br>385 390 395     |     |     | 1296 |
| agg aac aat gcg gcg atc cag tac ggc acc acc acc cag cgt tgg atc<br>Arg Asn Asn Ala Ala Ile Gln Tyr Gly Thr Thr Thr Gln Arg Trp Ile<br>400 405 410 415 |     |     | 1344 |
| aac aat gat gtt tac att tat gaa cgg aaa ttt ttc aac gat gtc gtg<br>Asn Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Phe Asn Asp Val Val<br>420 425 430     |     |     | 1392 |
| ttg gtg gcc atc aat cga aac acg caa tcc tcc tat tcg att tcc ggt<br>Leu Val Ala Ile Asn Arg Asn Thr Gln Ser Ser Tyr Ser Ile Ser Gly<br>435 440 445     |     |     | 1440 |
| ttg cag acg gcc ttg cca aat ggc agc tat gcg gat tat ctg tca ggg<br>Leu Gln Thr Ala Leu Pro Asn Gly Ser Tyr Ala Asp Tyr Leu Ser Gly<br>450 455 460     |     |     | 1488 |
| ctg ttg ggg ggg aac ggg att tcc gtt tcc aat gga agt gtc gct tcg<br>Leu Leu Gly Gly Asn Gly Ile Ser Val Ser Asn Gly Ser Val Ala Ser<br>465 470 475     |     |     | 1536 |
| ttc acg ctt gcg cct gga gcc gtg tct gtt tgg cag tac agc aca tcc<br>Phe Thr Leu Ala Pro Gly Ala Val Ser Val Trp Gln Tyr Ser Thr Ser<br>480 485 490 495 |     |     | 1584 |
| gct tca gcg ccg caa atc gga tcg gtt gct cca aat atg ggg att ccg<br>Ala Ser Ala Pro Gln Ile Gly Ser Val Ala Pro Asn Met Gly Ile Pro<br>500 505 510     |     |     | 1632 |

ggt aat gtg gtc acg atc gac ggg aaa ggt ttt ggg acg acg cag gga 1680  
 Gly Asn Val Val Thr Ile Asp Gly Lys Gly Phe Gly Thr Thr Gln Gly  
 515 520 525

acc gtg aca ttt ggc gga gtg aca gcg act gtg aaa tcc tgg aca tcc 1728  
 Thr Val Thr Phe Gly Gly Val Thr Ala Thr Val Lys Ser Trp Thr Ser  
 530 535 540

aat cgg att gaa gtg tac gtt ccc aac atg gcc gcc ggg ctg acc gat 1776  
 Asn Arg Ile Glu Val Tyr Val Pro Asn Met Ala Ala Gly Leu Thr Asp  
 545 550 555

gtg aaa gtc acc gcg ggt gga gtt tcc agc aat ctg tat tct tac aat 1824  
 Val Lys Val Thr Ala Gly Gly Val Ser Ser Asn Leu Tyr Ser Tyr Asn  
 560 565 570 575

att ttg agt gga acg cag aca tcg gtt gtg ttt act gtg aaa agt gcg 1872  
 Ile Leu Ser Gly Thr Gln Thr Ser Val Val Phe Thr Val Lys Ser Ala  
 580 585 590

cct ccg acc aac ctg ggg gat aag att tac ctg acg ggc aac ata ccg 1920  
 Pro Pro Thr Asn Leu Gly Asp Lys Ile Tyr Leu Thr Gly Asn Ile Pro  
 595 600 605

gaa ttg ggg aat tgg agc acg gat acg agc gga gcc gtt aac aat gcg 1968  
 Glu Leu Gly Asn Trp Ser Thr Asp Thr Ser Gly Ala Val Asn Asn Ala  
 610 615 620

caa ggg ccc ctg ctc gcg ccc aat tat ccg gat tgg ttt tat gta ttc 2016  
 Gln Gly Pro Leu Leu Ala Pro Asn Tyr Pro Asp Trp Phe Tyr Val Phe  
 625 630 635

agc gtt cca gca gga aag acg att caa ttc aag ttc ttc atc aag cgt 2064  
 Ser Val Pro Ala Gly Lys Thr Ile Gln Phe Lys Phe Phe Ile Lys Arg  
 640 645 650 655

gcg gat gga acg att caa tgg gag aat ggt tcg aac cac gtg gcc aca 2112  
 Ala Asp Gly Thr Ile Gln Trp Glu Asn Gly Ser Asn His Val Ala Thr  
 660 665 670

act ccc acg ggt gca acc ggt aac att act gtt acg tgg caa aac tag 2160  
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 675 680 685

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 <213> Bacillus sp.

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 -15 -10 -5

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

240                      245                      250                      255  
 Glu Trp Tyr Gly Asp Asp Pro Gly Thr Ala Asn His Leu Glu Lys Val  
                                  260                                   265                                   270  
 Arg Tyr Ala Asn Asn Ser Gly Val Asn Val Leu Asp Phe Asp Leu Asn  
                                  275                                   280                                   285  
 Thr Val Ile Arg Asn Val Phe Gly Thr Phe Thr Gln Thr Met Tyr Asp  
                                  290                                   295                                   300  
 Leu Asn Asn Met Val Asn Gln Thr Gly Asn Glu Tyr Lys Tyr Lys Glu  
                                  305                                   310                                   315  
 Asn Leu Ile Thr Phe Ile Asp Asn His Asp Met Ser Arg Phe Leu Ser  
                                  320                                   325                                   330                                   335  
 Val Asn Ser Asn Lys Ala Asn Leu His Gln Ala Leu Ala Phe Ile Leu  
                                  340                                   345                                   350  
 Thr Ser Arg Gly Thr Pro Ser Ile Tyr Tyr Gly Thr Glu Gln Tyr Met  
                                  355                                   360                                   365  
 Ala Gly Gly Asn Asp Pro Tyr Asn Arg Gly Met Met Pro Ala Phe Asp  
                                  370                                   375                                   380  
 Thr Thr Thr Thr Ala Phe Lys Glu Val Ser Thr Leu Ala Gly Leu Arg  
                                  385                                   390                                   395  
 Arg Asn Asn Ala Ala Ile Gln Tyr Gly Thr Thr Thr Gln Arg Trp Ile  
                                  400                                   405                                   410                                   415  
 Asn Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Phe Asn Asp Val Val  
                                  420                                   425                                   430  
 Leu Val Ala Ile Asn Arg Asn Thr Gln Ser Ser Tyr Ser Ile Ser Gly  
                                  435                                   440                                   445  
 Leu Gln Thr Ala Leu Pro Asn Gly Ser Tyr Ala Asp Tyr Leu Ser Gly  
                                  450                                   455                                   460  
 Leu Leu Gly Gly Asn Gly Ile Ser Val Ser Asn Gly Ser Val Ala Ser  
                                  465                                   470                                   475  
 Phe Thr Leu Ala Pro Gly Ala Val Ser Val Trp Gln Tyr Ser Thr Ser  
                                  480                                   485                                   490                                   495

Ala Ser Ala Pro Gln Ile Gly Ser Val Ala Pro Asn Met Gly Ile Pro  
500 505 510

Gly Asn Val Val Thr Ile Asp Gly Lys Gly Phe Gly Thr Thr Gln Gly  
515 520 525

Thr Val Thr Phe Gly Gly Val Thr Ala Thr Val Lys Ser Trp Thr Ser  
530 535 540

Asn Arg Ile Glu Val Tyr Val Pro Asn Met Ala Ala Gly Leu Thr Asp  
545 550 555

Val Lys Val Thr Ala Gly Gly Val Ser Ser Asn Leu Tyr Ser Tyr Asn  
560 565 570 575

Ile Leu Ser Gly Thr Gln Thr Ser Val Val Phe Thr Val Lys Ser Ala  
580 585 590

Pro Pro Thr Asn Leu Gly Asp Lys Ile Tyr Leu Thr Gly Asn Ile Pro  
595 600 605

Glu Leu Gly Asn Trp Ser Thr Asp Thr Ser Gly Ala Val Asn Asn Ala  
610 615 620

Gln Gly Pro Leu Leu Ala Pro Asn Tyr Pro Asp Trp Phe Tyr Val Phe  
625 630 635

Ser Val Pro Ala Gly Lys Thr Ile Gln Phe Lys Phe Phe Ile Lys Arg  
640 645 650 655

Ala Asp Gly Thr Ile Gln Trp Glu Asn Gly Ser Asn His Val Ala Thr  
660 665 670

Thr Pro Thr Gly Ala Thr Gly Asn Ile Thr Val Thr Trp Gln Asn  
675 680 685

&lt;210&gt; 10

&lt;211&gt; 514

&lt;212&gt; PRT

&lt;213&gt; Bacillus stearothermophilus

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)..(514)

&lt;400&gt; 10

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 Ala 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 Ser 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

Phe Ser Phe Phe Pro Asp Trp Leu Ser Asp 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 Met Lys Thr Asn 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 Thr  
 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 Val  
 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 Trp Ser Ile Thr Thr  
 485 490 495  
 Arg Pro Trp Thr Asp Glu Phe Val Arg Trp Thr Glu Pro Arg Leu Val  
 500 505 510

Ala Trp

<210> 11

<211> 484

<212> PRT

<213> Aspergillus niger

<220>

<221> mat\_peptide

<222> (1)..(484)

<400> 11

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

Val Glu Asp Cys Trp Glu Gly Asp Thr Ile Val Ser Leu Pro Asp Leu  
 165 170 175  
 Asp Thr Thr Glu Thr Ala Val Arg Thr Ile Trp Tyr Asp Trp Val Ala  
 180 185 190  
 Asp Leu Val Ser Asn Tyr Ser Val Asp Gly Leu Arg Ile Asp Ser Val  
 195 200 205  
 Leu Glu Val Gln Pro Asp Phe Phe Pro Gly Tyr Asn Lys Ala Ser Gly  
 210 215 220  
 Val Tyr Cys Val Gly Glu Ile Asp Asn Gly Asn Pro Ala Ser Asp Cys  
 225 230 235 240  
 Pro Tyr Gln Lys Val Leu Asp Gly Val Leu Asn Tyr Pro Ile Tyr Trp  
 245 250 255  
 Gln Leu Leu Tyr Ala Phe Glu Ser Ser Ser Gly Ser Ile Ser Asn Leu  
 260 265 270  
 Tyr Asn Met Ile Lys Ser Val Ala Ser Asp Cys Ser Asp Pro Thr Leu  
 275 280 285  
 Leu Gly Asn Phe Ile Glu Asn His Asp Asn Pro Arg Phe Ala Lys Tyr  
 290 295 300  
 Thr Ser Asp Tyr Ser Gln Ala Lys Asn Val Leu Ser Tyr Ile Phe Leu  
 305 310 315 320  
 Ser Asp Gly Ile Pro Ile Val Tyr Ala Gly Glu Glu Gln His Tyr Ala  
 325 330 335  
 Gly Gly Lys Val Pro Tyr Asn Arg Glu Ala Thr Trp Leu Ser Gly Tyr  
 340 345 350  
 Asp Thr Ser Ala Glu Leu Tyr Thr Trp Ile Ala Thr Thr Asn Ala Ile  
 355 360 365  
 Arg Lys Leu Ala Ile Ala Ala Asp Ser Ala Tyr Ile Thr Tyr Ala Asn  
 370 375 380  
 Asp Ala Phe Tyr Thr Asp Ser Asn Thr Ile Ala Met Ala Lys Gly Thr  
 385 390 395 400  
 Ser Gly Ser Gln Val Ile Thr Val Leu Ser Asn Lys Gly Ser Ser Gly  
 405 410 415  
 Ser Ser Tyr Thr Leu Thr Leu Ser Gly Ser Gly Tyr Thr Ser Gly Thr  
 420 425 430  
 Lys Leu Ile Glu Ala Tyr Thr Cys Thr Ser Val Thr Val Asp Ser Ser  
 435 440 445  
 Gly Asp Ile Pro Val Pro Met Ala Ser Gly Leu Pro Arg Val Leu Leu  
 450 455 460  
 Pro Ala Ser Val Val Asp Ser Ser Ser Leu Cys Gly Gly Ser Gly Arg  
 465 470 475 480  
 Leu Tyr Val Glu  
 <210> 12  
 <211> 435  
 <212> PRT  
 <213> Humicola insolens  
 <220>  
 <221> mat\_peptide

&lt;222&gt; (1)..(435)

&lt;400&gt; 12

Met Ala Arg Gly Thr Ala Leu Leu Gly Leu Thr Ala Leu Leu Gly  
1 5 10 15

Leu Val Asn Gly Gln Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln  
20 25 30

Leu Thr Thr Phe Arg Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr  
35 40 45

Asn Phe Ile Val Leu Asp Ser Leu Ser His Pro Ile His Arg Ala Glu  
50 55 60

Gly Leu Gly Pro Gly Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys  
65 70 75 80

Asp Val Cys Pro Asp Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu  
85 90 95

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

Leu Arg Leu Gln His Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg  
115 120 125

Val Tyr Leu Leu Asp Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu  
130 135 140

Thr Gly Phe Glu Phe Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys  
145 150 155 160

Gly Met Asn Ser Ala Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala  
165 170 175

Lys Ser Lys Tyr Asn Ser Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys  
180 185 190

Asp Ala Gln Cys Phe Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile  
195 200 205

Glu Gly Lys Gly Ser Cys Cys Asn Glu Met Asp Ile Trp Glu Val Asn  
210 215 220

Ser Arg Ala Ser His Val Val Pro His Thr Cys Asn Lys Lys Gly Leu  
225 230 235 240

Tyr Leu Cys Glu Gly Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys  
245 250 255

Asn Gly Cys Gly Trp Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr  
260 265 270

Gly Arg Gly Glu Glu Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val  
275 280 285

Val Thr Gln Phe Leu Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His  
290 295 300

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

Lys Glu Gly Val Pro Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu  
325 330 335

Ala Thr Gly Ser Arg Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met

|                                                                 |     |     |     |
|-----------------------------------------------------------------|-----|-----|-----|
| 340                                                             | 345 | 350 |     |
| Gly Glu Ala Leu Thr Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp |     |     |     |
| 355                                                             | 360 | 365 |     |
| Asp Gln Gly Gly Asn Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro |     |     |     |
| 370                                                             | 375 | 380 |     |
| Cys Ala Lys Gly Glu Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro |     |     |     |
| 385                                                             | 390 | 395 | 400 |
| Phe Pro Glu Val Thr Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser |     |     |     |
| 405                                                             | 410 | 415 |     |
| Thr Tyr Gln Glu Val Gln Lys Pro Lys Pro Lys Pro Gly His Gly Pro |     |     |     |
| 420                                                             | 425 | 430 |     |
| Arg Ser Asp                                                     |     |     |     |
| 435                                                             |     |     |     |
| <210> 13                                                        |     |     |     |
| <211> 1008                                                      |     |     |     |
| <212> DNA                                                       |     |     |     |
| <213> Thermoascus aurantiacus                                   |     |     |     |
| <220>                                                           |     |     |     |
| <221> CDS                                                       |     |     |     |
| <222> (1)..(1005)                                               |     |     |     |
| <220>                                                           |     |     |     |
| <221> sig_peptide                                               |     |     |     |
| <222> (1)..(90)                                                 |     |     |     |
| <220>                                                           |     |     |     |
| <221> mat_peptide                                               |     |     |     |
| <222> (91)..(1005)                                              |     |     |     |
| <400> 13                                                        |     |     |     |
| atg aag ctc ggc tct ctc gtg ctc gct ctc agc gca gct agg ctt aca |     | 48  |     |
| Met Lys Leu Gly Ser Leu Val Leu Ala Leu Ser Ala Ala Arg Leu Thr |     |     |     |
| -30 -25 -20 -15                                                 |     |     |     |
| ctg tcg gcc cct ctc gca gac aga aag cag gag acc aag cgt gcg aaa |     | 96  |     |
| Leu Ser Ala Pro Leu Ala Asp Arg Lys Gln Glu Thr Lys Arg Ala Lys |     |     |     |
| -10 -5 -1 1                                                     |     |     |     |
| gta ttc caa tgg ttc ggt tcg aac gag tcc ggt gct gaa ttc gga agc |     | 144 |     |
| Val Phe Gln Trp Phe Gly Ser Asn Glu Ser Gly Ala Glu Phe Gly Ser |     |     |     |
| 5 10 15                                                         |     |     |     |
| cag aac ctt cca gga gtc gag gga aag gat tat ata tgg cct gat ccc |     | 192 |     |
| Gln Asn Leu Pro Gly Val Glu Gly Lys Asp Tyr Ile Trp Pro Asp Pro |     |     |     |
| 20 25 30                                                        |     |     |     |

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aac acc att gac aca ttg atc agc aag ggg atg aac atc ttt cgt gtc      240
Asn Thr Ile Asp Thr Leu Ile Ser Lys Gly Met Asn Ile Phe Arg Val
35          40          45          50

ccc ttt atg atg gag aga ttg gtt ccc aac tca atg acc ggc tct ccg      288
Pro Phe Met Met Glu Arg Leu Val Pro Asn Ser Met Thr Gly Ser Pro
55          60          65

gat ccg aac tac ctg gca gat ctc ata gcg act gta aat gca atc acc      336
Asp Pro Asn Tyr Leu Ala Asp Leu Ile Ala Thr Val Asn Ala Ile Thr
70          75          80

cag aaa ggt gcc tac gcc gtc gtc gat cct cat aac tac ggc aga tac      384
Gln Lys Gly Ala Tyr Ala Val Val Asp Pro His Asn Tyr Gly Arg Tyr
85          90          95

tac aat tct ata atc tcg agc cct tcc gat ttc cag acc ttc tgg aaa      432
Tyr Asn Ser Ile Ile Ser Ser Pro Ser Asp Phe Gln Thr Phe Trp Lys
100         105         110

acg gtc gcc tca cag ttt gct tcg aat cca ctg gtc atc ttc gac act      480
Thr Val Ala Ser Gln Phe Ala Ser Asn Pro Leu Val Ile Phe Asp Thr
115         120         125         130

aat aac gaa tac cac gat atg gac cag acc tta gtc ctc aat ctc aac      528
Asn Asn Glu Tyr His Asp Met Asp Gln Thr Leu Val Leu Asn Leu Asn
135         140         145

cag gcc gct atc gac gcc atc cgt tcc gcc gga gcc act tcc cag tac      576
Gln Ala Ala Ile Asp Gly Ile Arg Ser Ala Gly Ala Thr Ser Gln Tyr
150         155         160

atc ttt gtc gag ggc aat tcg tgg acc ggg gca tgg acc tgg acg aac      624
Ile Phe Val Glu Gly Asn Ser Trp Thr Gly Ala Trp Thr Trp Thr Asn
165         170         175

gtg aac gat aac atg aaa agc ctg acc gac cca tct gac aag atc ata      672
Val Asn Asp Asn Met Lys Ser Leu Thr Asp Pro Ser Asp Lys Ile Ile
180         185         190

tac gag atg cac cag tac ctg gac tct gac gga tcc ggg aca tca gcg      720
Tyr Glu Met His Gln Tyr Leu Asp Ser Asp Gly Ser Gly Thr Ser Ala
195         200         205         210

acc tgc gta tct tcg acc atc ggt caa gag cga atc acc agc gca acg      768
Thr Cys Val Ser Ser Thr Ile Gly Gln Glu Arg Ile Thr Ser Ala Thr
215         220         225

cag tgg ctc agg gcc aac ggg aag aag ggc atc atc ggc gag ttt gcg      816
Gln Trp Leu Arg Ala Asn Gly Lys Lys Gly Ile Ile Gly Glu Phe Ala
230         235         240

ggc gga gcc aac gac gtc tgc gag acg gcc atc acg ggc atg ctg gac      864
Gly Gly Ala Asn Asp Val Cys Glu Thr Ala Ile Thr Gly Met Leu Asp
245         250         255

tac atg gcc cag aac aca gac gtc tgg act ggc gcc atc tgg tgg gcg      912
Tyr Met Ala Gln Asn Thr Asp Val Trp Thr Gly Ala Ile Trp Trp Ala
260         265         270

gcc ggg ccg tgg tgg gga gac tac ata ttc tcc atg gag ccg gac aat      960
Ala Gly Pro Trp Trp Gly Asp Tyr Ile Phe Ser Met Glu Pro Asp Asn
275         280         285         290

ggc atc gcg tat cag cag ata ctt cct att ttg act ccg tat ctt tga      1008
Gly Ile Ala Tyr Gln Gln Ile Leu Pro Ile Leu Thr Pro Tyr Leu
295         300         305

```

&lt;210&gt; 14

&lt;211&gt; 335

&lt;212&gt; PRT

<213> *Thermoascus aurantiacus*

&lt;400&gt; 14

```

Met Lys Leu Gly Ser Leu Val Leu Ala Leu Ser Ala Ala Arg Leu Thr
-30          -25          -20          -15

Leu Ser Ala Pro Leu Ala Asp Arg Lys Gln Glu Thr Lys Arg Ala Lys
          -10          -5          -1  1

Val Phe Gln Trp Phe Gly Ser Asn Glu Ser Gly Ala Glu Phe Gly Ser
      5          10          15

Gln Asn Leu Pro Gly Val Glu Gly Lys Asp Tyr Ile Trp Pro Asp Pro
      20          25          30

Asn Thr Ile Asp Thr Leu Ile Ser Lys Gly Met Asn Ile Phe Arg Val
35          40          45          50

Pro Phe Met Met Glu Arg Leu Val Pro Asn Ser Met Thr Gly Ser Pro
          55          60          65

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

Gln Lys Gly Ala Tyr Ala Val Val Asp Pro His Asn Tyr Gly Arg Tyr
      85          90          95

Tyr Asn Ser Ile Ile Ser Ser Pro Ser Asp Phe Gln Thr Phe Trp Lys
100          105          110

Thr Val Ala Ser Gln Phe Ala Ser Asn Pro Leu Val Ile Phe Asp Thr
115          120          125          130

Asn Asn Glu Tyr His Asp Met Asp Gln Thr Leu Val Leu Asn Leu Asn
135          140          145

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

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

Val Asn Asp Asn Met Lys Ser Leu Thr Asp Pro Ser Asp Lys Ile Ile
180          185          190

Tyr Glu Met His Gln Tyr Leu Asp Ser Asp Gly Ser Gly Thr Ser Ala
195          200          205          210

Thr Cys Val Ser Ser Thr Ile Gly Gln Glu Arg Ile Thr Ser Ala Thr
          215          220          225

Gln Trp Leu Arg Ala Asn Gly Lys Lys Gly Ile Ile Gly Glu Phe Ala
          230          235          240

Gly Gly Ala Asn Asp Val Cys Glu Thr Ala Ile Thr Gly Met Leu Asp
245          250          255

Tyr Met Ala Gln Asn Thr Asp Val Trp Thr Gly Ala Ile Trp Trp Ala
260          265          270

Ala Gly Pro Trp Trp Gly Asp Tyr Ile Phe Ser Met Glu Pro Asp Asn
275          280          285          290

Gly Ile Ala Tyr Gln Gln Ile Leu Pro Ile Leu Thr Pro Tyr Leu
295          300          305

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## REFERENCES CITED IN THE DESCRIPTION

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11323-DK-EPT

**PATENTKRAV**

1. Fremgangsmåde til fremstilling af en ølurt, hvilken fremgangsmåde omfatter dannelse af en mask ud fra en maltskrå og etablering af kontakt mellem masken og en pullulanase, hvor  
5 pullulanasen har en aminosyresekvens, som er mindst 98% identisk med aminosyresekvensen ifølge SEQ ID NO: 4.
2. Fremgangsmåde ifølge krav 1, hvor pullulanasen stammer fra *Bacillus acidopullulyticus*.
- 10 3. Fremgangsmåde ifølge krav 1 eller 2, hvilken fremgangsmåde endvidere omfatter etablering af kontakt mellem masken og en glucoamylase og/eller alfa-amylase.
4. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor glucoamylasen og/eller alfa-amylasen stammer fra *Aspergillus niger* eller *Talaromyces emersonii*.
- 15 5. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvilken fremgangsmåde endvidere omfatter etablering af kontakt mellem masken og et enzym valgt fra gruppen bestående af cellulase, isoamylase, xylanase og protease.
- 20 6. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor maltskråen omfatter maltede og/eller umaltede kerner.
7. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor de umaltede kerner og/eller de maltede kerner er valgt fra listen bestående af byg, hvede, rug, sorghum,  
25 hirse, majs og ris.
8. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor de maltede kerner omfatter maltede kerner valgt blandt maltet byg, hvede, rug, sorghum, hirse, majs og ris.
- 30 9. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor urten er koncentreret og/eller tørret.

11323-DK-EPT

10. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvilken fremgangsmåde endvidere omfatter fermentering af urten for at opnå en alkoholholdig drikkevare.

5 11. Fremgangsmåde ifølge krav 10, hvor den alkoholholdige drikkevare er en øl.

12. Fremgangsmåde ifølge krav 11, hvor øllen er ale, stærk ale, bitter øl, stout, porter, pilsner, eksportøl, maltøl, barley wine, happoushu, øl med højt alkoholindhold, øl med lavt alkoholindhold, kalorielet øl eller let øl.

10

13. Sammensætning, som er egnet til anvendelse ved fremgangsmåden ifølge et hvilket som helst af de foregående krav, hvilken sammensætning omfatter en pullulanase, en glucoamylase og eventuelt en alfa-amylase, hvor pullulanasen har en aminosyresekvens, som er mindst 98% identisk med aminosyresekvensen ifølge SEQ ID NO: 4.

15

14. Sammensætning ifølge krav 13, hvor glucoamylasen og/eller alfa-amylasen stammer fra *Aspergillus niger* eller *Talaromyces emersonii*.

20