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(54) Benævnelse: **Fremgangsmåde til fremstilling af et fermenteret mælkeprodukt**

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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to a process for producing a fermented milk product.

BACKGROUND OF THE INVENTION

[0002] WO 2009/071539 discloses a lactase originating from *Bifidobacterium bifidum,* which is capable of very efficient hydrolysis in milk, and which is active over a broad pH range, including low pH, e.g. a pH below 5. The lactase may be used in processes for producing milk and fermented milk products, such as cheese, yogurt, butter, butter milk, sour cream etc., for reducing the content of lactose.

[0003] WO 2013/160413 discloses a method of producing a fermented milk product using a combination of glucose-negative lactic acid bacteria strains and a conventional lactase with an object of reducing the content of lactose in the fermented milk product while increasing the content of glucose.

[0004] EP-A1 -2 957 180 in one embodiment discloses a method of producing a fermented milk product using a combination of a starter cultures and a conventional lactase with an object of reducing content of lactose and the level of post-acidification in the fermented milk product. EP-A1-2 957 180 in a second embodiment discloses a method of producing a fermented milk product using lactose-deficient lactic acid bacteria.

SUMMARY OF THE INVENTION

[0005] The object of the present invention is to provide an improved process for producing a fermented milk product.

[0006] The object of the present invention is obtained by a process for producing a fermented milk product comprising the steps of

1. 1) adding a starter culture comprising at least one lactic acid bacteria strain to a milk base,
2. 2) fermenting the milk for a period of time until a target pH is reached,
3. 3) wherein the starter culture comprises at least one lactose-deficient strain, which is capable of metabolizing a non-lactose carbohydrate, and
4. 4) adding a low pH stable lactase to the process either at the start, during or at the end of the fermentation step, wherein the low pH stable lactase retains its activity at a pH of

5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

[0007] Lactose-deficient lactic acid bacteria typically grow on a non-lactose carbohydrate source, such as sucrose, galactose and glucose, added to the milk in an amount measured so as stop the fermentation process and the growth of the lactic acid bacteria by depletion of the added carbohydrate source. Hereby, the post-acidification during subsequent storage is lowered significantly or even fully prevented. A low pH stable lactase will be active during the full course of a fermentation process and hence allows conversion of most or all of the lactose present in milk to glucose and galactose. Hereby, it is possible to produce a fermented milk product with a reduced content of lactose or a lactose-free product. Also, it is possible to produce a fermented milk product with an increased natural sweetness, as glucose and galactose has a much higher sweetness than lactose.

[0008] The present invention is based on the recognition that by using a combination of a low pH stable lactase and lactose-deficient lactic acid bacteria, it is possible to obtain a fermented milk product, which at the same time has reduced lactose content, an increased sweetness and reduced post-acidification. Furthermore, it has surprisingly been found that the said combination results in a fermented milk product having an increased texture as compared to using lactose-deficient lactic acid bacteria and no lactase and as compared to using a low pH stable lactase and lactose-positive lactic acid bacteria.

[0009] Furthermore, when the lactase is added before the fermentation step the process of the invention has provided a possibility of reducing or even eliminating the amount of non-lactose carbohydrate to be added to the milk. Thus, the glucose and galactose formed by the enzymatic action of lactase will be available for the growth of the lactose-deficient lactic acid bacteria.

[0010] Finally, when the lactase is added after the fermentation step, it is possible to take full advantage of the ability of the lactose-deficient lactic acid bacteria to avoid post-acidification.

[0011] Worldwide, a significant numbers of consumers are intolerant or sensitive to lactose. Therefore, there is presently a high demand for dairy products, including fermented milk products, with a reduced content of lactose or which is substantially free of lactose. The present invention has provided a new approach for producing such product in a simple and cost-efficient manner.

DETAILED DISCLOSURE OF THE INVENTION

Lactase

[0012] The lactase of the fermented milk product of the invention may be any lactase, which retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

[0013] In relation to the present invention the activity in LAU of the lactase is measured as specified in the "Definitions" section below.

[0014] In a preferred embodiment of the invention, the lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 10 %, preferably at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum pH of the lactase.

[0015] In a preferred embodiment of the invention, the lactase retains its activity at a pH of 4.0 and a temperature of 37 °C at a level of at least 5 %, preferably at least 10 %, more preferably at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum pH of the lactase.

[0016] In a preferred embodiment of the invention, the lactase retains its activity at a pH of 3.0 and a temperature of 37 °C at a level of at least 5 %, preferably at least 10 %, more preferably at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum pH of the lactase.

[0017] In connection with the present invention the optimum pH of the lactase is determined by measuring the lactase activity at pH using the method indicated in the "Definitions" section below and determining the pH with optimum activity.
In a preferred embodiment of the invention, the lactase retains its activity at a temperature of 10 °C and a pH of 6.0 at a level of at least 10 % as compared to its activity at the optimum temperature of the lactase. Preferably, the lactase retains its activity at a temperature of 10 °C and a pH of 6.0 at a level of at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum temperature of the lactase.

[0018] In connection with the present invention the optimum temperature of the lactase is determined by measuring the lactase activity at different temperatures using the method indicated in the "Definitions" section below and determining the temperature with optimum activity.

[0019] In a preferred embodiment, the lactase to be used in the product of the present invention has a lactase activity at 37°C and pH 5 which is at least 55%, such as at least 60%, at

least 65%, at least 70% or at least 75%, of its lactase activity at 37°C and pH 6.

[0020] In another preferred embodiment, the lactase to be used in the product of the present invention has a lactase activity at 37°C and pH 4.5 which is at least 10%, such as at least 20%, at least 30%, at least 35% or at least 40%, of its lactase activity at 37°C and pH 6.

[0021] In another preferred embodiment, the lactase to be used in the product of the present invention has a pH optimum of the lactase activity at 37°C which is above pH 5.5.

[0022] In another preferred embodiment, the lactase to be used in the product of the present invention has a lactase activity at a temperature of 52°C and a pH of 6.5 which is at least 50%, such as at least 55%, at least 60%, at least 65%, at least 70%, at least 75% or at least 80%, of its lactase activity at a temperature of 38°C and a pH of 6.5.

[0023] In a preferred embodiment of the present invention, Km of the lactase at 5°C is below 25 mM, such as below 20 mM, below 15 mM or below 10 mM. In another preferred embodiment, Km of the lactase at 37°C is below 25 mM, such as below 20 mM or below 15 mM. The skilled person will know how to determine Km for the lactase activity at a specific temperature. Km may be determined by the method described in WO2009/071539.

[0024] In another preferred embodiment, the enzyme when hydrolyzing the lactose in the milk product has a ratio of lactase to transgalactosylase activity of more than 1:1, such as more than 2:1 or more than 3:1. In another preferred embodiment, the enzyme treatment is performed under conditions where the lactase activity of the enzyme is higher than the transgalactosylase activity, such as at least two times higher or at least three times higher.

[0025] The ratio of lactase to transgalactosylase activity in the milk product may, e.g., be determined by HPLC analysis. In another preferred embodiment, the enzyme treatment is performed under conditions where at least 50% (w/w%) of the hydrolyzed lactose is converted into free galactose. In another preferred embodiment, the enzyme treatment is performed under conditions where the hydrolyzed lactose is converted into equal amounts of free glucose and free galactose.

[0026] A lactase in the context of the present invention is a glycoside hydrolase having the ability to hydrolyze the disaccharide lactose into constituent galactose and glucose monomers. The group of lactases, to which the lactase of the invention belongs, comprises but is not limited to enzymes assigned to subclass EC 3.2.1.108. Enzymes assigned to other subclasses, such as, e.g., EC 3.2.1.23, may also be lactases in the context of the present invention. A lactase in the context of the invention may have other activities than the lactose hydrolyzing activity, such as for example a transgalactosylating activity. In the context of the invention, the lactose hydrolyzing activity of the lactase may be referred to as its lactase activity or its beta-galactosidase activity.

[0027] Enzymes having lactase activity to be used in a method of the present invention may be

of animal, of plant or of microbial origin. Preferred lactases are obtained from microbial sources, in particular from a filamentous fungus or yeast, or from a bacterium.

[0028] The enzyme may, e.g., be derived from a strain of *Agaricus,* e.g. *A. bisporus; Ascovaginospora; Aspergillus,* e.g. *A. niger, A. awamori, A. foetidus, A. japonicus, A. oryzae; Candida; Chaetomium; Chaetotomastia; Dictyostelium,* e.g. *D. discoideum; Kluveromyces,* e.g. *K. fragilis, K. lactis; Mucor,* e.g. *M. javanicus, M. mucedo, M. subtilissimus; Neurospora,* e.g. *N. crassa; Rhizomucor,* e.g. *R. pusillus; Rhizopus,* e.g. *R. arrhizus, R. japonicus, R. stolonifer; Sclerotinia,* e.g. *S. libertiana; Torula; Torulopsis; Trichophyton,* e.g. *T. rubrum; Whetzelinia,* e.g. *W. sclerotiorum*; *Bacillus,* e.g. *B. coagulans, B. circulans, B. megaterium, B. novalis, B. subtilis, B. pumilus, B. stearothermophilus, B. thuringiensis*; *Bifidobacterium,* e.g. *B. longum, B. bifidum, B. animalis; Chryseobacterium; Citrobacter,* e.g. *C. freundii; Clostridium,* e.g. *C. perfringens; Diplodia,* e.g. *D. gossypina; Enterobacter,* e.g. *E. aerogenes, E. cloacae Edwardsiella, E. tarda; Erwinia,* e.g. *E. herbicola; Escherichia,* e.g. *E. coli; Klebsiella,* e.g. *K. pneumoniae; Miriococcum; Myrothesium; Mucor; Neurospora,* e.g. *N. crassa; Proteus,* e.g. *P. vulgaris; Providencia,* e.g. *P. stuartii; Pycnoporus,* e.g. *Pycnoporus cinnabarinus, Pycnoporus sanguineus; Ruminococcus,* e.g. *R. torques; Salmonella,* e.g. *S. typhimurium*; *Serratia,* e.g. *S. liquefasciens, S. marcescens; Shigella,* e.g. *S. flexneri; Streptomyces,* e.g. *S. antibioticus, S. castaneoglobisporus, S. violeceoruber; Trametes; Trichoderma,* e.g. *T. reesei, T. viride; Yersinia,* e.g. *Y. enterocolitica.*

[0029] In a preferred embodiment, the lactase originates from a bacterium, e.g. from the family Bifidobacteriaceae, such as from the genus *Bifidobacterium,* such as from a strain of *B. bifidum, B. animalis* or *B. longum.* In a more preferred embodiment, the lactase originates from *Bifidobacterium bifidum.*

[0030] In a preferred embodiment, an enzyme having lactase activity to be used in the product of the present invention comprises an amino acid sequence which is at least 50% identical to a sequence selected from the group consisting of amino acids 28-1931 of SEQ ID NO: 1, amino acids 28-1331 of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and lactase active fragments thereof. In a more preferred embodiment, the enzyme comprises an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to a sequence selected from the group consisting of amino acids 28-1931 of SEQ ID NO: 1, amino acids 28-1331 of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and lactase active fragments thereof.

[0031] A preferred enzyme is a lactase having a sequence which is at least 50%, such as at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to amino acids 28-1931 of SEQ ID NO: 1 or to a lactase active fragment thereof. Such lactase active fragment of SEQ ID NO: 1 may be any fragment of SEQ ID NO: 1 having lactase activity. A lactase active fragment of SEQ ID NO: 1 may be, e.g., amino acids 28-979, amino acids 28-1170, amino acids 28-1323, amino acids 28-1331, or amino acids 28-1600 of SEQ ID NO: 1.

[0032] In a preferred embodiment, an enzyme having lactase activity to be used in the product

of the present invention comprises an amino acid sequence which is at least 50% identical to amino acids 28-1331 of SEQ ID NO: 2. In a more preferred embodiment, the enzyme comprises an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to amino acids 28-1331 of SEQ ID NO: 2.

[0033] In another embodiment, an enzyme having lactase activity to be used in product of the present invention has an amino acid sequence which is at least 50% identical to SEQ ID NO: 3. Preferably, the enzyme has an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to SEQ ID NO: 3.

[0034] In another embodiment, an enzyme having lactase activity to be used in the product of the present invention has an amino acid sequence which is at least 50% identical to SEQ ID NO: 4. Preferably, the enzyme has an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to SEQ ID NO: 4.

[0035] For purposes of the present invention, the degree of identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch (1970) J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al. (2000) Trends in Genetics 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labelled "longest identity" (obtained using the -no brief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

[0036] A particular commercial lactase suitable for use in the present invention is Lactase F "Amano" 100SD available from Amano Enzyme Europe.

[0037] Lactases to be used in a method of the present invention may be extracellular. They may have a signal sequence at their N-terminus, which is cleaved off during secretion.

[0038] Lactases to be used in a method of the present invention may be derived from any of the sources mentioned herein. The term "derived" means in this context that the enzyme may have been isolated from an organism where it is present natively, i.e. the identity of the amino acid sequence of the enzyme are identical to a native enzyme. The term "derived" also means that the enzymes may have been produced recombinantly in a host organism, the recombinantly produced enzyme having either an identity identical to a native enzyme or having a modified amino acid sequence, e.g. having one or more amino acids which are deleted, inserted and/or substituted, i.e. a recombinantly produced enzyme which is a mutant and/or a fragment of a native amino acid sequence. Within the meaning of a native enzyme are included natural variants. Furthermore, the term "derived" includes enzymes produced

synthetically by, e.g., peptide synthesis. The term "derived" also encompasses enzymes which have been modified e.g. by glycosylation, phosphorylation etc., whether *in vivo* or *in vitro.* With respect to recombinantly produced enzyme the term "derived from" refers to the identity of the enzyme and not the identity of the host organism in which it is produced recombinantly.

[0039] The lactase may be obtained from a microorganism by use of any suitable technique. For instance, a lactase enzyme preparation may be obtained by fermentation of a suitable microorganism and subsequent isolation of a lactase preparation from the resulting fermented broth or microorganism by methods known in the art. The lactase may also be obtained by use of recombinant DNA techniques. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector comprising a DNA sequence encoding the lactase in question and the DNA sequence being operationally linked with an appropriate expression signal such that it is capable of expressing the lactase in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may also be incorporated into the genome of the host cell. The DNA sequence may be of genomic, cDNA or synthetic origin or any combinations of these, and may be isolated or synthesized in accordance with methods known in the art.

[0040] Lactases to be used in a method of the present invention may be purified. The term "purified" as used herein covers lactase enzyme protein essentially free from insoluble components from the production organism. The term "purified" also covers lactase enzyme protein essentially free from insoluble components from the native organism from which it is obtained. Preferably, it is also separated from some of the soluble components of the organism and culture medium from which it is derived. More preferably, it is separated by one or more of the unit operations: filtration, precipitation, or chromatography.

[0041] Accordingly, the enzyme having lactase activity may be purified, viz. only minor amounts of other proteins being present. The expression "other proteins" relate in particular to other enzymes. The term "purified" as used herein also refers to removal of other components, particularly other proteins and most particularly other enzymes present in the cell of origin of the lactase. The lactase may be "substantially pure", i.e. free from other components from the organism in which it is produced, i.e., e.g., a host organism for recombinantly produced lactase. Preferably, the lactase is an at least 40% (w/w) pure enzyme protein preparation, more preferably at least 50%, 60%, 70%, 80% or even at least 90% pure.

[0042] The term enzyme having lactase activity includes whatever auxiliary compounds that may be necessary for the enzyme's catalytic activity, such as, e.g., an appropriate acceptor or cofactor, which may or may not be naturally present in the reaction system.

[0043] The enzyme may be in any form suited for the use in question, such as, e.g., in the form of a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a protected enzyme.

Lactose-deficient lactic acid bacteria

[0044] The terms "deficiency in lactose metabolism" and "lactose deficient" are used in the context of the present invention to characterize LAB which either partially or completely lost the ability to use lactose as a source for cell growth or maintaining cell viability. Respective LAB are capable of metabolizing one or several carbohydrates selected from sucrose, galactose and/or glucose or another fermentable carbohydrate. Since these carbohydrates are not naturally present in milk in sufficient amounts to support fermentation by lactose deficient mutants, it is necessary to add these carbohydrates to the milk. Lactose deficient and partially deficient LAB can be characterized as white colonies on a medium containing lactose and X-Gal.

[0045] In a particular embodiment of the invention, the lactose-deficient strain is capable of metabolizing a non-lactose carbohydrate selected from the group consisting of sucrose, galactose and glucose, preferably sucrose. In a particular embodiment of the invention, the lactose-deficient strain is capable of metabolizing galactose.

[0046] In a particular embodiment of the invention, the lactose-deficient strain is selected from the group consisting of lactose-deficient *Streptococcus thermophilus* and lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus.*

[0047] In a particular embodiment of the invention, the lactose-deficient strain is selected from the group consisting of:

1. (a) a *Streptococcus thermophilus* strain, which strain is:
    1. (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28952;
    2. (ii) or a strain derived from DSM 28952, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal;
2. (b) a *Streptococcus thermophilus* strain, which strain is:
    1. (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28953;
    2. (ii) or a strain derived from DSM 28953, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal;
3. (c) a *Lactobacillus delbrueckii* ssp. *bulgaricus* strain, which strain is:
    1. (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28910;
    2. (ii) or a strain derived from DSM 28910, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal.

[0048] In a particular embodiment of the invention, the starter culture contains both at least one lactose-deficient *Streptococcus thermophilus* and at least one lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus.*

[0049] In a particular embodiment of the invention, the starter culture contains both at least one *Streptococcus thermophilus* and at least one *Lactobacillus delbrueckii* subsp. *bulgaricus,* and wherein all *Streptococcus thermophilus* and all *Lactobacillus delbrueckii* subsp. *bulgaricus* strains are lactose-deficient.

Steps of the process of the invention

[0050] In a particular embodiment of the invention, the fermentation step is terminated by a method selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, 2) cooling treatment and 3) depletion of the non-lactose carbohydrate.

[0051] In a particular embodiment of the invention, non-lactose carbohydrate is added to the milk base at the start of the fermentation step.

[0052] Preferably, the non-lactose carbohydrate is added to the milk base in an amount measured so as to become depleted and hence result in stopping the growth of lactic acid bacteria and in stopping the fermentation. Preferably, the non-lactose carbohydrate is added to the milk base in an amount measured so as to become depleted and hence result in stopping the growth of lactic acid bacteria and in stopping the fermentation at a selected target pH value.

[0053] The amount of non-lactose carbohydrate to be added to the milk base depends on a number of parameters, including the lactic acid bacteria strains used in the starter culture, the composition of the milk base, the fermentation temperature and the desired target pH. Also, the amount of non-lactose carbohydrate to be added to the milk base may depend on the type and amount of lactase used in the process. The amount of non-lactose carbohydrate to be added to the milk base can be determined by experimentation, and it is well within the skills of a skilled person to carry out such experimentation.

[0054] In a particular embodiment of the invention, the added non-lactose carbohydrate is selected from the group consisting of sucrose, galactose and glucose, preferably sucrose.

[0055] In a first aspect of the invention, the low pH stable lactase is added to the milk base at the start of the fermentation step. In this aspect the added lactase will convert the lactose of the milk base to glucose and galactose, which will be available for metabolization for the starter culture in addition to the added non-lactose carbohydrate. In this case, it will not be possible to

stop the fermentation by depletion of the added non-lactose carbohydrate. Thus, in a particular embodiment of the first aspect of the invention, the fermentation step is terminated by a method selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, and 2) cooling treatment.

[0056] In a particular embodiment of the first aspect of the invention no non-lactose carbohydrate is added to the fermentation step and at least one lactose-deficient lactic acid strain of the starter culture is capable of metabolizing a carbohydrate selected from the group consisting of glucose and galactose. In this embodiment the lactose-deficient lactic acid strain of the starter culture grows solely on the glucose and/or galactose formed by the enzymatic action of the low pH lactase. In this embodiment of the invention, the fermentation is stopped at a target pH value by a method selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, 2) cooling treatment and 3) depletion of the glucose and/or galactose formed by the low pH stable lactase.

[0057] In a second aspect of the invention, the low pH stable lactase is added to the fermented milk at the end of the fermentation. In this case, the lactase-containing fermented milk product is preferably stored at a temperature of at least 2 °C for at least 1 day. In a particular embodiment of the process of the invention, the lactase-containing fermented milk product is stored for at least two days, preferably at least 3 days, more preferably at least 4 days, more preferably at least 5 days, more preferably at least 6 days, and most preferably at least 7 days.

[0058] In a particular embodiment of the second aspect of the invention, the fermentation step is terminated by depletion of the non-lactose carbohydrate.

[0059] In a particular embodiment of the invention the target pH is between 3.2 and 4.8, more preferably between 3.6 and 4.6, more preferably between 3.8 and 4.5 and most preferably between 4.0 and 4.4.

[0060] In a particular embodiment of the invention the fermentation temperature is between 35 °C and 45 °C, preferably between 37 °C and 43 °C, and more preferably between 40 °C and 43 °C.

[0061] In another particular embodiment of the invention the fermentation is between 15 °C and 35 °C, preferably between 25 °C and 35 °C, and more preferably between 30 °C and 33 °C.

[0062] In a particular embodiment of the invention, the fermented milk product is packaged at a temperature between 15 and 45 °C.

[0063] In a particular embodiment of the invention, the pH value of the fermented milk product is maintained within a range of 0.3 pH units, preferably within a range of 0.2 pH units and most preferably within a range of 0.1 pH units, when stored after termination of the fermentation at

the temperature used for fermentation over a period of 20 hours.

[0064] In a particular embodiment of the invention, the amount of added non-lactose carbohydrate is from 1 mg/g to 30 mg/g, preferably from 2 mg/g to 20 mg/g, and more preferably from 3 mg/g to 10 mg/g milk base.

[0065] In a particular embodiment of the invention, the amount of added non-lactose carbohydrate is from 0.1 % to 10 %, preferably from 0.2 % to 8 %, preferably from 0.3 % to 2 %, preferably from 0.4 % to 1.5 %, and more preferably from 0.5 % to 1.2 %, wherein % is (w/w) based on milk base.

[0066] The starter culture may have the strain composition of any conventional starter culture of lactic acid bacteria, including single strain culture and culture blends, used for producing a specific type of fermented milk product. Other useful bacteria, which may be added to the product in addition to the starter culture, include the probiotic bacteria *Bifidobacterium spp.*

[0067] In a particular embodiment of the invention, the fermented milk product after fermentation is subjected to a heat treatment so as to reduce the level of bacteria of the starter culture to no more than 1X10exp02 CFU per g fermented milk product. In this case a particular embodiment is characterized in that the lactase is added after the heat treatment. In this case the lactase-containing fermented milk product is stored at a temperature of at least 2 °C for at least 1 day. In a particular embodiment of the process of the invention, the lactase-containing fermented milk product is stored for at least two days, preferably at least 3 days, more preferably at least 4 days, more preferably at least 5 days, more preferably at least 6 days, and most preferably at least 7 days.

[0068] The heat treatment so as to reduce the level of bacteria of the starter culture to no more than 1.0X10exp02 CFU per g fermented milk is preferably carried out by subjecting the starter culture fermented milk product to a temperature of between 50 °C and 110 °C, preferably between 50 °C and 100 °C, preferably between 50 °C and 90 °C, preferably between 60 °C and 85 °C, more preferably between 65 °C and 82 °C, and most preferably between 70 °C and 80 °C. The heat treatment is preferably carried out for a period of between 5 seconds and 180 seconds, preferably between 5 seconds and 120 seconds, more preferably between 5 seconds and 90 seconds, more preferably between 5 seconds and 60 seconds, more preferably between 8 seconds and 50 seconds and most preferably between 10 and 40 seconds. Preferably, the level of bacteria of the starter culture is reduced to no more than 1.0X10exp01 CFU per g fermented milk, more preferably 0 CFU per g. Fermented milk products subjected to a heat treatment so as to reduce the level of bacteria to no more than 1X10exp02 CFU per g are suitable for use storage at ambient temperature, such as storage at a temperature of at least 5 °C, preferably at least 10 °C, more preferably at least 15 °C, and most preferably at least 20 °C.

[0069] The low pH stable lactase is added in a suitable amount to achieve the desired degree of lactose hydrolysis under the chosen reaction conditions. In a particular embodiment of the

invention, lactase is added in an amount of between 100 and 20000 LAU per liter milk base, preferably between 100 and 10000 LAU per liter milk base, preferably between 100 and 5000 LAU per liter milk base, preferably less than 3000, such as less than 1500, less than 1000, less than 750 or less than 500, LAU per liter milk base.

[0070] In a preferred embodiment, the lactase is added at a concentration of between 5 and 400 LAU per g lactose in the milk base, preferably between 5 and 200 LAU per g lactose in the milk base, preferably between 5 and 100 LAU per g lactose in the milk base, preferably less than 50, such as less than 40, less than 30, less than 20 or less than 10, LAU per g lactose in the milk base.

[0071] In a preferred embodiment of the invention, the lactase is added to the milk base in an amount of between 2.0 mg/ml and 50 mg/ml, preferably between 5 mg/ml and 48 mg/ml, more preferably between 10 mg/ml and 46 mg/ml, and most preferably between 20 mg/ml and 45 mg/ml.

[0072] In a preferred embodiment of the invention, the residual level of lactose at the end of fermentation is less than 40 mg/ml, preferably less than 35 mg/ml, more preferably less than 30 mg/ml, more preferably less than 25 mg/ml, more preferably less than 20 mg/ml, more preferably less than 15 mg/ml, more preferably less than 10 mg/ml, more preferably less than 5 mg/ml, more preferably less than 3 mg/ml, and most preferably less than 1.5 mg/ml.

[0073] In a preferred embodiment of the invention, the milk base at the start of the fermentation step has a content of lactose of between 30.0 mg/ml and 70 mg/ml, preferably between 35 mg/ml and 65 mg/ml, more preferably between 40 mg/ml and 60 mg/ml, and most preferably between 50 mg/ml and 60 mg/ml.

[0074] In a preferred embodiment of the invention, wherein the low pH-stable lactase is added to the process at the end of the fermentation step, the fermented milk product, to which the lactase is to be added, has a viscosity, which allows easy distribution of the lactase in the fermented milk product, e.g. by mixing.

[0075] In a preferred embodiment of the process of the invention, the lactase to be added to the process is provided in a sterile formulation. In another preferred embodiment of the process of the invention the lactase is added to the process under aseptic conditions, e.g. by sterile filtration of a solution of the lactase.

Fermented milk product

[0076] The present disclosure further relates to a fermented milk product produced by the process of the invention.

[0077] In a particular aspect the fermented milk product produced by the process of the

invention comprises the starter culture of step 3) of the said process and the low pH stable lactase added in step 4) of the said process.

[0078] In a particular aspect the fermented milk product is a product, which may be produced using a lactic acid bacteria strain selected from the group consisting of lactose-deficient *Streptococcus thermophilus* and lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus.*

[0079] In a particular aspect the fermented milk product is selected form the group consisting of yogurt, cream cheese, sour milk, sour cream, buttermilk, fermented whey, cultured milk, Smetana, Kefir, drinking yogurt, and Yakult. Preferably, the yogurt is selected from the group consisting of set yogurt, stirred yogurt and drinking yogurt.

[0080] In a preferred aspect the fermented milk product contains a further food product selected from the group consisting of fruit beverage, fermented cereal products, chemically acidified cereal products, soy milk products and any mixture thereof.

[0081] The fermented milk product typically contains protein in a level of between 2.0 % by weight to 3.5 % by weight. The fermented milk product may also be a low protein product with a protein level of between 1.0 % by weight and 2.0 % by weight. Alternatively, the fermented milk product may be a high protein product with a protein level of above 3.5 % by weight.

Use

[0082] The present invention further relates to the use in a process for producing a fermented milk product comprising the steps of

1. 1) adding a starter culture comprising at least one lactic acid bacteria strain to a milk base, and
2. 2) fermenting the milk for a period of time until a target pH is reached, of
3. 3) the starter culture comprising at least one lactose-deficient strain, which is capable of metabolizing a non-lactose carbohydrate, and
4. 4) a low pH stable lactase added to the process either at the start, during or at the end of the fermentation step, wherein the low pH stable lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

Definitions

[0083] In connection with the present invention the following definitions apply:
"LAU" means "Lactose Units" and 1 lactase unit (1 LAU) is the amount of enzyme which

releases 1 micromole glucose per minute in M-buffer at pH 6.5 and 37°C with a lactose concentration of 4.75% w/v. M-buffer is prepared by dissolving 3.98 g $C_6H_5Na_3O_7$-$2H_2O$, 8.31 g citric acid, 0.9 g K_2SO_4, 2.6 g K_2HPO_4, 7.35 g KH_2PO_4, 5.45 g KOH, 4.15 g $MgCl_2$-$6H_2O$, 3.75 g $CaCl_2$-$2H_2O$ and 1.4 g $NaHCO_3$ in 4 liter water, adding 12.5 ml 4N NaOH, adjusting to pH 6.5 using HCl, and adding water up to a total volume of 5 liter.

[0084] The activity in LAU of a specific lactase may be determined by direct measurement of glucose released from lactose under the conditions described above. The skilled person will know how to determine such activity. Alternatively, the activity may be determined by using the lactase activity assay described in Example 1 of the present application. Here, the activity is obtained by comparing to a standard curve run with a lactase of known activity, and the activity of the unknown sample calculated from this. The lactase of known activity may, e.g., be Lactozym obtained from Novozymes A/S, Denmark.

[0085] The expression "heat treatment" means any treatment using any temperature, for any period of time and by any means or equipment, which inactivates at least a portion of the bacteria of the starter culture. In this connection the term "inactivate" means any stop, reduction or inhibition of growth of the bacteria, e.g. cell lysing.

[0086] The expression "lactic acid bacteria" ("LAB") designates a gram-positive, microaerophilic or anaerobic bacteria, which ferment sugars with the production of acids including lactic acid as the predominantly produced acid, acetic acid and propionic acid. The industrially most useful lactic acid bacteria are found within the order "Lactobacillales" which includes *Lactococcus spp., Streptococcus spp., Lactobacillus spp., Leuconostoc spp.,* Pseudoleuconostoc spp., *Pediococcus spp., Brevibacterium spp., Enterococcus spp.* and *Propionibacterium spp.* These are frequently used as food cultures alone or in combination with other lactic acid bacteria.

[0087] Lactic acid bacteria, including bacteria of the species Lactobacillus sp. and Lactococcus sp., are normally supplied to the dairy industry either as frozen or freeze-dried cultures for bulk starter propagation or as so-called "Direct Vat Set" (DVS) cultures, intended for direct inoculation into a fermentation vessel or vat for the production of a dairy product, such as a fermented milk product or a cheese. Such lactic acid bacterial cultures are in general referred to as "starter cultures" or "starters". Typically, a starter culture for yogurt comprises *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* and in most countries a yogurt is by legislation defined as a fermented milk product produced using a starter culture comprising the two said strains.

[0088] The term "milk" is to be understood as the lacteal secretion obtained by milking of any mammal, such as cows, sheep, goats, buffaloes or camels. In a preferred embodiment, the milk is cow's milk. The term milk also includes protein/fat solutions made of plant materials, e.g. soy milk.

[0089] The term "milk base" may be any raw and/or processed milk material that can be

subjected to fermentation according to the method of the invention. Thus, useful milk bases include, but are not limited to, solutions/-suspensions of any milk or milk like products comprising protein, such as whole or low fat milk, skim milk, buttermilk, reconstituted milk powder, condensed milk, dried milk, whey, whey permeate, lactose, mother liquid from crystallization of lactose, whey protein concentrate, or cream. Obviously, the milk base may originate from any mammal, e.g. being substantially pure mammalian milk, or reconstituted milk powder.

[0090] Prior to fermentation, the milk base may be homogenized and pasteurized according to methods known in the art.

[0091] "Homogenizing" as used herein means intensive mixing to obtain a soluble suspension or emulsion. If homogenization is performed prior to fermentation, it may be performed so as to break up the milk fat into smaller sizes so that it no longer separates from the milk. This may be accomplished by forcing the milk at high pressure through small orifices.

[0092] "Pasteurizing" as used herein means treatment of the milk base to reduce or eliminate the presence of live organisms, such as microorganisms. Preferably, pasteurization is attained by maintaining a specified temperature for a specified period of time. The specified temperature is usually attained by heating. The temperature and duration may be selected in order to kill or inactivate certain bacteria, such as harmful bacteria. A rapid cooling step may follow.

[0093] "Fermentation" in the methods of the present invention means the conversion of carbohydrates into alcohols or acids through the action of a microorganism. Preferably, fermentation in the methods of the invention comprises conversion of lactose to lactic acid.

[0094] Fermentation processes to be used in production of dairy products are well known and the person of skill in the art will know how to select suitable process conditions, such as temperature, oxygen, amount and characteristics of microorganism(s) and process time. Obviously, fermentation conditions are selected so as to support the achievement of the present invention, i.e. to obtain a dairy product in solid (such as a cheese) or liquid form (such as a fermented milk product).

[0095] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising", "having", "including" and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly

contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0096] The expression "fermented milk product" means a food or feed product wherein the preparation of the food or feed product involves fermentation of a milk base with a lactic acid bacteria. "Fermented milk product" as used herein includes but is not limited to products such as thermophilic fermented milk products, e.g. yoghurt, mesophilic fermented milk products, e.g. sour cream and buttermilk, as well as fermented whey.

[0097] The term "thermophile" herein refers to microorganisms that thrive best at temperatures above 35°C. The industrially most useful thermophilic bacteria include Streptococcus spp. and Lactobacillus spp. The term "thermophilic fermentation" herein refers to fermentation at a temperature above about 35°C, such as between about 35°C to about 45°C. The term "thermophilic fermented milk product" refers to fermented milk products prepared by thermophilic fermentation of a thermophilic starter culture and include such fermented milk products as set-yoghurt, stirred-yoghurt and drinking yoghurt, e.g. Yakult.

[0098] The term "mesophile" herein refers to microorganisms that thrive best at moderate temperatures (15°C-35°C). The industrially most useful mesophilic bacteria include Lactococcus spp. and Leuconostoc spp. The term "mesophilic fermentation" herein refers to fermentation at a temperature between about 22°C and about 35°C. The term "mesophilic fermented milk product" refers to fermented milk products prepared by mesophilic fermentation of a mesophilic starter culture and include such fermented milk products as buttermilk, sour milk, cultured milk, smetana, sour cream, Kefir and fresh cheese, such as quark, tvarog and cream cheese.

[0099] In connection with the present invention, "shear stress" may be measured by the following method:
Seven days after production, the fermented milk product was brought to 13°C and manually stirred gently by means of a spoon (5 times) until homogeneity of the sample. The rheological properties of the sample were assessed on a rheometer (Anton Paar Physica Rheometer with ASC, Automatic Sample Changer, Anton Paar® GmbH, Austria) by using a bob-cup. The rheometer was set to a constant temperature of 13 °C during the time of measurement. Settings were as follows:

Holding time (to rebuild to somewhat original structure)

5 minutes without any physical stress (oscillation or rotation) applied to the sample. Oscillation step (to measure the elastic and viscous modulus, G' and G", respectively, therefore calculating the complex modulus G*)

Constant strain = 0.3 %, frequency (f) = [0.5..8] Hz

6 measuring points over 60 s (one every 10 s)

Rotation step (to measure shear stress at 300 1/s)

Two steps were designed:

1. 1) Shear rate = [0.3-300] 1/s and 2) Shear rate = [275-0.3] 1/s.

Each step contained 21 measuring points over 210 s (on every 10 s).

The shear stress at 300 1/s was chosen for further analysis, as this correlates to mouth thickness when swallowing a fermented milk product.

[0100] In connection with the present invention, "gel firmness" may be measured by the following method:
Gel firmness is measured by a back extrusion test with a texture analyzer (TA.XT Plus, Stable Micro System, Surrey, UK) supplied with a 35mm parallel plate. The travel distance is set to 15 mm, and the travel speed to 2 mm/s. The test is performed after 7 days from production. The fermented milk product was brought to 13°C and manually stirred gently, and measured in a 250 g plastic container. The maximal force (N or g) obtained by force versus distance curves is used as "gel firmness" parameter, the positive area (N* mm) as degree of deformation, the maximal negative force (N) as ropiness.

[0101] The term "low pH stable lactase" herein refers to a lactase, which retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

[0102] The term "activity at the optimum pH" means the lactase activity at the pH, where the lactase has its optimum activity.

[0103] The term "non-lactose carbohydrate" means any carbohydrate, which is not lactose, and which a lactose-deficient lactic acid bacterium used in the process of the invention is capable of metabolizing.

[0104] The expression "at the start of the fermentation step" means shortly before, at the same time as or shortly after addition of the starter culture to the milk base. Here, the term "shortly" means less than 30 minutes".

[0105] The expression "during the fermentation step" means at any time during the fermentation after the start and before the end of the fermentation.

[0106] The expression "at the end of the fermentation step" means shortly before, at the same time as or shortly after the target pH is reached. Here, the term "shortly" means less than 30 minutes".

[0107] The term "target pH" means the pH at which the fermentation step ends. Depending on various parameters of the process, the fermentation step is terminated by a method selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, 2) cooling treatment and 3) depletion of the non-lactose carbohydrate.

DEPOSITS AND EXPERT SOLUTION

[0108] The Applicant requests that a sample of the deposited microorganism should be made available only to an expert approved by the Applicant.

[0109] *Streptococcus thermophilus* strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28952.

[0110] *Streptococcus thermophilus* strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28953.

[0111] *Lactobacillus delbrueckii* ssp. *bulgaricus* strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28910;
The deposits were made according to the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

EXAMPLES

Example 1

Lactase activity-assay in Eppendorf tubes at 37°C, pH 6.5

Principle:

[0112] Lactase hydrolyses lactose into glucose and galactose. Glucose is measured after a modified version of the common glucose oxidase / peroxidase assay (Werner, W. et al. (1970) Z. analyt. Chem. 252: 224.).

[0113] LAU is defined as the amount of enzyme liberating 1 micromole of glucose per min at 37°C, pH 6.5 in M-buffer (M-buffer is defined in the description of the present patent application). Alternatively, the activity in LAU for a specific lactase may be determined by the method described here. The value obtained is compared to a standard curve run with a lactase of known activity, and the activity of the unknown sample calculated from this. The lactase of known activity may, e.g., be Lactozym obtained from Novozymes A/S, Denmark.

Solutions:

[0114] Assay buffer: 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01% Triton X100, pH 6.5

[0115] GOD-Perid solution: 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP (Horse Radish Peroxidase), 0.65 g/l ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)).

Substrate:

[0116] 160 mM lactose, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM $CaCl_2$, 1 mom $MgCl_2$, pH 6.5.

Standard:

[0117] Lactozym (available from Novozymes A/S, Denmark) with a known activity in LAU/g is used as standard, diluted in assay buffer in the range from 0.09 - 0.7 LAU/g.

Sam ples:

[0118] Enzyme samples are diluted appropriately in assay buffer.

Procedure:

[0119]

1. 1. 375 µl substrate is incubated 5 minutes at 37°C.
2. 2. 25 µl enzyme diluted in assay buffer is added.
3. 3. The reaction is stopped after 30 minutes by adding 60 µl 1 M NaOH

4. 4. 20 µl is transferred to a 96 well microtiter plate and 200 µl GOD-Perid solution is added. After 30 minutes at room temperature, the absorbance is measured at 420 nm.

Example 2

[0120] 100 ml 15 or 30%(w/w) whey permeate containing primarily lactose and ions was made by mixing 15 or 30 g spray-dried whey permeate powder (Variolac, Aria) in 85 or 70 ml ionic water respectively. The solution was poured in a flask containing a magnetic stirring bar and placed in a water bath at 37°C. After 15 min, enzyme was added. Enzymes tested were Lactozym, a commercially available lactase from Novozymes A/S, Denmark, having an activity of 3060 LAU/g, and an experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 and an activity of 295 LAU/g.

[0121] Dosages were 4225 LAU/l milk of Lactozym and 2025 LAU/l milk of the *Bifidobacterium* lactase. Milk samples were taken at regular intervals up till 5.5 hrs. and the enzyme was inactivated by heating to 99°C for 10 min in a thermomixer. Samples were diluted appropriately and filtered through a 0.20 µm filter.

[0122] Lactose hydrolysis was measured using a Dionex BioLC equipped with a Dionex PA1 column and a Pulsed Amperiometrisk Detektor (PAD). Peaks were identified and quantified by comparing with known standards of lactose, glucose and galactose.

[0123] Results are given below.

Table 1: Lactose, glucose and galactose in 15% DS whey permeate after treatment with Lactozym or *Bifidobacterium* lactase.

	Lactozym			*Bifidobacterium* lactase		
Time min	Lactose mM	Glucose mM	Galactose mM	Lactose mM	Glucose mM	Galactose mM
0	499	1	2	499	1	2
30	312	135	106	410	61	63
60	211	224	155	349	119	122
120	110	295	221	220	199	202
180	66	324	249	149	281	290
240	50	346	279	84	336	348
330	37	372	312	31	350	368

Table 2: Lactose, glucose and galactose in 30% DS whey permeate after treatment with Lactozym or *Bifidobacterium* lactase.

	Lactozym			*Bifidobacterium* lactase		
Time min	Lactose mM	Glucose mM	Galactose mM	Lactose mM	Glucose mM	Galactose mM
0	848	1	4	848	1	4
30	824	109	75	819	43	45
60	615	253	150	788	86	83
120	420	370	242	651	159	158
180	291	459	300	625	232	230
240	246	559	373	501	283	273
330	154	544	367	391	333	324
1440	54	649	545	20	727	739

[0124] Also when tested at higher lactose concentrations as in 15% or 30% whey permeate no or very little galactooligosaccharides are produced. Again, the produced galactose and glucose levels are equal and match the amount of lactose hydrolyzed. For comparison, Lactozym produces less galactose than glucose, clearly showing that galactooligosaccharides have been produced.

Example 3

[0125] *pH profile (at 37°C) and temperature profile (at pH 6.5) of experimental lactase from Bifidobacterium bifidum using lactose as substrate.*

Principle:

[0126] Lactase hydrolyses lactose and glucose + galactose is formed. Glucose is measured after a modified version of the common glucose oxidase / peroxidase assay (Werner, W. et al. (1970) Z. analyt. Chem. 252: 224.)

pH profile

Substrate:

[0127] 167 mM lactose, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and pH adjusted to pH 3, 4, 5, 6, 7, 8, 9 and 10 with NaOH.

Enzyme Sample:

[0128] Experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 was diluted appropriately in 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01% Triton X100.

Procedure:

[0129]

- 10 µl enzyme sample diluted in enzyme dilution buffer was added to PCR tubes at room temp.
- 90 µl substrate was added at room temp. and quickly placed in a Peltier Thermal Cycler (PCT-200, MJ research) at 37ºC and incubated for 30 min and then placed on ice.
- The reaction was stopped by adding 100 µl 0.25 M NaOH.
- 20 µl was transferred to a 96 well microtitre plate and 230 µl 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP, 0.65 g/l ABTS solution was added. After 30 minutes at room temperature, the absorbance was measured at 420 nm.

Table 3:

pH	*B. bifidum* lactase relative activity (% of activity at pH6)
3	0
4	4
5	75
6	100
7	85
8	39
9	10
10	4

Temperature profile

Substrate:

[0130] 167 mM lactose, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM

$CaCl_2$, 1 mM $MgCl_2$ and pH adjusted to pH 6.5 with NaOH.

Enzyme Sample:

[0131] Experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 was diluted appropriately in 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01% Triton X100 and pH adjusted to pH 6.5.

Procedure:

[0132]

- 10 µl enzyme sample diluted in enzyme dilution buffer was added to PCR tubes at room temp.
- 90 µl preheated (in a Peltier Thermal Cycler 30-70°C) substrate was added and incubation was performed with a temp. gradient from 30-70°C for 30 min. and then placed on ice.
- The reaction was stopped by adding 100 µl 0.25 M NaOH.
- 20 µl was transferred to a 96 well microtitre plate and 230 µl 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP, 0.65 g/l ABTS solution was added. After 30 minutes at room temperature, the absorbance was measured at 420 nm.

Table 4:

Temp. °C	*B. bifidum* lactase relative activity (% of activity at 38.1 °C)
20	54
21	63
22	64
24	68
26	73
29	81
31	88
34	94
36	96
38	100
43	96
48	91
52	83
57	76

Temp. °C	*B. bifidum* lactase relative activity (% of activity at 38.1 °C)
62	58
66	32
69	20
70	17

Example 4

Determination of Km for lactase enzymes at 5°C

Principle:

[0133] Lactase hydrolyses lactose and glucose + galactose is formed. Glucose is measured after a modified version of the common glucose oxidase / peroxidase assay (Werner, W. et al. (1970) Z. analyt. Chem. 252: 224.)

Substrate:

[0134] Different lactose concentrations ranging from Km/5 to 10* Km, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and pH adjusted to pH 6.5 with NaOH.

Enzyme Sample:

[0135] Experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 was diluted appropriately in 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01% Triton X100, pH adjusted to pH 6.5 with NaOH.

[0136] 12 g/l Lactozym (commercially available lactase from Novozymes A/S, Denmark) was diluted 6000 times in the same buffer.

Procedure:

[0137]

- 10 µl enzyme sample (5°C) was added to a 96 well microtitre plate on ice.
- 90 µl substrate (5°C) was added and incubated for 2 hours at 5°C.
- The reaction was stopped by adding 100 µl 0.25 M NaOH.
- 20 µl was transferred to a 96 well microtitre plate and 230 µl 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP, 0.65 g/l ABTS solution was added. After 30 minutes at room temperature, the absorbance was measured at 420 nm.

Km determination:

[0138] Computerized nonlinear least-squares fitting and the Michaelis-Menten equation:

$$v = (Vmax * S)/(Km + S)$$

was used. Km for the *Bifidobacterium* lactase and Lactozym were determined to be 8 mM and 30 mM, respectively.

[0139] In a similar test performed at 37°C, Km for the *Bifidobacterium* lactase and Lactozym were determined to be 13 mM and 30 mM, respectively.

Example 5

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - Different levels of sucrose and lactase

Experimental plan

[0140] For the selected combination of a low pH stable lactase and a lactose-deficient starter culture, two levels of added sucrose (0.5 % and 0.7 %), and two levels of added lactase (800 LAU/L and 1000 LAU/L), were tested. As reference fermentations with no added lactase and two levels of sucrose (1.0 % and 1.5 %) were carried out. The lactase was added at the start of the fermentation together with the starter culture.

Milk base

[0141]

Table 5: Composition of milk base

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Skim milk	1000	3.5	4.8	0.1
1.5 % milk	2000	3.4	4.7	1.5
Protein powder "Promilk 802FB"	14	80.0		
Protein powder "Milex 240"	95	34.0		
Total milk base	3109	4.71	4.57	1.00

Starter culture

[0142] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0143] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Measurements

[0144] Fat, protein and lactose levels were determined using MilkoScan analysis. Post-acidification was measured over a period of 28 days storage at 5 °C.

[0145] The level of sucrose, glucose, galactose, fructose and lactose in the fermented milk at day 1 and day 14 after production was measured by HPLC.

Gel firmness

[0146] Gel firmness is measured by a back extrusion test with a texture analyzer (TA.XT Plus, Stable Micro System, Surrey, UK) supplied with a 35mm parallel plate. The travel distance is set to 15 mm, and the travel speed to 2 mm/s. The test is performed after 7 days from production. The fermented milk product was brought to 13°C and manually stirred gently, and measured in a 250 g plastic container. The maximal force (g) obtained by force versus distance curves is used as "gel firmness" parameter.

Shear stress

[0147] Seven days after incubation, the fermented milk product was brought to 13°C and manually stirred gently by means of a spoon (5 times) until homogeneity of the sample. The rheological properties of the sample were assessed on a rheometer (Anton Paar Physica Rheometer with ASC, Automatic Sample Changer, Anton Paar® GmbH, Austria) by using a bob-cup. The rheometer was set to a constant temperature of 13 °C during the time of measurement. Settings were as follows:

Holding time (to rebuild to somewhat original structure)

5 minutes without any physical stress (oscillation or rotation) applied to the sample. Oscillation step (to measure the elastic and viscous modulus, G' and G", respectively, therefore calculating the complex modulus G*)

Constant strain = 0.3 %, frequency (f) = [0.5..8] Hz

6 measuring points over 60 s (one every 10 s)

Rotation step (to measure shear stress at 300 1/s)

Two steps were designed:

1. 1) Shear rate = [0.3-300] 1/s and 2) Shear rate = [275-0.3] 1/s.

Each step contained 21 measuring points over 210 s (on every 10 s).

The shear stress at 300 1/s was chosen for further analysis, as this correlates to mouth thickness when swallowing a fermented milk product.

Procedure

[0148] The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 5 °C. The milk base was then pasteurized at 90 °C for 20 minutes. The fermentation was carried out at 43 °C to an end pH of 4.55 to form yogurt. The yogurt was cooled in a PTU (Post Treatment Unit) at a cooling temperature of 25 °C at 2 bars. The cooled yogurt was stored at 6 °C.

Results

Post-acidification

[0149]

Table 6: Post-acidification

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%)	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Ferment. time (Hours: Min.)	7:25	7:05	7:15	7:15	7:00	7:00
End pH	4.58	4.55	4.55	4.55	4.55	4.55
pH Day 1	4.58	4.53	4.54	4.53	4.50	4.51
pH Day 7	4.54	4.49	4.50	4.50	4.47	4.49
pH Day 14	4.49	4.43	4.49	4.49	4.46	4.47
pH Day 21	4.35	4.34	4.45	4.45	4.42	4.43
pH Day 28	4.35	4.34	4.45	4.42	4.43	4.42
pH drop	0.23	0.19	0.09	0.11	0.07	0.09

[0150] For all six samples, i.e. 2 reference samples and 4 samples produced according to the invention (test samples), a pH of about 4.55 was reached in 7 hours.

[0151] As will appear from Table 6, the post-acidification over a period of 28 days was strongly reduced for the samples produced according to the invention as compared to the reference samples.

Carbohydrate analysis

[0152]

Table 7: Residual carbohydrate levels after 1 day from production

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%) start ferment.	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Fructose (mg/g)	1.62	1.91	0.80*	0.74*	1.27	1.18
Galactose (mg/g)	< 0.9	1.78*	29.01	29.87	28.78	29.29
Glucose (mg/g)	0.77*	1.73	27.60	27.84	28.08	28.19
Lactose (mg/g)	54.16	53.37	1.18	0.40	1.20	0.40

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (mg/g)	< 0.3	4.6	< 0.3	< 0.3	1.3	0.7
*value is between Limit of Detection and Limit of Quantification.						

[0153] All levels of Table 7 are mean values of two samples.

[0154] For the 4 test samples, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference samples indicating high activity of the added lactase. For the test samples using a lactase level of 1000 LAU/L a residual lactose level of approx. 0.04 % was obtained, and for the test samples using a lactase level of 800 LAU/L a residual lactose level of approx. 0.1 % was obtained.

[0155] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of perceived sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness index than lactose.

Gel firmness

[0156]

Table 8: Gel firmness

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%) start ferment.	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Gel firmness (g)	45.76	47.42	48.19	54.57	50.19	46.60

[0157] All levels of Table 8 are mean values of two samples.

[0158] As will appear from Table 8, 3 out of the 4 test samples had significantly higher gel firmness than the 2 reference samples.

Shear stress

[0159]

Table 9: Shear stress measured at 300 s^{-1}

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%) start ferment.	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Shear stress (Pa)	67.1	65.3	78.2	84.4	84.7	77.6

[0160] All levels of Table 9 are mean values of two samples.

[0161] As will appear from Table 9, the 4 test samples had significantly higher shear stress measured at 300 s^{-1} than the 2 reference samples. In the group of 4 test samples, the combinations of 1) a level of added sucrose of 0.5 % and a lactase level of 1000 LAU/L, and 2) a level of added sucrose of 0.7 % and a lactase level of 800 LAU/L, had the highest shear stress.

Example 6

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of excess level of sucrose before fermentation I

Experimental plan

[0162] Fermentations were carried out with a reference sample containing no lactase and 9.5 % sucrose and 2 test samples containing 800 LAU/L of lactase and 6.5 % and 7.0 % sucrose. The lactase was added at the start of the fermentation together with the starter culture.

Milk base

[0163]

Table 10: Composition of basic milk base 1

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Skim milk	984	3.5	4.8	0.1
1.5 % milk	1911	3.4	4.7	1.5
Protein powder "Promilk 802FB"	13.3	80.0		

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Protein powder "Milex 240"	91.5	34.0	54.00	1.00
Total milk base	3000	4.71	4.57	1.02

Table 11: Composition of basic milk base 2

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Skim milk	984	3.5	4.8	0.1
1.5 % milk	1911	3.4	4.7	1.5
Protein powder "Promilk 802FB"	17.40	80.0		
Protein powder "Milex 240"	109.50	34.0	5400	1.00
Sucrose	180.00		100.0	
Total milk base	3202	4.70	11.75	0.96

[0164] Basic milk base 2 was used to prepare the final milk bases for the reference sample and the two test samples by addition of additional sucrose.

Starter culture

[0165] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0166] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Measurements

[0167] All measurements were carried out using the same methods as in Example 5.

Procedure

[0168] The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 5

°C. The milk base was then pasteurized at 95 °C for 5 minutes. The milk base was then homogenized at 65 °C at 200 bar. The fermentation was carried out at 43 °C to an end pH of 4.55 to form yogurt. The yogurt was cooled in a PTU (Post Treatment Unit) at a cooling temperature of 25 °C at 2 bars. The cooled yogurt was stored at 6 °C.

Results

Post-acidification

[0169]

Table 12: Post-acidification

Sample	1	2	3
Lactase (LAU/L)	0	800	800
Fermentation time	6 h 15 min	6 h 15 min	6 h 15 min
End pH	4.55	4.51	4.51
pH Day 4	4.54	4.44	4.44
pH Day 7	4.51	4.42	4.42
pH Day 14	4.40	4.38	4.37
pH Day 21	4.38	4.38	4.37
pH Day 28	4.36	4.37	4.36
pH drop	0.19	0.14	0.15

Carbohydrate analysis

[0170]

Table 13: Carbohydrate analysis

Sample	1		2		3	
Lactase (LAU/L)	0		800		800	
Sucrose (%) start ferment.	9.5		6.5		7.0	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Fructose (mg/g)	2.4	3.07	1.7	2.01	1.9	2.00
Galactose (mg/g)	< 2.0	8.99	26.4	29.03	26.4	28.72
Glucose (mg/g)	< 1.0	8.84	26.2	27.52	26.2	27.22
Lactose (mg/g)	51.4	38.04	2.8	< 2	2.7	< 2

Sample	1		2		3	
Sucrose (mg/g	64.3	64.23	41.7	42.38	46.0	46.26

[0171] All levels of Table 13 are mean values of two samples.

[0172] As will appear from Table 13, for the 2 test samples, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference sample indicating high activity of the added lactase.

[0173] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness than lactose.

Gel firmness and shear stress

[0174]

Table 14: Gel firmness and shear stress at 300 s^{-1}

Sample	1		2		3	
Lactase (LAU/L)	0		800		800	
Sucrose (%) start ferment.	9.5		6.5		7.0	
Gel firmness (g)	46.36		46.76		45.75	
Shear stress at 1 s-1 (Pa)	9.4	9.2	9.3	8.8	10.3	10.2
Shear stress at 30.2 s-1 (Pa)	39.2	38.8	41.6	39.2	44.7	45.1
Shear stress at 135 s-1 (Pa)	58.3	58.0	67.2	64.5	72.3	72.2
Shear stress at 300 s-1 (Pa)	69.4	69.1	80.6	78.2	84.4	84.4

[0175] As will appear from Table 14, the shear stress at 300 s^{-1} of the two test samples containing lactase was significantly increased as compared to the reference sample with no lactase.

Example 7

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of excess level of sucrose before fermentation II

Experimental plan

[0176] Fermentations were carried out with two reference samples containing no lactase and 1.5 % and 9.5 % sucrose and 3 test samples containing 1000 LAU/L of lactase and 0.5 %, 6.5 % and 7.0 % sucrose. The lactase was added at the start of the fermentation together with the starter culture.

Milk base

[0177] The two basic milk bases of Example 6 were used to prepare the final milk bases for the reference samples and the three test samples by addition of additional sucrose.

Starter culture

[0178] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0179] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Measurements

[0180] All measurements were carried out using the same methods as in Example 5.

Procedure

[0181] The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 5 °C. The milk base was then pasteurized at 90 °C for 20 minutes. The fermentation was carried out at 43 °C to an end pH of 4.55 to form yogurt. The yogurt was cooled in a PTU (Post Treatment Unit) at a cooling temperature of 25 °C at 2 bars. The cooled yogurt was stored at 6 °C.

Results

Post-acidification

[0182]

Table 15: Post-acidification

Sam ple	1	2	3	4	5
Lactase (LAU/L)	0	1000	0	1000	1000
Fermentation time	6 h 50 min	6 h 55 min	6 h 35 min	6 h 10 min	5 h 40 min
End pH	4.55*	4.55*	4.55*	4.55*	4.55*
pH Day 1	4.54	4.51	4.52	4.48	4.47
pH Day 7	4.45	4.45	4.40	4.34	4.35
pH Day 14	4.37	4.44	4.37	4.32	4.34
pH Day 21	4.35	4.39	4.35	4.29	4.29
pH Day 28	4.34	4.37	4.33	4.27	4.26
pH drop	0.21	0.18	0.22	0.28	0.29
*The fermentation is stopped by cooling at pH = 4.55.					

[0183] As will appear from Table 15, the post-acidification was at the same level for the reference samples containing no lactase and the test samples containing lactase.

Carbohydrate analysis

[0184]

Table 16: Carbohydrate analysis Day 1

Sam ple	1	2	3	4	5
Lactase (LAU/ L)	0	1000	0	1000	1000
Sucrose (%) start ferment.	1.5	0.5	9.5	6.5	7.0
Fructose (mg/g)	1.96	< 1	2.70	1.74	1.88
Galactose (mg/g)	< 2	29.19	< 2	27.83	27.89
Glucose (mg/g)	< 1	27.37	< 1	27.29	27.40
Lactose (mg/g)	56.11	< 2	54.56	< 2	< 2
Sucrose (mg/g	5.45	< 4	63.44	39.79	45.44

Table 17: Carbohydrate analysis Day 28

Sam ple	1	2	3	4	5
Lactase (LAU/ L)	0	1000	0	1000	1000
Sucrose (%) start ferment.	1.5	0.5	9.5	6.5	7.0
Fructose (mg/g)	1.88	< 1	4.72	2.95	3.39
Galactose (mg/g)	3.90	28.83	< 2	28.79	29.15
Glucose (mg/g)	2.69	26.78	1.66	26.83	27.27
Lactose (mg/g)	50.71	< 2	52.42	< 2	< 2
Sucrose (mg/g	4.18	< 4	62.07	39.91	46.17

[0185] As will appear from Tables 16 and 17, for the 3 test samples, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference sample indicating high activity of the added lactase.

[0186] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness than lactose.

Gel firmness and shear stress

[0187]

Table 18: Gel firmness and shear stress

Sam ple	1		2		3		4		5	
Lactase (LAU/L)	0		1000		0		1000		1000	
Sucrose (%) start ferment.	1.5		0.5		9.5		6.5		7.0	
Gel firmness (g)	51.13		49.41		45.04		45.94		47.89	
Shear stress at 1 s-1 (Pa)	9.4	9.3	8.4	8.5	8.1	8.5	7.7	7.8	8.3	8.5
Shear stress at 30.2 s-1 (Pa)	39.4	38.5	38.2	37.6	34.3	34.7	37.3	37.1	38.8	38.9
Shear stress at 135 s-1 (Pa)	61.0	59.5	65.8	65.1	56.8	56.8	72.7	72.3	74.8	74.9
Shear stress at 300 s-1 (Pa)	72.1	70.5	78.8	77.6	69.1	69.1	86.7	86.6	89.4	89.4

[0188] As will appear from Table 18, the shear stress of the three test samples containing

lactase was significantly increased as compared to the reference sample with no lactase.

Example 8

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of lactase before and after fermentation

Experimental plan

[0189]

Table 19: Experimental plan

Sample	Timing of lactase addition	Milk Base	Sucrose (%)	Lactase (LAU/L)
1	None	1	0.70	0
2	Together with culture	1	0.70	600
3	Together with culture	1	0.70	800
4	Together with culture	1	0.70	1000
5	Together with culture	1	0.70	1400
6	None	2	0.97	0
7	After fermentation	2	0.97	800
8	After fermentation	2	0.97	1600
9	After fermentation	2	0.97	2400
10	After fermentation	2	0.97	3200

[0190] The fermentation temperature was 43 °C. The end pH was 4.50. The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 6 °C. The milk base was then pasteurized at 95 °C for 5 minutes and homogenized at 200/50 bar at 65 °C. The fermentation was carried out at 43 °C to an end pH of 4.50 to form yogurt. The yogurt was cooled to 6 °C. The cooled yogurt was stored at 6 °C.

Starter culture

[0191] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0192] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Milk base

[0193]

Table 20: Composition of milk base 1

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Sucrose	22	0.0	100.0	0.0
0.5 % milk	1500	3.8	4.8	0.5
1.5 % milk	1500	3.6	4.7	1.5
Arla Skimmed Milk Powder	103	34.0	54.0	1.0
Total milk base	3125	4.67	7.05	0.98

Table 21: Composition of milk base 2

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Sucrose	30	0.0	100.0	0.0
0.5 % milk	1500	3.8	4.8	0.5
1.5 % milk	1500	3.6	4.7	1.5
Arla Skimmed Milk Powder	103	34.0	54.0	1.0
Total milk base	3133	4.66	7.30	1.00

Measurem ents

[0194] All measurements were carried out using the same methods as in Example 5.

Results

Post-acidification

[0195]

Table 22: Post-acidification

Sample	Timing of lactase addition	Milk Base	Lactase (LAU/L)	pH Day 0	pH Day 7	pH Day 28	pH Day 42	pH drop
1	None	1	0	4.89*	4.89*	4.87	4.84	0.05
2	Together with culture	1	600	4.26	4.23	4.23	4.22	0.04
3	Together with culture	1	800	4.22	4.26	4.24	4.24	-0.02
4	Together with culture	1	1000	4.32	4.26	4.26	4.26	0.06
5	Together with culture	1	1400	4.36	4.30	4.28	4.27	0.09
6	None	2	0	4.47	4.47	4.47	4.44	0.03
7	After fermentation	2	800	4.43	4.40	4,42	4.38	0.05
8	After fermentation	2	1600	4.44	4.40	4,42	4.39	0.05
9	After fermentation	2	2400	4.41	4.43	4.40	4.40	0.01
10	After fermentation	2	3200	NA	4.43	4.41	4.40	NA

* Insufficient sucrose to obtain fermentation to target pH. Therefore, this sample cannot be used as reference.

[0196] As will appear from Table 22, the post-acidification over a period of 42 days was at a low level of below 0.09 for all lactase levels tested with lactase addition at the start of fermentation and at an even lower level of below 0.05 for all lactase levels tested with lactase addition at the end of fermentation. The addition of lactase at the end of the fermentation did not result in a statistically different post-acidification as compared to the reference sample with no added lactase. Thus, the present experiment shows that the use of a low pH stable lactase in combination with a lactose-deficient starter culture does not result in an unacceptable increase of the low post-acidification, which may be obtained with such a starter culture. This is true both when the lactase is added at the start and at the end of fermentation.

Gel firmness and shear stress

[0197]

Table 23: Gel firmness and Shear Stress

Sample	Timing of lactase addition	Milk Base	Sucrose (%)	Lactase (LAU/L)	Gel firmness (g)	Shear Stress (Pa)
1	None	1	0.70	0	0.187*	46.4*
2	Together with culture	1	0.70	600	0.281	63.9
3	Together with culture	1	0.70	800	0.302	65.3
4	Together with culture	1	0.70	1000	0.318	64.5
5	Together with culture	1	0.70	1400	0.307	63.7
6	None	2	0.97	0	0.304	53.1
7	After fermentation	2	0.97	800	0.312	51.4
8	After fermentation	2	0.97	1600	0.322	52.8
9	After fermentation	2	0.97	2400	0.323	52.9
10	After fermentation	2	0.97	3200	0.320	51.7
* Insufficient sucrose to obtain fermentation to target pH. Therefore, this sample cannot be used as reference.						

[0198] As will appear from Table 23, high levels of both gel firmness and shear stress was obtained for all lactase levels tested with lactase addition at the start of fermentation. For lactase levels tested with lactase addition at the end of fermentation, the levels of gel firmness and shear stress are somewhat lower, which is due to the mixing of the lactase into the yogurt, which partly disrupts the texture of the yogurt. For all lactase levels tested with lactase addition at the end of fermentation, the gel firmness is slightly higher than the reference sample with no added lactase. For all lactase levels tested with lactase addition at the end of fermentation, the shear stress is maintained at the same level or slightly lower.

Carbohydrate analysis

[0199]

Table 24: Carbohydrate analysis

Sam-ple	Lactase (LAU/L)	Galactose Day 20 (mg/g)	Glucose Day 20 (mg/g)	Sucrose Day 20 (mg/g)	Lactose Day 20 (mg/g)	Lactose 24 hours (%)	Lactose Day 20 (%)
1	0	2.4	2.3	< 1.0	50.7	ND	ND
2	600	29.5	27.7	< 1.0	< 0.5	> 0.1	0.023
3	800	30.2	28.1	< 1.0	< 0.5	0.028	0.016
4	1000	30.0	27.8	< 1.0	< 0.5	0.014	0.014
5	1400	29.5	27.4	< 1.0	< 0.5	0.006	0.013
6	0	4.2	4.2	< 1.0	49.2	ND	ND
7	800	31.2	30.7	< 1.0	< 0.5	> 0.2	0.014
8	1600	30.7	30.2	< 1.0	< 0.5	> 0.1	0.012
9	2400	31.2	30.6	< 1.0	< 0.5	0.029	0.011
10	3200	31.8	31.2	< 1.0	< 0.5	0.015	0.011

[0200] As will appear from Table 24, at Day 20 high concentrations of galactose and glucose are formed for all lactase levels tested both with lactase addition at the start and at the end of fermentation. Also, at Day 20 the level of glucose was below the detection limit of 0.5 mg/g, which qualifies as lactose free in some countries. For the higher levels of lactase, most of the lactose removal has been obtained 24 hours after the end of fermentation.

Example 9

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of lactase before fermentation II

Experimental plan

[0201]

Table 25: Experimental plan

Sample	Sucrose (%)	Lactase (LAU/L)
1	0	0
2	0	1000
3	0	1200
4	0	1600
5	0.2	0

Sample	Sucrose (%)	Lactase (LAU/L)
6	0.2	1000
7	0.2	1200
8	0.2	1600
9	0.7	0
10	0.7	1000
11	0.7	1200
12	0.7	1600

[0202] The fermentation temperature was 43 °C. The end pH was 4.45. The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 6 °C. The milk base was then pasteurized at 95 °C for 5 minutes and homogenized at 200/50 bar at 65 °C. The fermentation was carried out at 43 °C to an end pH of 4.45 to form yogurt. The yogurt was cooled to 5 °C. The cooled yogurt was stored at 6 °C.

Starter culture

[0203] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0204] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Milk base

[0205]

Table 26: Composition of milk base

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
0.5 % milk	1500	3.8	4.8	0.5
1.5 % milk	1500	3.6	4.7	1.5
Arla Skimmed Milk Powder	103	34.0	54.0	1.0

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Total milk base	3103	4.70	6.39	0.99

Measurements

[0206] All measurements were carried out using the same methods as in Example 5.

Results

Post-acidification

[0207]

Table 27: Post-acidification

Sample	Sucrose (%)	Lactase (LAU/L)	pH Day 0	pH Day 1	pH Day 14	pH Day 28	pH Day 42	pH drop
1	0	0	ND	ND	ND	ND	ND	ND
2	0	1000	4.45	4.39	4.36	4.33	4.39	0.06
3	0	1200	4.45	4.39	4.38	4.34	4.39	0.06
4	0	1600	4.46	4.39	4.38	4.34	4.39	0.07
5	0.2	0	ND	ND	ND	ND	ND	ND
6	0.2	1000	4.50	4.44	4.40	4.40	4.43	0.07
7	0.2	1200	4.53	4.47	4.46	4.42	4.46	0.07
8	0.2	1600	4.53	4.49	4.46	4.44	4.47	0.06
9	0.7	0	4.76	4.73	4.69	4.68	4.70	0.06
10	0.7	1000	4.44	4.40	4.39	4.33	4.38	0.06
11	0.7	1200	4.46	4.40	4.39	4.33	4.39	0.07
12	0.7	1600	4.47	4.41	4.40	4.34	4.41	0.06

[0208] As will appear from Table 27, the post-acidification over a period of 42 days was at a low very level of approx. 0.06 for all lactase and sucrose levels tested. Thus, the present experiment shows that the use of a low pH stable lactase in combination with a lactose-deficient starter culture does not result in an unacceptable increase of the low post-acidification, which may be obtained with such a starter culture.

Gel *firmness and shear stress*

[0209]

Table 28: Gel firmness and Shear Stress

Sample	Sucrose (%)	Lactase (LAU/L)	Gel firmness (g)	Shear Stress (Pa)
1	0	0	ND	ND
2	0	1000	0.386	70.3
3	0	1200	0.377	69.8
4	0	1600	0.388	69.5
5	0.20	0	ND	ND
6	0.20	1000	0.380	70.7
7	0.20	1200	0.378	69.5
8	0.20	1600	0.397	70.2
9	0.70	0	ND	ND
10	0.70	1000	0.362	71.2
11	0.70	1200	0.375	72.2
12	0.70	1600	0.362	71.9

[0210] As will appear from Table 28, high levels of both gel firmness and shear stress was obtained for all lactase levels tested. The gel firmness and shear strength are at the same order of magnitude for all three levels of lactase tested.

Carbohydrate analysis

[0211]

Table 29: Carbohydrate analysis

Sam - ple	Sucrose (%)	Lactase (LAU/L)	Fructose (mg/g)	Galactose (mg/g)	Glucose (mg/g)	Lactose (mg/g)	Sucrose (mg/g)
1	0	0	< 0.5	6.3	< 0.5	45.5	<2
2	0	1000	< 0.5	30.7	26.0	< 0.9	<2
3	0	1200	< 0.5	31.5	26.6	< 0.9	<2
4	0	1600	< 0.5	30.9	25.8	< 0.9	<2
5	0.20	0	< 0.5	11.7	6.7	36.1	<2
6	0.20	1000	< 0.5	30.8	27.8	< 0.9	<2
7	0.20	1200	< 0.5	31.6	28.3	< 0.9	<2

Sam - ple	Sucrose (%)	Lactase (LAU/L)	Fructose (mg/g)	Galactose (mg/g)	Glucose (mg/g)	Lactose (mg/g)	Sucrose (mg/g)
8	0.20	1600	< 0.5	31.5	28.0	< 0.9	<2
9	0.70	0	1.1	9.1	9.1	43.4	<2
10	0.70	1000	1,1	31.1	29.5	< 0.9	<2
11	0.70	1200	1.0	32.1	30.3	< 0.9	1.8
12	0.70	1600	0.9	31.8	29.9	< 0.9	1.9

[0212] As will appear from Table 29, for the test samples containing lactase, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference sample indicating high activity of the added lactase.

[0213] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness than lactose.

Sequence listing

[0214]

SEQ ID NO.: 1 shows the sequence of a mutant of SEQ ID NO. 4.

SEQ ID NO.: 2 shows the sequence of a mutant of SEQ ID NO. 4.

SEQ ID NO.: 3 shows the sequence of a lactase from *Bifidobacterium bifidum* DSM20215.

SEQ ID NO.: 4 shows the sequence of a lactase from *Bifidobacterium bifidum* NCI MB41171, the nucleotide sequence of which is listed in NCBI with the accession number DQ448279.

[0215] SEQ ID NO: 4 is discussed in the following references, wherein it is referred to as bbgIII:

Appl Microbiol Biotechnol (2007) 76:1365-1372, T K Goulas et al.

Appl Microbiol Biotechnol (2009) 82: 1079-1088, T Goulas et al.

Appl Microbiol Biotechnol (2009) 84:899-907, T Goulas et al.

SEQUENCE LISTING

[0216]

<110> Chr. Hansen A/S

<120> Process for producing a fermented milk product

<130> P6170PC00

<160> 4

<170> PatentIn version 3.5

<210> 1
<211> 1931
<212> PRT
<213> Bifidobacterium bifidum

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            20                  25                  30

Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr Pro Glu Val Ala
        35                  40                  45

Tyr Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr Ser Asp Phe Asp
    50                  55                  60

Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln Ala Gln Asp Pro
65                  70                  75                  80

Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu Pro His Asp Tyr
                85                  90                  95

Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala Glu Ser Ala Tyr
            100                 105                 110

Leu Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe Thr Ile Asp Arg
        115                 120                 125

Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp Gly Val Tyr Met
    130                 135                 140

Asn Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly Thr His Pro Tyr
145                 150                 155                 160

Gly Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn Ala Lys Phe Gly
                165                 170                 175
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```
Gly Glu Asn Thr Ile Val Val Lys Val Glu Asn Arg Leu Pro Ser Ser
            180                 185                 190

Arg Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val Thr Leu Thr Val
        195                 200                 205

Thr Asp Gly Val His Val Gly Asn Asn Gly Val Ala Ile Lys Thr Pro
    210                 215                 220

Ser Leu Ala Thr Gln Asn Gly Gly Asp Val Thr Met Asn Leu Thr Thr
225                 230                 235                 240

Lys Val Ala Asn Asp Thr Glu Ala Ala Ala Asn Ile Thr Leu Lys Gln
                245                 250                 255

Thr Val Phe Pro Lys Gly Gly Lys Thr Asp Ala Ala Ile Gly Thr Val
            260                 265                 270

Thr Thr Ala Ser Lys Ser Ile Ala Ala Gly Ala Ser Ala Asp Val Thr
        275                 280                 285

Ser Thr Ile Thr Ala Ala Ser Pro Lys Leu Trp Ser Ile Lys Asn Pro
    290                 295                 300

Asn Leu Tyr Thr Val Arg Thr Glu Val Leu Asn Gly Gly Lys Val Leu
305                 310                 315                 320

Asp Thr Tyr Asp Thr Glu Tyr Gly Phe Arg Trp Thr Gly Phe Asp Ala
                325                 330                 335

Thr Ser Gly Phe Ser Leu Asn Gly Glu Lys Val Lys Leu Lys Gly Val
            340                 345                 350

Ser Met His His Asp Gln Gly Ser Leu Gly Ala Val Ala Asn Arg Arg
        355                 360                 365

Ala Ile Glu Arg Gln Val Glu Ile Leu Gln Lys Met Gly Val Asn Ser
    370                 375                 380

Ile Arg Thr Thr His Asn Pro Ala Ala Lys Ala Leu Ile Asp Val Cys
385                 390                 395                 400

Asn Glu Lys Gly Val Leu Val Val Glu Glu Val Phe Asp Met Trp Asn
                405                 410                 415

Arg Ser Lys Asn Gly Asn Thr Glu Asp Tyr Gly Lys Trp Phe Gly Gln
            420                 425                 430

Ala Ile Ala Gly Asp Asn Ala Val Leu Gly Gly Asp Lys Asp Glu Thr
        435                 440                 445

Trp Ala Lys Phe Asp Leu Thr Ser Thr Ile Asn Arg Asp Arg Asn Ala
    450                 455                 460

Pro Ser Val Ile Met Trp Ser Leu Gly Asn Glu Met Met Glu Gly Ile
465                 470                 475                 480
```

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Ser Gly Ser Val Ser Gly Phe Pro Ala Thr Ser Ala Lys Leu Val Ala
                485                 490                 495

Trp Thr Lys Ala Ala Asp Ser Thr Arg Pro Met Thr Tyr Gly Asp Asn
            500                 505                 510

Lys Ile Lys Ala Asn Trp Asn Glu Ser Asn Thr Met Gly Asp Asn Leu
        515                 520                 525

Thr Ala Asn Gly Gly Val Val Gly Thr Asn Tyr Ser Asp Gly Ala Asn
    530                 535                 540

Tyr Asp Lys Ile Arg Thr Thr His Pro Ser Trp Ala Ile Tyr Gly Ser
545                 550                 555                 560

Glu Thr Ala Ser Ala Ile Asn Ser Arg Gly Ile Tyr Asn Arg Thr Thr
                565                 570                 575

Gly Gly Ala Gln Ser Ser Asp Lys Gln Leu Thr Ser Tyr Asp Asn Ser
            580                 585                 590

Ala Val Gly Trp Gly Ala Val Ala Ser Ser Ala Trp Tyr Asp Val Val
        595                 600                 605

Gln Arg Asp Phe Val Ala Gly Thr Tyr Val Trp Thr Gly Phe Asp Tyr
    610                 615                 620

Leu Gly Glu Pro Thr Pro Trp Asn Gly Thr Gly Ser Gly Ala Val Gly
625                 630                 635                 640

Ser Trp Pro Ser Pro Lys Asn Ser Tyr Phe Gly Ile Val Asp Thr Ala
                645                 650                 655

Gly Phe Pro Lys Asp Thr Tyr Tyr Phe Tyr Gln Ser Gln Trp Asn Asp
            660                 665                 670

Asp Val His Thr Leu His Ile Leu Pro Ala Trp Asn Glu Asn Val Val
        675                 680                 685

Ala Lys Gly Ser Gly Asn Asn Val Pro Val Val Val Tyr Thr Asp Ala
    690                 695                 700

Ala Lys Val Lys Leu Tyr Phe Thr Pro Lys Gly Ser Thr Glu Lys Arg
705                 710                 715                 720

Leu Ile Gly Glu Lys Ser Phe Thr Lys Lys Thr Thr Ala Ala Gly Tyr
                725                 730                 735

Thr Tyr Gln Val Tyr Glu Gly Ser Asp Lys Asp Ser Thr Ala His Lys
            740                 745                 750

Asn Met Tyr Leu Thr Trp Asn Val Pro Trp Ala Glu Gly Thr Ile Ser
        755                 760                 765

Ala Glu Ala Tyr Asp Glu Asn Asn Arg Leu Ile Pro Glu Gly Ser Thr
    770                 775                 780

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Glu Gly Asn Ala Ser Val Thr Thr Thr Gly Lys Ala Ala Lys Leu Lys
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Ala Asp Ala Asp Arg Lys Thr Ile Thr Ala Asp Gly Lys Asp Leu Ser
                805                 810                 815

Tyr Ile Glu Val Asp Val Thr Asp Ala Asn Gly His Ile Val Pro Asp
            820                 825                 830

Ala Ala Asn Arg Val Thr Phe Asp Val Lys Gly Ala Gly Lys Leu Val
        835                 840                 845

Gly Val Asp Asn Gly Ser Ser Pro Asp His Asp Ser Tyr Gln Ala Asp
    850                 855                 860

Asn Arg Lys Ala Phe Ser Gly Lys Val Leu Ala Ile Val Gln Ser Thr
865                 870                 875                 880

Lys Glu Ala Gly Glu Ile Thr Val Thr Ala Lys Ala Asp Gly Leu Gln
                885                 890                 895

Ser Ser Thr Val Lys Ile Ala Thr Thr Ala Val Pro Gly Thr Ser Thr
            900                 905                 910

Glu Lys Thr Val Arg Ser Phe Tyr Tyr Ser Arg Asn Tyr Tyr Val Lys
        915                 920                 925

Thr Gly Asn Lys Pro Ile Leu Pro Ser Asp Val Glu Val Arg Tyr Ser
    930                 935                 940

Asp Gly Thr Ser Asp Arg Gln Asn Val Thr Trp Asp Ala Val Ser Asp
945                 950                 955                 960

Asp Gln Ile Ala Lys Ala Gly Ser Phe Ser Val Ala Gly Thr Val Ala
                965                 970                 975

Gly Gln Lys Ile Ser Val Arg Val Thr Met Ile Asp Glu Ile Gly Ala
            980                 985                 990

Leu Leu Asn Tyr Ser Ala Ser Thr  Pro Val Gly Thr Pro  Ala Val Leu
        995                 1000                1005

Pro Gly  Ser Arg Pro Ala Val  Leu Pro Asp Gly Thr  Val Thr Ser
    1010                1015                1020

Ala Asn  Phe Ala Val His Trp  Thr Lys Pro Ala Asp  Thr Val Tyr
    1025                1030                1035

Asn Thr  Ala Gly Thr Val Lys  Val Pro Gly Thr Ala  Thr Val Phe
    1040                1045                1050

Gly Lys  Glu Phe Lys Val Thr  Ala Thr Ile Arg Val  Gln Arg Ser
    1055                1060                1065

Gln Val  Thr Ile Gly Ser Ser  Val Ser Gly Asn Ala  Leu Arg Leu
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1070                1075                1080

Thr Gln  Asn Ile Pro Ala Asp  Lys Gln Ser Asp Thr  Leu Asp Ala
1085                1090                1095

Ile Lys  Asp Gly Ser Thr Thr  Val Asp Ala Asn Thr  Gly Gly Gly
1100                1105                1110

Ala Asn  Pro Ser Ala Trp Thr  Asn Trp Ala Tyr Ser  Lys Ala Gly
1115                1120                1125

His Asn  Thr Ala Glu Ile Thr  Phe Glu Tyr Ala Thr  Glu Gln Gln
1130                1135                1140

Leu Gly  Gln Ile Val Met Tyr  Phe Phe Arg Asp Ser  Asn Ala Val
1145                1150                1155

Arg Phe  Pro Asp Ala Gly Lys  Thr Lys Ile Gln Ile  Ser Ala Asp
1160                1165                1170

Gly Lys  Asn Trp Thr Asp Leu  Ala Ala Thr Glu Thr  Ile Ala Ala
1175                1180                1185

Gln Glu  Ser Ser Asp Arg Val  Lys Pro Tyr Thr Tyr  Asp Phe Ala
1190                1195                1200

Pro Val  Gly Ala Thr Phe Val  Lys Val Thr Val Thr  Asn Ala Asp
1205                1210                1215

Thr Thr  Thr Pro Ser Gly Val  Val Cys Ala Gly Leu  Thr Glu Ile
1220                1225                1230

Glu Leu  Lys Thr Ala Thr Ser  Lys Phe Val Thr Asn  Thr Ser Ala
1235                1240                1245

Ala Leu  Ser Ser Leu Thr Val  Asn Gly Thr Lys Val  Ser Asp Ser
1250                1255                1260

Val Leu  Ala Ala Gly Ser Tyr  Asn Thr Pro Ala Ile  Ile Ala Asp
1265                1270                1275

Val Lys  Ala Glu Gly Glu Gly  Asn Ala Ser Val Thr  Val Leu Pro
1280                1285                1290

Ala His  Asp Asn Val Ile Arg  Val Ile Thr Glu Ser  Glu Asp His
1295                1300                1305

Val Thr  Arg Lys Thr Phe Thr  Ile Asn Leu Gly Thr  Glu Gln Glu
1310                1315                1320

Phe Pro  Ala Asp Ser Asp Glu  Arg Asp Tyr Pro Ala  Ala Asp Met
1325                1330                1335

Thr Val  Thr Val Gly Ser Glu  Gln Thr Ser Gly Thr  Ala Thr Glu
1340                1345                1350

Gly Pro  Lys Lys Phe Ala Val  Asp Gly Asn Thr Ser  Thr Tyr Trp
1355                1360                1365
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1355 1360 1365

His Ser Asn Trp Thr Pro Thr Thr Val Asn Asp Leu Trp Ile Ala
1370 1375 1380

Phe Glu Leu Gln Lys Pro Thr Lys Leu Asp Ala Leu Arg Tyr Leu
1385 1390 1395

Pro Arg Pro Ala Gly Ser Lys Asn Gly Ser Val Thr Glu Tyr Lys
1400 1405 1410

Val Gln Val Ser Asp Asp Gly Thr Asn Trp Thr Asp Ala Gly Ser
1415 1420 1425

Gly Thr Trp Thr Thr Asp Tyr Gly Trp Lys Leu Ala Glu Phe Asn
1430 1435 1440

Gln Pro Val Thr Thr Lys His Val Arg Leu Lys Ala Val His Thr
1445 1450 1455

Tyr Ala Asp Ser Gly Asn Asp Lys Phe Met Ser Ala Ser Glu Ile
1460 1465 1470

Arg Leu Arg Lys Ala Val Asp Thr Thr Asp Ile Ser Gly Ala Thr
1475 1480 1485

Val Thr Val Pro Ala Lys Leu Thr Val Asp Arg Val Asp Ala Asp
1490 1495 1500

His Pro Ala Thr Phe Ala Thr Lys Asp Val Thr Val Thr Leu Gly
1505 1510 1515

Asp Ala Thr Leu Arg Tyr Gly Val Asp Tyr Leu Leu Asp Tyr Ala
1520 1525 1530

Gly Asn Thr Ala Val Gly Lys Ala Thr Val Thr Val Arg Gly Ile
1535 1540 1545

Asp Lys Tyr Ser Gly Thr Val Ala Lys Thr Phe Thr Ile Glu Leu
1550 1555 1560

Lys Asn Ala Pro Ala Pro Glu Pro Thr Leu Thr Ser Val Ser Val
1565 1570 1575

Lys Thr Lys Pro Ser Lys Leu Thr Tyr Val Val Gly Asp Ala Phe
1580 1585 1590

Asp Pro Ala Gly Leu Val Leu Gln Leu Asn Tyr Asp Asp Asp Ser
1595 1600 1605

Thr Gly Thr Val Thr Trp Asn Thr Gln Thr Ala Gly Asp Phe Thr
1610 1615 1620

Phe Lys Pro Ala Leu Asp Ala Lys Leu Lys Val Thr Asp Lys Thr
1625 1630 1635

Val Thr Val Thr Tyr Gln Gly Lys Ser Ala Val Ile Asp Ile Thr
1640 1645 1650

Val Ser Gln Pro Ala Pro Thr Val Ser Lys Thr Asp Leu Asp Lys
1655 1660 1665

Ala Ile Lys Ala Ile Glu Ala Lys Asn Pro Asp Ser Ser Lys Tyr
1670 1675 1680

Thr Ala Asp Ser Trp Lys Thr Phe Ala Asp Ala Met Ala His Ala
1685 1690 1695

Lys Ala Val Ile Ala Asp Asp Ser Ala Thr Gln Gln Asp Val Asp
1700 1705 1710

Asn Ala Leu Lys Ala Leu Thr Asp Ala Tyr Ala Gly Leu Thr Glu
1715 1720 1725

Lys Thr Pro Glu Pro Ala Pro Val Ser Lys Ser Glu Leu Asp Lys
1730 1735 1740

Lys Ile Lys Ala Ile Glu Ala Glu Lys Leu Asp Gly Ser Lys Tyr
1745 1750 1755

Thr Ala Glu Ser Trp Lys Ala Phe Glu Thr Ala Leu Ala His Ala
1760 1765 1770

Lys Ala Val Ile Ala Ser Asp Ser Ala Thr Gln Gln Asn Val Asp
1775 1780 1785

Ala Ala Leu Gly Ala Leu Thr Ser Ala Arg Asp Gly Leu Thr Glu
1790 1795 1800

Lys Gly Glu Val Lys Pro Asp Pro Lys Pro Glu Pro Gly Thr Val
1805 1810 1815

Asp Lys Ala Ala Leu Asp Lys Ala Val Lys Lys Val Glu Ala Glu
1820 1825 1830

Lys Leu Asp Gly Ser Lys Tyr Thr Ala Asp Ser Trp Lys Ala Phe
1835 1840 1845

Glu Thr Ala Leu Ala His Ala Lys Ala Val Ile Gly Asn Ala Asn
1850 1855 1860

Ser Thr Gln Phe Asp Ile Asp Asn Ala Leu Ser Met Leu Asn Asp
1865 1870 1875

Ala Arg Ala Ala Leu Lys Glu Lys Pro Gly Arg Ile Ile Ala Ile
1880 1885 1890

Ile Asp Gly Ser Ala Leu Ser Lys Thr Gly Ala Ser Val Ala Ile
1895 1900 1905

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1910 1915 1920

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Met Ala  Leu Arg Arg Lys Arg  Ser
    1925                  1930
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<211> 1341

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<213> Bifidobacterium bifidum

<400> 2

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            20                  25                  30

Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr Pro Glu Val Ala Tyr
        35                  40                  45

Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr Ser Asp Phe Asp Ala
    50                  55                  60

Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln Ala Gln Asp Pro Ala
65                  70                  75                  80

Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu Pro His Asp Tyr Ser
                85                  90                  95

Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala Glu Ser Ala Tyr Leu
            100                 105                 110

Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe Thr Ile Asp Arg Asp
        115                 120                 125

Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp Gly Val Tyr Met Asn
    130                 135                 140

Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly Thr His Pro Tyr Gly
145                 150                 155                 160

Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn Ala Lys Phe Gly Gly
                165                 170                 175

Glu Asn Thr Ile Val Val Lys Val Glu Asn Arg Leu Pro Ser Ser Arg
            180                 185                 190

Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val Thr Leu Thr Val Thr
        195                 200                 205

Asp Gly Val His Val Gly Asn Asn Gly Val Ala Ile Lys Thr Pro Ser
    210                 215                 220

Leu Ala Thr Gln Asn Gly Gly Asp Val Thr Met Asn Leu Thr Thr Lys
225                 230                 235                 240
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Val Ala Asn Asp Thr Glu Ala Ala Ala Asn Ile Thr Leu Lys Gln Thr
                245                 250                 255

Val Phe Pro Lys Gly Gly Lys Thr Asp Ala Ala Ile Gly Thr Val Thr
            260                 265                 270

Thr Ala Ser Lys Ser Ile Ala Ala Gly Ala Ser Ala Asp Val Thr Ser
        275                 280                 285

Thr Ile Thr Ala Ala Ser Pro Lys Leu Trp Ser Ile Lys Asn Pro Asn
    290                 295                 300

Leu Tyr Thr Val Arg Thr Glu Val Leu Asn Gly Gly Lys Val Leu Asp
305                 310                 315                 320

Thr Tyr Asp Thr Glu Tyr Gly Phe Arg Trp Thr Gly Phe Asp Ala Thr
                325                 330                 335

Ser Gly Phe Ser Leu Asn Gly Glu Lys Val Lys Leu Lys Gly Val Ser
            340                 345                 350

Met His His Asp Gln Gly Ser Leu Gly Ala Val Ala Asn Arg Arg Ala
        355                 360                 365

Ile Glu Arg Gln Val Glu Ile Leu Gln Lys Met Gly Val Asn Ser Ile
    370                 375                 380

Arg Thr Thr His Asn Pro Ala Ala Lys Ala Leu Ile Asp Val Cys Asn
385                 390                 395                 400

Glu Lys Gly Val Leu Val Val Glu Glu Val Phe Asp Met Trp Asn Arg
                405                 410                 415

Ser Lys Asn Gly Asn Thr Glu Asp Tyr Gly Lys Trp Phe Gly Gln Ala
            420                 425                 430

Ile Ala Gly Asp Asn Ala Val Leu Gly Gly Asp Lys Asp Glu Thr Trp
        435                 440                 445

Ala Lys Phe Asp Leu Thr Ser Thr Ile Asn Arg Asp Arg Asn Ala Pro
    450                 455                 460

Ser Val Ile Met Trp Ser Leu Gly Asn Glu Met Met Glu Gly Ile Ser
465                 470                 475                 480

Gly Ser Val Ser Gly Phe Ser Ala Thr Ser Ala Lys Leu Val Ala Trp
                485                 490                 495

Thr Lys Ala Ala Asp Ser Thr Arg Pro Met Thr Tyr Gly Asp Asn Lys
            500                 505                 510

Ile Lys Ala Asn Trp Asn Glu Ser Asn Thr Met Gly Asp Asn Leu Thr
        515                 520                 525

Ala Asn Gly Gly Val Val Gly Thr Asn Tyr Ser Asp Gly Ala Asn Tyr
    530                 535                 540
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```
Asp Lys Ile Arg Thr Thr His Pro Ser Trp Ala Ile Tyr Gly Ser Glu
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Thr Ala Ser Ala Ile Asn Ser Arg Gly Ile Tyr Asn Arg Thr Thr Gly
                565                 570                 575

Gly Ala Gln Ser Ser Asp Lys Gln Leu Thr Ser Tyr Asp Asn Ser Ala
            580                 585                 590

Val Gly Trp Gly Ala Val Ala Ser Ser Ala Trp Tyr Asp Val Val Gln
        595                 600                 605

Arg Asp Phe Val Ala Gly Thr Tyr Val Trp Thr Gly Phe Asp Tyr Leu
    610                 615                 620

Gly Glu Pro Thr Pro Trp Asn Gly Thr Gly Ser Gly Ala Val Gly Ser
625                 630                 635                 640

Trp Pro Ser Pro Lys Asn Ser Tyr Phe Gly Ile Val Asp Thr Ala Gly
                645                 650                 655

Phe Pro Lys Asp Thr Tyr Tyr Phe Tyr Gln Ser Gln Trp Asn Asp Asp
            660                 665                 670

Val His Thr Leu His Ile Leu Pro Ala Trp Asn Glu Asn Val Val Ala
        675                 680                 685

Lys Gly Ser Gly Asn Asn Val Pro Val Val Val Tyr Thr Asp Ala Ala
    690                 695                 700

Lys Val Lys Leu Tyr Phe Thr Pro Lys Gly Ser Thr Glu Gln Arg Leu
705                 710                 715                 720

Ile Gly Glu Lys Ser Phe Thr Lys Lys Thr Thr Ala Ala Gly Tyr Thr
                725                 730                 735

Tyr Gln Val Tyr Glu Gly Ser Asp Lys Asp Ser Thr Ala His Lys Asn
            740                 745                 750

Met Tyr Leu Thr Trp Asn Val Pro Trp Ala Glu Gly Thr Ile Ser Ala
        755                 760                 765

Glu Ala Tyr Asp Glu Asn Asn Arg Leu Ile Pro Glu Gly Ser Thr Glu
    770                 775                 780

Gly Asn Ala Ser Val Thr Thr Thr Gly Lys Ala Ala Lys Leu Lys Ala
785                 790                 795                 800

Asp Ala Asp Arg Lys Thr Ile Thr Ala Asp Gly Lys Asp Leu Ser Tyr
                805                 810                 815

Ile Glu Val Asp Val Thr Asp Ala Asn Gly His Ile Val Pro Asp Ala
            820                 825                 830

Ala Asn Arg Val Thr Phe Asp Val Lys Gly Ala Gly Lys Leu Val Gly
        835                 840                 845
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```
Val Asp Asn Gly Ser Ser Pro Asp His Asp Ser Tyr Gln Ala Asp Asn
    850                 855                 860

Arg Lys Ala Phe Ser Gly Lys Val Leu Ala Ile Val Gln Ser Thr Lys
865                 870                 875                 880

Glu Ala Gly Glu Ile Thr Val Thr Ala Lys Ala Asp Gly Leu Gln Ser
                885                 890                 895

Ser Thr Val Lys Ile Ala Thr Thr Ala Val Pro Gly Thr Ser Thr Glu
            900                 905                 910

Lys Thr Val Arg Ser Phe Tyr Tyr Ser Arg Asn Tyr Tyr Val Lys Thr
        915                 920                 925

Gly Asn Lys Pro Ile Leu Pro Ser Asp Val Glu Val Arg Tyr Ser Asp
    930                 935                 940

Gly Thr Ser Asp Arg Gln Asn Val Thr Trp Asp Ala Val Ser Asp Asp
945                 950                 955                 960

Gln Ile Ala Lys Ala Gly Ser Phe Ser Val Ala Gly Thr Val Ala Gly
                965                 970                 975

Gln Lys Ile Ser Val Arg Val Thr Met Ile Asp Glu Ile Gly Ala Leu
            980                 985                 990

Leu Asn Tyr Ser Ala Ser Thr Pro  Val Gly Thr Pro Ala  Val Leu Pro
        995                 1000                1005

Gly Ser  Arg Pro Ala Val Leu  Pro Asp Gly Thr Val  Thr Ser Ala
    1010                1015                1020

Asn Phe  Ala Val His Trp Thr  Lys Pro Ala Asp Thr  Val Tyr Asn
    1025                1030                1035

Thr Ala  Gly Thr Val Lys Val  Pro Gly Thr Ala Thr  Val Phe Gly
    1040                1045                1050

Lys Glu  Phe Lys Val Thr Ala  Thr Ile Arg Val Gln  Arg Ser Gln
    1055                1060                1065

Val Thr  Ile Gly Ser Ser Val  Ser Gly Asn Ala Leu  Arg Leu Thr
    1070                1075                1080

Gln Asn  Ile Pro Ala Asp Lys  Gln Ser Asp Thr Leu  Asp Ala Ile
    1085                1090                1095

Lys Asp  Gly Ser Thr Thr Val  Asp Ala Asn Thr Gly  Gly Gly Ala
    1100                1105                1110

Asn Pro  Ser Ala Trp Thr Asn  Trp Ala Tyr Ser Lys  Ala Gly His
    1115                1120                1125

Asn Thr  Ala Glu Ile Thr Phe  Glu Tyr Ala Thr Glu  Gln Gln Leu
    1130                1135                1140
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Gly Gln  Ile Val Met Tyr Phe  Phe Arg Asp Ser Asn  Ala Val Arg
    1145                 1150                 1155

Phe Pro  Asp Ala Gly Lys Thr  Lys Ile Gln Ile Ser  Ala Asp Gly

    1160                 1165                 1170

Lys Asn  Trp Thr Asp Leu Ala  Ala Thr Glu Thr Ile  Ala Ala Gln
    1175                 1180                 1185

Glu Ser  Ser Asp Arg Val Lys  Pro Tyr Thr Tyr Asp  Phe Ala Pro
    1190                 1195                 1200

Val Gly  Ala Thr Phe Val Arg  Val Thr Val Thr Asn  Ala Asp Thr
    1205                 1210                 1215

Thr Thr  Pro Ser Gly Val Val  Cys Ala Gly Leu Thr  Glu Ile Glu
    1220                 1225                 1230

Leu Lys  Thr Ala Thr Ser Lys  Phe Val Ala Asn Thr  Ser Ala Ala
    1235                 1240                 1245

Leu Ser  Ser Leu Thr Val Asn  Gly Thr Lys Val Ser  Asp Ser Val
    1250                 1255                 1260

Leu Ala  Ala Gly Ser Tyr Asn  Thr Pro Ala Ile Ile  Ala Asp Val
    1265                 1270                 1275

Lys Ala  Glu Gly Glu Gly Asn  Ala Ser Val Thr Val  Leu Pro Ala
    1280                 1285                 1290

His Asp  Asn Val Ile Arg Val  Ile Thr Glu Ser Glu  Asp His Val
    1295                 1300                 1305

Thr Arg  Lys Thr Phe Thr Ile  Asn Leu Gly Thr Glu  Gln Glu Phe
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Pro Ala  Asp Ser Asp Glu Arg  Asp Gln His Gln His  Gln His Gln

    1325                 1330                 1335

His Gln  Gln
    1340
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<211> 1752

<212> PRT

<213> Bifidobacterium bifidum

<400> 3

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Val Ala Leu Ser Ile Pro Leu Gly Leu Leu Thr Asn Ser Ala Trp Ala
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            20                  25                  30

Val Glu Asp Ala Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr
        35                  40                  45

Pro Glu Val Val Tyr Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr
    50                  55                  60

Ser Asp Phe Asp Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln
65                  70                  75                  80

Ala Gln Asp Pro Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu
                85                  90                  95

Pro His Asp Tyr Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala
            100                 105                 110

Glu Ser Ala Tyr Leu Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe
        115                 120                 125

Thr Ile Asp Arg Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp
    130                 135                 140

Gly Val Tyr Met Asn Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly
145                 150                 155                 160

Thr His Pro Tyr Gly Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn
                165                 170                 175

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

Leu Pro Ser Ser Arg Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val
        195                 200                 205

Thr Leu Thr Val Thr Asp Gly Val His Val Gly Asn Asn Gly Val Ala
    210                 215                 220

Ile Lys Thr Pro Ser Leu Ala Thr Gln Asn Gly Gly Asp Val Thr Met
225                 230                 235                 240

Asn Leu Thr Thr Lys Val Ala Asn Asp Thr Glu Ala Ala Ala Asn Ile
                245                 250                 255

Thr Leu Lys Gln Thr Val Phe Pro Lys Gly Gly Lys Thr Asp Ala Ala
            260                 265                 270

Ile Gly Thr Val Thr Thr Ala Ser Lys Ser Ile Ala Ala Gly Ala Ser
        275                 280                 285

Ala Asp Val Thr Ser Thr Ile Thr Ala Ala Ser Pro Lys Leu Trp Ser
    290                 295                 300

Ile Lys Asn Pro Asn Leu Tyr Thr Val Arg Thr Glu Val Leu Asn Gly
305                 310                 315                 320
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Gly Lys Val Leu Asp Thr Tyr Asp Thr Glu Tyr Gly Phe Arg Trp Thr
                325                 330                 335

Gly Phe Asp Ala Thr Ser Gly Phe Ser Leu Asn Gly Glu Lys Val Lys
            340                 345                 350

Leu Lys Gly Val Ser Met His His Asp Gln Gly Ser Leu Gly Ala Val
        355                 360                 365

Ala Asn Arg Arg Ala Ile Glu Arg Gln Val Glu Ile Leu Gln Lys Met
    370                 375                 380

Gly Val Asn Ser Ile Arg Thr Thr His Asn Pro Ala Ala Lys Ala Leu
385                 390                 395                 400

Ile Asp Val Cys Asn Glu Lys Gly Val Leu Val Val Glu Glu Val Phe
                405                 410                 415

Asp Met Trp Asn Arg Ser Lys Asn Gly Asn Thr Glu Asp Tyr Gly Lys
            420                 425                 430

Trp Phe Gly Gln Ala Ile Ala Gly Asp Asn Ala Val Leu Gly Gly Asp
        435                 440                 445

Lys Asp Glu Thr Trp Ala Lys Phe Asp Leu Thr Ser Thr Ile Asn Arg
    450                 455                 460

Asp Arg Asn Ala Pro Ser Val Ile Met Trp Ser Leu Gly Asn Glu Met
465                 470                 475                 480

Met Glu Gly Ile Ser Gly Ser Val Ser Gly Phe Pro Ala Thr Ser Ala
                485                 490                 495

Lys Leu Val Ala Trp Thr Lys Ala Ala Asp Ser Thr Arg Pro Met Thr
            500                 505                 510

Tyr Gly Asp Asn Lys Ile Lys Ala Asn Trp Asn Glu Ser Asn Thr Met
        515                 520                 525

Gly Asp Asn Leu Thr Ala Asn Gly Gly Val Val Gly Thr Asn Tyr Ser
    530                 535                 540

Asp Gly Ala Asn Tyr Asp Lys Ile Arg Thr Thr His Pro Ser Trp Ala
545                 550                 555                 560

Ile Tyr Gly Ser Glu Thr Ala Ser Ala Ile Asn Ser Arg Gly Ile Tyr
                565                 570                 575

Asn Arg Thr Thr Gly Gly Ala Gln Ser Ser Asp Lys Gln Leu Thr Ser
            580                 585                 590

Tyr Asp Asn Ser Ala Val Gly Trp Gly Ala Val Ala Ser Ser Ala Trp
        595                 600                 605

Tyr Asp Val Val Gln Arg Asp Phe Val Ala Gly Thr Tyr Val Trp Thr
    610                 615                 620
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Gly Phe Asp Tyr Leu Gly Glu Pro Thr Pro Trp Asn Gly Thr Gly Ser
625 630 635 640

Gly Ala Val Gly Ser Trp Pro Ser Pro Lys Asn Ser Tyr Phe Gly Ile
645 650 655

Val Asp Thr Ala Gly Phe Pro Lys Asp Thr Tyr Tyr Phe Tyr Gln Ser
660 665 670

Gln Trp Asn Asp Asp Val His Thr Leu His Ile Leu Pro Ala Trp Asn
675 680 685

Glu Asn Val Val Ala Lys Gly Ser Gly Asn Asn Val Pro Val Val Val
690 695 700

Tyr Thr Asp Ala Ala Lys Val Lys Leu Tyr Phe Thr Pro Lys Gly Ser
705 710 715 720

Thr Glu Lys Arg Leu Ile Gly Glu Lys Ser Phe Thr Lys Lys Thr Thr
725 730 735

Ala Ala Gly Tyr Thr Tyr Gln Val Tyr Glu Gly Ser Asp Lys Asp Ser
740 745 750

Thr Ala His Lys Asn Met Tyr Leu Thr Trp Asn Val Pro Trp Ala Glu
755 760 765

Gly Thr Ile Ser Ala Glu Ala Tyr Asp Glu Asn Asn Arg Leu Ile Pro
770 775 780

Glu Gly Ser Thr Glu Gly Asn Ala Ser Val Thr Thr Thr Gly Lys Ala
785 790 795 800

Ala Lys Leu Lys Ala Asp Ala Asp Arg Lys Thr Ile Thr Ala Asp Gly
805 810 815

Lys Asp Leu Ser Tyr Ile Glu Val Asp Val Thr Asp Ala Asn Gly His
820 825 830

Ile Val Pro Asp Ala Ala Asn Arg Val Thr Phe Asp Val Lys Gly Ala
835 840 845

Gly Lys Leu Val Gly Val Asp Asn Gly Ser Ser Pro Asp His Asp Ser
850 855 860

Tyr Gln Ala Asp Asn Arg Lys Ala Phe Ser Gly Lys Val Leu Ala Ile
865 870 875 880

Val Gln Ser Thr Lys Glu Ala Gly Glu Ile Thr Val Thr Ala Lys Ala
885 890 895

Asp Gly Leu Gln Ser Ser Thr Val Lys Ile Ala Thr Thr Ala Val Pro
900 905 910

Gly Thr Ser Thr Glu Lys Thr Val Arg Ser Phe Tyr Tyr Ser Arg Asn
915 920 925

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Tyr Tyr Val Lys Thr Gly Asn Lys Pro Ile Leu Pro Ser Asp Val Glu
    930                 935                 940

Val Arg Tyr Ser Asp Gly Thr Ser Asp Arg Gln Asn Val Thr Trp Asp
945                 950                 955                 960

Ala Val Ser Asp Asp Gln Ile Ala Lys Ala Gly Ser Phe Ser Val Ala
                965                 970                 975

Gly Thr Val Ala Gly Gln Lys Ile Ser Val Arg Val Thr Met Ile Asp
            980                 985                 990

Glu Ile Gly Ala Leu Leu Asn Tyr  Ser Ala Ser Thr Pro  Val Gly Thr
        995                 1000                1005

Pro Ala  Val Leu Pro Gly Ser  Arg Pro Ala Val Leu  Pro Asp Gly
    1010                1015                1020

Thr Val  Thr Ser Ala Asn Phe  Ala Val His Trp Thr  Lys Pro Ala

    1025                1030                1035

Asp Thr  Val Tyr Asn Thr Ala  Gly Thr Val Lys Val  Pro Gly Thr
    1040                1045                1050

Ala Thr  Val Phe Gly Lys Glu  Phe Lys Val Thr Ala  Thr Ile Arg
    1055                1060                1065

Val Gln  Arg Ser Gln Val Thr  Ile Gly Ser Ser Val  Ser Gly Asn
    1070                1075                1080

Ala Leu  Arg Leu Thr Gln Asn  Ile Pro Ala Asp Lys  Gln Ser Asp
    1085                1090                1095

Thr Leu  Asp Ala Ile Lys Asp  Gly Ser Thr Thr Val  Asp Ala Asn
    1100                1105                1110

Thr Gly  Gly Gly Ala Asn Pro  Ser Ala Trp Thr Asn  Trp Ala Tyr
    1115                1120                1125

Ser Lys  Ala Gly His Asn Thr  Ala Glu Ile Thr Phe  Glu Tyr Ala
    1130                1135                1140

Thr Glu  Gln Gln Leu Gly Gln  Ile Val Met Tyr Phe  Phe Arg Asp
    1145                1150                1155

Ser Asn  Ala Val Arg Phe Pro  Asp Ala Gly Lys Thr  Lys Ile Gln
    1160                1165                1170

Ile Ser  Ala Asp Gly Lys Asn  Trp Thr Asp Leu Ala  Ala Thr Glu
    1175                1180                1185

Thr Ile  Ala Ala Gln Glu Ser  Ser Asp Arg Val Lys  Pro Tyr Thr
    1190                1195                1200

Tyr Asp  Phe Ala Pro Val Gly  Ala Thr Phe Val Lys  Val Thr Val
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Thr Asn Ala Asp Thr Thr Thr Pro Ser Gly Val Val Cys Ala Gly
1220 1225 1230

Leu Thr Glu Ile Glu Leu Lys Thr Ala Thr Ser Lys Phe Val Thr
1235 1240 1245

Asn Thr Ser Ala Ala Leu Ser Ser Leu Thr Val Asn Gly Thr Lys
1250 1255 1260

Val Ser Asp Ser Val Leu Ala Ala Gly Ser Tyr Asn Thr Pro Ala
1265 1270 1275

Ile Ile Ala Asp Val Lys Ala Glu Gly Glu Gly Asn Ala Ser Val
1280 1285 1290

Thr Val Leu Pro Ala His Asp Asn Val Ile Arg Val Ile Thr Glu
1295 1300 1305

Ser Glu Asp His Val Thr Arg Lys Thr Phe Thr Ile Asn Leu Gly
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Thr Glu Gln Glu Phe Pro Ala Asp Ser Asp Glu Arg Asp Tyr Pro
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Ala Ala Asp Met Thr Val Thr Val Gly Ser Glu Gln Thr Ser Gly
1340 1345 1350

Thr Ala Thr Glu Gly Pro Lys Lys Phe Ala Val Asp Gly Asn Thr
1355 1360 1365

Ser Thr Tyr Trp His Ser Asn Trp Thr Pro Thr Thr Val Asn Asp
1370 1375 1380

Leu Trp Ile Ala Phe Glu Leu Gln Lys Pro Thr Lys Leu Asp Ala
1385 1390 1395

Leu Arg Tyr Leu Pro Arg Pro Ala Gly Ser Lys Asn Gly Ser Val
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Thr Glu Tyr Lys Val Gln Val Ser Asp Asp Gly Thr Asn Trp Thr
1415 1420 1425

Asp Ala Gly Ser Gly Thr Trp Thr Thr Asp Tyr Gly Trp Lys Leu
1430 1435 1440

Ala Glu Phe Asn Gln Pro Val Thr Thr Lys His Val Arg Leu Lys
1445 1450 1455

Ala Val His Thr Tyr Ala Asp Ser Gly Asn Asp Lys Phe Met Ser
1460 1465 1470

Ala Ser Glu Ile Arg Leu Arg Lys Ala Val Asp Thr Thr Asp Ile
1475 1480 1485

Ser Gly Ala Thr Val Thr Val Pro Ala Lys Leu Thr Val Asp Arg
1490 1495 1500

1490 1495 1500

Val Asp Ala Asp His Pro Ala Thr Phe Ala Thr Lys Asp Val Thr
1505 1510 1515

Val Thr Leu Gly Asp Ala Thr Leu Arg Tyr Gly Val Asp Tyr Leu
1520 1525 1530

Leu Asp Tyr Ala Gly Asn Thr Ala Val Gly Lys Ala Thr Val Thr
1535 1540 1545

Val Arg Gly Ile Asp Lys Tyr Ser Gly Thr Val Ala Lys Thr Phe
1550 1555 1560

Thr Ile Glu Leu Lys Asn Ala Pro Ala Pro Glu Pro Thr Leu Thr
1565 1570 1575

Ser Val Ser Val Lys Thr Lys Pro Ser Lys Leu Thr Tyr Val Val
1580 1585 1590

Gly Asp Ala Phe Asp Pro Ala Gly Leu Val Leu Gln His Asp Arg
1595 1600 1605

Gln Ala Asp Arg Pro Pro Gln Pro Leu Val Gly Glu Gln Ala Asp
1610 1615 1620

Glu Arg Gly Leu Thr Cys Gly Thr Arg Cys Asp Arg Val Glu Gln
1625 1630 1635

Leu Arg Lys His Glu Asn Arg Glu Ala His Arg Thr Gly Leu Asp
1640 1645 1650

His Leu Glu Phe Val Gly Ala Ala Asp Gly Ala Val Gly Glu Gln
1655 1660 1665

Ala Thr Phe Lys Val His Val His Ala Asp Gln Gly Asp Gly Arg
1670 1675 1680

His Asp Asp Ala Asp Glu Arg Asp Ile Asp Pro His Val Pro Val
1685 1690 1695

Asp His Ala Val Gly Glu Leu Ala Arg Ala Ala Cys His His Val
1700 1705 1710

Ile Gly Leu Arg Val Asp Thr His Arg Leu Lys Ala Ser Gly Phe
1715 1720 1725

Gln Ile Pro Ala Asp Asp Met Ala Glu Ile Asp Arg Ile Thr Gly
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Phe His Arg Phe Glu Arg His Val Gly
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Met Ala Val Arg Arg Leu Gly Gly Arg Ile Val Ala Phe Ala Ala Thr
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Val Glu Asp Ala Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr
35 40 45

Pro Glu Val Val Tyr Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr
50 55 60

Ser Asp Phe Asp Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln
65 70 75 80

Ala Gln Asp Pro Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu
85 90 95

Pro His Asp Tyr Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala
100 105 110

Glu Ser Ala Tyr Leu Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe
115 120 125

Thr Ile Asp Arg Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp
130 135 140

Gly Val Tyr Met Asn Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly
145 150 155 160

Thr His Pro Tyr Gly Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn
165 170 175

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

Leu Pro Ser Ser Arg Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val
195 200 205

Thr Leu Thr Val Thr Asp Gly Val His Val Gly Asn Asn Gly Val Ala
210 215 220

Ile Lys Thr Pro Ser Leu Ala Thr Gln Asn Gly Gly Asn Val Thr Met
225 230 235 240

Asn Leu Thr Thr Lys Val Ala Asn Asp Thr Lys Ala Ala Ala Asn Ile
245 250 255

Thr Leu Lys Gln Thr Val Phe Pro Lys Gly Gly Lys Thr Asp Ala Ala
260 265 270

Ile Gly Thr Val Thr Thr Ala Ser Lys Ser Ile Ala Ala Gly Ala Ser
275 280 285

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Ala Asp Val Thr Ser Thr Ile Thr Ala Ala Ser Pro Lys Leu Trp Ser
    290                 295                 300

Ile Lys Asn Pro Asn Leu Tyr Thr Val Arg Thr Glu Val Leu Asn Gly
305                 310                 315                 320

Gly Lys Val Leu Asp Thr Tyr Asp Thr Glu Tyr Gly Phe Arg Trp Thr
                325                 330                 335

Gly Phe Asp Ala Thr Ser Gly Phe Ser Leu Asn Gly Glu Lys Val Lys
            340                 345                 350

Leu Lys Gly Val Ser Met His His Asp Gln Gly Ser Leu Gly Ala Val
        355                 360                 365

Ala Asn Arg Arg Ala Ile Glu Arg Gln Val Glu Ile Leu Gln Lys Met
    370                 375                 380

Gly Val Asn Ser Ile Arg Thr Thr His Asn Pro Ala Ala Lys Ala Leu
385                 390                 395                 400

Ile Asp Val Cys Asn Glu Lys Gly Val Leu Val Val Glu Glu Val Phe
                405                 410                 415

Asp Met Trp Asn Arg Ser Lys Asn Gly Asn Thr Glu Asp Tyr Gly Lys
            420                 425                 430

Trp Phe Gly Gln Ala Ile Ala Gly Asp Asn Ala Val Leu Gly Gly Asp
        435                 440                 445

Lys Asp Glu Thr Trp Ala Lys Phe Asp Leu Thr Ser Thr Ile Asn Arg
    450                 455                 460

Asp Arg Asn Ala Pro Ser Val Ile Met Trp Ser Leu Gly Asn Glu Met
465                 470                 475                 480

Met Glu Gly Ile Ser Gly Ser Val Ser Gly Phe Pro Ala Thr Ser Ala
                485                 490                 495

Lys Leu Val Ala Trp Thr Lys Ala Ala Asp Ser Thr Arg Pro Met Thr
            500                 505                 510

Tyr Gly Asp Asn Lys Ile Lys Ala Asn Trp Asn Glu Ser Asn Thr Met
        515                 520                 525

Gly Asp Asn Leu Thr Ala Asn Gly Gly Val Val Gly Thr Asn Tyr Ser
    530                 535                 540

Asp Gly Ala Asn Tyr Asp Lys Ile Arg Thr Thr His Pro Ser Trp Ala
545                 550                 555                 560

Ile Tyr Gly Ser Glu Thr Ala Ser Ala Ile Asn Ser Arg Gly Ile Tyr
                565                 570                 575

Asn Arg Thr Thr Gly Gly Ala Gln Ser Ser Asp Lys Gln Leu Thr Ser
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Tyr Asp Asn Ser Ala Val Gly Trp Gly Ala Val Ala Ser Ser Ala Trp
595 600 605

Tyr Asp Val Val Gln Arg Asp Phe Val Ala Gly Thr Tyr Val Trp Thr
610 615 620

Gly Phe Asp Tyr Leu Gly Glu Pro Thr Pro Trp Asn Gly Thr Gly Ser
625 630 635 640

Gly Ala Val Gly Ser Trp Pro Ser Pro Lys Asn Ser Tyr Phe Gly Ile
645 650 655

Val Asp Thr Ala Gly Phe Pro Lys Asp Thr Tyr Tyr Phe Tyr Gln Ser
660 665 670

Gln Trp Asn Asp Asp Val His Thr Leu His Ile Leu Pro Ala Trp Asn
675 680 685

Glu Asn Val Val Ala Lys Gly Ser Gly Asn Asn Val Pro Val Val Val
690 695 700

Tyr Thr Asp Ala Ala Lys Val Lys Leu Tyr Phe Thr Pro Lys Gly Ser
705 710 715 720

Thr Glu Lys Arg Leu Ile Gly Glu Lys Ser Phe Thr Lys Lys Thr Thr
725 730 735

Ala Ala Gly Tyr Thr Tyr Gln Val Tyr Glu Gly Ala Asp Lys Asp Ser
740 745 750

Thr Ala His Lys Asn Met Tyr Leu Thr Trp Asn Val Pro Trp Ala Glu
755 760 765

Gly Thr Ile Ser Ala Glu Ala Tyr Asp Glu Asn Asn Arg Leu Ile Pro
770 775 780

Glu Gly Ser Thr Glu Gly Asn Ala Ser Val Thr Thr Thr Gly Lys Ala
785 790 795 800

Ala Lys Leu Lys Ala Asp Ala Asp Arg Lys Thr Ile Thr Ala Asp Gly
805 810 815

Lys Asp Leu Ser Tyr Ile Glu Val Asp Val Thr Asp Ala Asn Gly His
820 825 830

Ile Val Pro Asp Ala Ala Asn Arg Val Thr Phe Asp Val Lys Gly Ala
835 840 845

Gly Lys Leu Val Gly Val Asp Asn Gly Ser Ser Pro Asp His Asp Ser
850 855 860

Tyr Gln Ala Asp Asn Arg Lys Ala Phe Ser Gly Lys Val Leu Ala Ile
865 870 875 880

Val Gln Ser Thr Lys Glu Ala Gly Glu Ile Thr Val Thr Ala Lys Ala

```
                885                 890                 895

Asp Gly Leu Gln Ser Ser Thr Val Lys Ile Ala Thr Thr Ala Val Pro
            900                 905                 910

Gly Thr Ser Thr Glu Lys Thr Val Arg Ser Phe Tyr Tyr Ser Arg Asn
        915                 920                 925

Tyr Tyr Val Lys Thr Gly Asn Lys Pro Ile Leu Pro Ser Asp Val Glu
    930                 935                 940

Val Arg Tyr Ser Asp Gly Thr Ser Asp Arg Gln Asn Val Thr Trp Asp
945                 950                 955                 960

Ala Val Ser Asp Asp Gln Ile Ala Lys Ala Gly Ser Phe Ser Val Ala
                965                 970                 975

Gly Thr Val Ala Gly Gln Lys Ile Ser Val Arg Val Thr Met Ile Asp
            980                 985                 990

Glu Ile Gly Ala Leu Leu Asn Tyr  Ser Ala Ser Thr Pro  Val Gly Thr
        995                 1000                1005

Pro Ala  Val Leu Pro Gly Ser  Arg Pro Ala Val Leu  Pro Asp Gly
    1010                1015                1020

Thr Val  Thr Ser Ala Asn Phe  Ala Val Asp Trp Thr  Lys Pro Ala
    1025                1030                1035

Asp Thr  Val Tyr Asn Thr Ala  Gly Thr Val Lys Val  Pro Gly Thr
    1040                1045                1050

Ala Thr  Val Phe Gly Lys Glu  Phe Lys Val Thr Ala  Thr Ile Arg
    1055                1060                1065

Val Gln  Arg Ser Gln Val Thr  Ile Gly Ser Ser Val  Ser Gly Asn
    1070                1075                1080

Ala Leu  Arg Leu Thr Gln Asn  Ile Pro Ala Asp Lys  Gln Ser Asp
    1085                1090                1095

Thr Leu  Asp Ala Ile Lys Asp  Gly Ser Thr Thr Val  Asp Ala Asn
    1100                1105                1110

Thr Gly  Gly Gly Ala Asn Pro  Ser Ala Trp Thr Asn  Trp Ala Tyr
    1115                1120                1125

Ser Lys  Ala Gly His Asn Thr  Ala Glu Ile Thr Phe  Glu Tyr Ala
    1130                1135                1140

Thr Glu  Gln Gln Leu Gly Gln  Ile Val Met Tyr Phe  Phe Arg Asp
    1145                1150                1155

Ser Asn  Ala Val Arg Phe Pro  Asp Ala Gly Lys Thr  Lys Ile Gln
    1160                1165                1170

Ile Ser  Ala Asp Gly Lys Asn  Trp Thr Asp Leu Ala  Ala Thr Glu
    1175                1180                1185
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Thr Ile Ala Ala Gln Glu Ser Ser Asp Arg Val Lys Pro Tyr Thr
1190 1195 1200

Tyr Asp Phe Ala Pro Val Gly Ala Thr Phe Val Lys Val Thr Val
1205 1210 1215

Thr Asn Ala Asp Thr Thr Thr Pro Ser Gly Val Val Cys Ala Gly
1220 1225 1230

Leu Thr Glu Ile Glu Leu Lys Thr Ala Thr Ser Lys Phe Val Thr
1235 1240 1245

Asn Thr Ser Ala Ala Leu Ser Ser Leu Thr Val Asn Gly Thr Lys
1250 1255 1260

Val Ser Asp Ser Val Leu Ala Ala Gly Ser Tyr Asn Thr Pro Ala
1265 1270 1275

Ile Ile Ala Asp Val Lys Ala Glu Gly Glu Gly Asn Ala Ser Val
1280 1285 1290

Thr Val Leu Pro Ala His Asp Asn Val Ile Arg Val Ile Thr Glu
1295 1300 1305

Ser Glu Asp His Val Thr Arg Lys Thr Phe Thr Ile Asn Leu Gly
1310 1315 1320

Thr Glu Gln Glu Phe Pro Ala Asp Ser Asp Glu Arg Asp Tyr Pro
1325 1330 1335

Ala Ala Asp Met Thr Val Thr Ala Gly Ser Glu Gln Thr Ser Gly
1340 1345 1350

Thr Ala Thr Glu Gly Pro Lys Lys Phe Ala Val Asp Gly Asn Thr
1355 1360 1365

Ser Thr Tyr Trp His Ser Asn Trp Thr Pro Thr Thr Val Asn Asp
1370 1375 1380

Leu Trp Ile Ala Phe Glu Leu Gln Lys Pro Thr Lys Leu Asp Ala
1385 1390 1395

Leu Arg Tyr Leu Pro Arg Pro Ala Gly Ser Lys Asn Gly Ser Val
1400 1405 1410

Thr Glu Tyr Lys Val Gln Val Ser Asp Asp Gly Thr Asn Trp Thr
1415 1420 1425

Asp Ala Gly Ser Gly Thr Trp Thr Thr Asp Tyr Gly Trp Lys Leu

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Ala Glu Phe Asn Gln Pro Val Thr Thr Lys His Val Arg Leu Lys
1445 1450 1455

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Ala Val  His Thr Tyr Ala Asp  Ser Gly Asn Asp Lys  Phe Met Ser
    1460                  1465                  1470

Ala Ser  Glu Ile Arg Leu Arg  Lys Ala Val Asp Thr  Thr Asp Ile
    1475                  1480                  1485

Ser Gly  Ala Thr Val Thr Val  Pro Ala Lys Leu Thr  Val Asp Arg
    1490                  1495                  1500

Val Asp  Ala Asp His Pro Ala  Thr Phe Ala Thr Lys  Asp Val Thr
    1505                  1510                  1515

Val Thr  Leu Gly Asp Ala Thr  Leu Arg Tyr Gly Val  Asp Tyr Leu
    1520                  1525                  1530

Leu Asp  Tyr Ala Gly Asn Thr  Ala Val Gly Lys Ala  Thr Val Thr
    1535                  1540                  1545

Val Arg  Gly Ile Asp Lys Tyr  Ser Gly Thr Val Ala  Lys Thr Phe
    1550                  1555                  1560

Thr Ile  Glu Leu Lys Asn Ala  Pro Ala Pro Glu Pro  Thr Leu Thr
    1565                  1570                  1575

Ser Val  Ser Val Lys Thr Lys  Pro Ser Lys Leu Thr  Tyr Val Val
    1580                  1585                  1590

Gly Asp  Ala Phe Asp Pro Ala  Gly Leu Val Leu Gln  Leu Asn Tyr
    1595                  1600                  1605

Asp Asp  Asp Ser Thr Gly Thr  Val Thr Trp Asn Thr  Gln Thr Ala
    1610                  1615                  1620

Gly Asp  Phe Thr Phe Lys Pro  Ala Leu Asp Ala Lys  Leu Lys Val
    1625                  1630                  1635

Thr Asp  Lys Thr Val Thr Val  Thr Tyr Gln Gly Lys  Ser Ala Val
    1640                  1645                  1650

Ile Asp  Ile Thr Val Ser Gln  Pro Ala Pro Thr Val  Ser Lys Thr
    1655                  1660                  1665

Asp Leu  Asp Lys Ala Ile Lys  Ala Ile Glu Ala Lys  Asn Pro Asp
    1670                  1675                  1680

Ser Ser  Lys Tyr Thr Ala Asp  Ser Trp Lys Thr Phe  Ala Asp Ala
    1685                  1690                  1695

Met Ala  His Ala Lys Ala Val  Ile Ala Asp Asp Ser  Ala Thr Gln
    1700                  1705                  1710

Gln Asp  Val Asp Lys Ala Leu  Lys Ala Leu Thr Asp  Ala Tyr Ala
    1715                  1720                  1725

Gly Leu  Thr Glu Lys Thr Pro  Glu Pro Ala Pro Val  Ser Lys Ser
    1730                  1735                  1740
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Glu Leu  Asp Lys Lys Ile Lys  Ala Ile Glu Ala Glu  Lys Leu Asp
    1745                 1750                 1755

Gly Ser  Lys Tyr Thr Ala Glu  Ser Trp Lys Ala Phe  Glu Thr Ala
    1760                 1765                 1770

Leu Ala  His Ala Lys Ala Val  Ile Ala Ser Asp Ser  Ala Thr Gln
    1775                 1780                 1785

Gln Asp  Val Asp Ala Ala Leu  Gly Ala Leu Thr Ser  Ala Arg Asp
    1790                 1795                 1800

Gly Leu  Thr Glu Lys Gly Glu  Val Lys Pro Asp Pro  Lys Pro Glu
    1805                 1810                 1815

Pro Gly  Thr Val Asp Lys Ala  Ala Leu Asp Lys Ala  Val Lys Lys
    1820                 1825                 1830

Val Glu  Ala Glu Lys Leu Asp  Gly Ser Lys Tyr Thr  Ala Asp Ser
    1835                 1840                 1845

Trp Lys  Ala Phe Glu Thr Ala  Leu Ala His Ala Lys  Ala Val Ile
    1850                 1855                 1860

Gly Asn  Ala Asn Ser Thr Gln  Phe Asp Ile Asp Asn  Ala Leu Ser
    1865                 1870                 1875

Met Leu  Asn Asp Ala Arg Ala  Ala Leu Lys Glu Lys  Pro Gly Arg
    1880                 1885                 1890

Ile Ile  Ala Ile Ile Asp Gly  Gly Ala Leu Ser Lys  Thr Gly Ala
    1895                 1900                 1905

Ser Val  Ala Ile Ile Ala Ser  Val Ala Ala Ala Met  Lys Ala Val
    1910                 1915                 1920

Gly Ala  Gly Val Met Ala Leu  Arg Pro Pro Lys Trp
    1925                 1930                 1935
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REFERENCES CITED IN THE DESCRIPTION

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- WO2013160413A [0003]
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PATENTKRAV

1. Fremgangsmåde til fremstilling af et fermenteret mælkeprodukt omfattende trinnene

1) tilsætning af en startkultur omfattende mindst en mælkesyrebakteriestamme til en mælkebase,

2) fermentering af mælken i et tidsrum, indtil en mål-pH er nået,

3) hvor startkulturen omfatter mindst én laktosedeficient stamme, som er i stand til at metabolisere et ikke-laktosecarbonhydrat, og

4) tilsætning af en laktase, som er stabil ved lav pH, til processen enten ved starten, under eller ved slutningen af fermenteringstrinnet, hvor laktasen, som er stabil ved lav pH, bevarer sin aktivitet ved en pH på 5,0 og en temperatur på 37°C ved et niveau på mindst 5% sammenlignet med dets aktivitet ved laktasens optimale pH.

2. Fremgangsmåde ifølge krav 1, hvor laktasen, som er stabil ved lav pH, bevarer sin aktivitet ved en temperatur på 10°C og en pH på 6,0 ved et niveau på mindst 10% sammenlignet med dens aktivitet ved laktasens optimale temperatur.

3. Fremgangsmåde ifølge krav 1 eller 2, hvor den laktosedeficiente stamme er i stand til at metabolisere et ikke-laktosecarbonhydrat valgt fra gruppen bestående af sakkarose, galaktose og glukose.

4. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor ikke-laktose-carbonhydrat tilsættes til mælkebasen ved starten af fermenteringstrinnet.

5. Fremgangsmåde ifølge krav 4, hvor ikke-laktose-carbonhydratet tilsættes til mælkebasen i en mængde målt for at blive udtømt og dermed resultere i standsning af væksten af mælkesyrebakterier og standsning af fermenteringen.

6. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor laktasen, som er stabil ved lav pH, tilsættes til mælkebasen ved starten af fermenteringstrinnet.

7. Fremgangsmåde ifølge krav 6, hvor intet ikke-laktosecarbonhydrat tilsættes til fermenteringstrinnet, og hvor mindst én laktosedeficient mælkesyrestamme fra startkulturen er i stand til at metabolisere et carbonhydrat valgt fra gruppen bestående af glukose og galaktose. .

8. Fremgangsmåde ifølge et hvilket som helst af kravene 1-5, hvor laktasen, som er stabil ved lav pH, tilsættes til mælkebasen ved slutningen af fermenteringstrinnet.

9. Fremgangsmåde ifølge et hvilket som helst af kravene 1-8, hvor den laktosedeficiente stamme er valgt fra gruppen bestående af laktosedeficient *Streptococcus thermophilus* og laktosedeficient *Lactobacillus delbrueckii* subsp. *bulgaricus.*

10. Fremgangsmåde ifølge krav 9, hvor den laktosedeficiente stamme er valgt fra gruppen bestående af:

(a) en *Streptococcus thermophilus*-stamme, hvilken stamme er:

(i) stammen deponeret hos DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, den 12-06-2014 under deponeringsnr. DSM 28952;

(ii) eller en stamme afledt fra DSM 28952, hvor den afledte stamme yderligere er karakteriseret som havende evne til at danne hvide kolonier på et medium indeholdende laktose og X-Gal;

(b) en *Streptococcus thermophilus*-stamme, hvilken stamme er:

(i) stammen deponeret hos DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, den 12-06-2014 under deponeringsnr. DSM 28953;

(ii) eller en stamme afledt af DSM 28953, hvor den afledte stamme yderligere er karakteriseret som havende evne til at danne hvide kolonier på et medium indeholdende laktose og X-Gal;

(c) en *Lactobacillus delbrueckii* ssp. *Bulgaricus*-stamme, hvilken stamme er:

(i) stammen deponeret hos DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, den 12-06-2014 under deponeringsnr. DSM 28910;

(ii) eller en stamme afledt af DSM 28910, hvor den afledte stamme yderligere er karakteriseret som havende evne til at danne hvide kolonier på et medium indeholdende laktose og X-Gal.

11. Fremgangsmåde ifølge krav 9 eller 10, hvor startkulturen både indeholder mindst én laktosedeficient *Streptococcus thermophilus* og mindst én laktosedeficient *Lactobacillus delbrueckii* subsp. *bulgaricus.*

12. Fremgangsmåde ifølge et hvilket som helst af kravene 9-11, hvor startkulturen indeholder mindst én *Streptococcus thermophilus* og mindst én *Lactobacillus delbrueckii* subsp. *bulgaricus,* og hvor alle *Streptococcus thermophilus*- og alle *Lactobacillus delbrueckii* subsp. *Bulgaricus*-stammerne er laktosedeficiente.

13. Anvendelse i en fremgangsmåde til fremstilling af et fermenteret mælkeprodukt omfattende trinnene

1) tilsætning af en startkultur omfattende mindst én mælkesyrebakteriestamme til en mælkebase,

2) fermentering af mælken i et tidsrum, indtil en mål-pH er nået,
af

3) startkulturen omfattende mindst én laktosedeficient stamme, som er i stand til at metabolisere et ikke-laktosecarbonhydrat, og

4) en laktase, som er stabil ved lav pH, tilsat til processen enten ved starten, under eller ved slutningen af fermenteringstrinnet, hvor laktasen, som er stabil ved lav pH, bevarer sin aktivitet ved en pH på 5,0 og en temperatur på 37°C ved et niveau på mindst 5% sammenlignet med dets aktivitet ved laktasens optimale pH.

14. Anvendelse ifølge krav 13 til at øge teksturen af det fermenterede mælkeprodukt sammenlignet med anvendelse af laktosedeficiente mælkesyrebakterier og ingen laktase og sammenlignet med anvendelse af en laktase, som er stabil ved lav pH, og laktosepositive mælkesyrebakterier.