

Product and Process

The present invention relates to a novel α -galactosidase with transgalactosylating activity capable of converting mellibiose to α -galactobiose disaccharides. In particular it
5 relates to an α -galactosidase isolated from a recently discovered strain of *Bifidobacterium bifidum*.

The invention particularly relates to DNA sequences encoding the isolated α -galactosidase enzyme, to the enzyme encoded by such a DNA sequence and to a host cell
10 comprising a DNA sequence or containing a recombinant vector incorporating the DNA sequence. The invention also relates to the use of the enzyme encoded by DNA sequence, or of the host cell containing a DNA sequence or recombinant vector, to produce α -galactobiose.

15 Bifidobacteria naturally colonise the lower intestinal tract, an environment which is poor in mono and disaccharides since such sugars are preferentially consumed by the host and microbes present in the upper intestinal tract. In order to survive in the lower intestinal tract bifidobacteria produce various kinds of exo- and endoglycosidases in surface bound and/or extracellular forms, by which they can utilise diverse carbohydrates.

20 Besides hydrolase activity, some enzymes from bifidobacteria show transferase activity. This transglycosylation activity of glycosidases is extensively used for the enzymatic synthesis of various oligosaccharides, which have proven to act as bifidobacteria growth promoting factors.

25 It is known that members of bifidobacteria produce β -galactosidase enzymes that are involved in the bacterial metabolism of lactose. Moller, P.L. et al in Appl & Environ. Microbial., (2001), 62, (5), 2276-2283 describe the isolation and characterisation of three β -galactosidase genes from a strain of *Bifidobacterium bifidum*. They found that all three β -galactosidases were able to catalyse the formation of beta-linked galactooligosaccharides
30

by transgalactosylation.

It is known that some species of bifidobacteria, but not *B. bifidum* produce α -galactosidases as well as β -galactosidases. α -Galactosidases belong to the group of glycosyl hydrolases and can be classified into two groups based on their substrate specificity, i.e. one group is specific for small saccharides such as p-nitrophenyl- α -D-galactopyranoside, melibiose and raffinose, and the other group can liberate galactose from galactomannans such as guar gum, in addition to small substrates.

A strain of *Bifidobacterium bifidum* has been found that is capable of producing a galactosidase enzyme activity that converts lactose to a novel mixture of galactooligosaccharides which unexpectedly contains up to 35% of disaccharides including galabiose (Gal (α 1-6) - Gal). This disaccharide is known (see Paton, J.C. & Paton, A.W. (1998), Clin. Microbiol. Revs., 11, 450-479; Carlsson, K.A. (1989), Ann. Reviews Biochem., 58, 309-350) to be an antiadhesive capable of preventing the adhesion of toxins, e.g. Shiga toxin and pathogens such as E. coli, to the wall of the gut.

This strain of *B. bifidum* was deposited under accession number NCIMB 41171 at the National Collection of Industrial & Marine Bacteria, Aberdeen, UK on 31 March 2003. It is also described in UK Patent No. 2 412 380.

It has been found that this strain of *B. bifidum* produces an α -galactosidase that is capable of converting melibiose to α -galactobiose disaccharides.

According to the invention there is provided a DNA sequence which encodes a protein with an amino acid sequence as given in SEQ. ID NO: 2 or hybridises under stringent conditions to the DNA sequence which encodes this protein. The DNA sequence is given in SEQ. ID NO: 1 or may comprise a fragment or degenerative thereof.

The phrase "degenerative" is construed to mean a DNA sequence which is at least

50% homologous to SEQ. ID NO: 1, preferably from 50 to 98% homologous, most preferably from 75 to 95% homologous.

Such a DNA sequence may comprise nucleotide substitutions, additions or deletions
5 which result in less than 60%, preferably less than 45%, more preferably less than 25% change in the amino acid sequence shown in SEQ. ID NO: 2. Nucleotide substitutions may result in conservative amino acid substitutions.

According to a second aspect of the invention there is provided an enzyme encoded
10 by a DNA sequence as defined above. Such an enzyme may comprise the amino acid sequence given in SEQ. ID NO: 2 or a fragment thereof.

According to a third aspect of the invention there is provided a recombinant vector, preferably an expression vector, comprising a DNA sequence as defined above. Such a
15 vector may be incorporated into a host cell such as a bacterial, yeast or fungal cell. Alternatively, the DNA sequence may be incorporated into such a host cell. A suitable host cell may be selected from Bifidobacterium, Lactococcus, Lactobacillus, Bacillus for example Bacillus subtilis or Bacillus circulans, Escherichia and Aspergillus for example Aspergillus niger.

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Using mellibiose as a substrate, the enzyme encoded by the DNA sequence as defined above produces a mixture of oligosaccharides, in particular α -galactobiose disaccharides.

25 The enzyme or the host cell as described above may be used to produce α -galactobiose disaccharides, which may form part of a product for improving gut health. Such a product may be selected from the group consisting of dairy products (for example liquid milk, dried milk powder such as whole milk powder, skimmed milk powder, fat filled milk powders, whey powders, baby milks, baby formula, ice cream, yoghurt, cheese,
30 fermented dairy products), beverages such as

fruit juice, infant foods, cereals, bread, biscuits, confectionery, cakes, food supplements, dietary supplements, probiotic comestible products, prebiotic comestible products, animal feeds, poultry feeds or indeed any other food or beverage.

5 Alternatively, the disaccharides so produced may be used for the preparation of a medicament for example in tablet or capsule form for preventing the adhesion of pathogens or toxins produced by pathogens to the gut wall. The medicament may be administered to a patient, for example following a course of antibiotic treatment, which often alters or even destroys the normal healthy gut flora.

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According to yet a further aspect of the invention there is provided a process for producing an enzyme as defined above which comprises culturing a host cell as defined above in a suitable culture medium under conditions permitting expression of the enzyme and recovering the resulting enzyme or enzyme products from the culture.

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The invention is also directed to a process for producing the galactobiose disaccharides which comprises contacting the enzyme as defined above or a host cell as defined above with a mellibiose-containing material under conditions that lead to the formation of the disaccharides.

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Suitable mellibiose containing material may be selected from commercially available mellibiose, raffinose, stachyose or as an extract of soybeans.

Brief Description of the Drawings

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Figure 1 shows the nucleotide sequence (SEQ. ID NO: 1) of *Bifidobacterium bifidum* α -galactosidase with the start and stop codon indicated in bold letters;

Figure 2 shows the nucleotide sequence of Figure 1 with the amino acid sequence (SEQ. ID NO: 2) of the enzyme;

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Figure 3 shows the first 540 amino acids of the amino acid sequence (SEQ. ID NO: 2) of Figure 2;

Figure 4 is a graph showing the time course reaction during α -galactooligosaccharide synthesis with the α -galactosidase and 40% (w/w) melliboise in 0.1M phosphate buffer at pH 6.0 as substrate; and

Figure 5 shows a high performance anion exchange chromatogram of the α -galactooligosaccharide mixture synthesized by the α -galactosidase from *B. bifidum* NCIMB 41171 using 40% (w/w) mellibiose in 0.1 M phosphate buffer at pH 6.0 as substrate. (Glc = glucose, Gal = galactose, Mel = mellibiose, DP = degree of polymerization). The dashed arrows denote the galactooligosaccharide products.

Genomic DNA was isolated from the *Bifidobacterium bifidum* strain (NCIMB 41171) using the method of Lawson *et al.* (1989) *Fems Microbiol Letters*, 65, (1-2), 41-45. The DNA was digested with restriction enzymes and fragments having a maximum size of 15 kbp were ligated with pBluescript KS(+) vector. *E. coli* cells were transformed with a vector containing insertions consisting of PstI digested chromosomal DNA from the *B. bifidum*. Clones with α -galactosidase activity were selected on Luria Bertani agar plates containing p-nitrophenyl α -D-galactopyranoside and isopropyl- β -D-thiogalactoside (IPTG). Two α -galactosidase positive clones (pMelA1 and pMelA2) were identified.

The two positive clones were digested with EcoRI, PstI and Bam HI and showed a similar restriction pattern indicating that both contained the same inserted DNA fragment. DNA sequencing of the inserted DNA fragment MelA1 was performed using the dideoxy chain-termination method of Sanger (Russel P., 2002 iGenetics, Pearson Education, Inc., San Francisco, 187-189) using the BigDye Terminator V.3.0 cycle sequencing kit (Applied Biosystems, USA). The DNA sequence of MelA1 is shown in Figure 1 (SEQ. ID NO: 1).

The open reading frame (ORF) was located by using the ORF finder from NCBI (National Center of Biotechnology Information). The bacterial genetic code was used and the frame length was determined to be 300 bp. The nucleotide sequence of Figure 1 was translated in all six possible reading frames and one open reading frame of 759 amino acids

encoding a putative α -galactosidase was identified. The translation is shown in Figure 2 (SEQ. ID NO: 2).

5 The present invention will be further described by way of reference to the following examples.

Example 1

Materials and Methods

10 All chemicals and media preparations used throughout this study were obtained from Sigma (Dorset, UK), Invitrogen (Paisley, UK), Oxoid (Basingstoke, UK), Qiagen (West Sussex, UK) and Promega (Southampton, UK).

Bacterial Strains

15 The *Bifidobacterium bifidum* strain (NCIMB 41171) was maintained on cryogenic beads in Microbank tubes at -70°C . For later experiments, the strain was revived on Wilkinson Chalgren (WC) agar (Oxoid, UK) and TPY medium (trypticase phytone yeast extract medium) and grown anaerobically (CO_2 and N_2 composition 80% and 20% respectively) at 37°C for 48 hours. The colony morphology and the absence of
20 contamination were tested by gram staining.

E. coli strains

Escherichia coli strains RA11r and DH5a used in this study were commonly incubated under aerobic conditions at 37°C in Luria Bertani (LB) agar or broth (Sambrook
25 J. and Russell, W.D., (2001) Molecular Cloning: A Laboratory Manual. Cold Spring Harbour Laboratory Press, New York) and when necessary was supplemented with antibiotics (100 $\mu\text{g}/\text{ml}$ ampicillin and/or 15 $\mu\text{g}/\text{ml}$ chloramphenicol) and 40 μl of 2% X- α -galactopyranoside (X- α -Gal), 7 μl of 20% (isopropyl- β -D-thiogalactoside) IPTG which were applied on the surface of a pre-made 90mm agar plate.

The α -galactosidase deficient strain *E. coli* RA11r (Hanatani et al, 1983, J. Biol. Chem., 259, (3), 1807-1812) (genotype: *melA*⁻*B*⁺, *recA*⁻, *lacZ*⁻*Y*⁻) is a derivative of *E. coli* K12 and was used in expression experiments. *E. coli* DH5a strain (Invitrogen, Paisley, UK) (genotype: F⁻ ϕ 80*lacZ* Δ M Δ (*lacZ*YA-argF)U169 *recA*1 *endA*1 *hsdR*17(*r*_k⁻, *m*_k⁻) *phoA* *supE*44 *thi*-1 *gyrA*96 *relA*1 λ ⁻) is an α -galactosidase positive strain and was used for all other genetic manipulations.

The choice of *E. coli* strain RA11r, for expression experiments, was done according to its genotype. This strain does not encode an active α -galactosidase due to *melA* mutation on its chromosomal DNA. However, this strain has an active melibiose transporter which is necessary for the transport of sugars (melibiose) into the cytoplasm and hence the metabolism of them by active α -galactosidases. It was not known whether the *Bifidobacterium bifidum* α -galactosidase was expressed intracellularly or extracellularly. So the existence of an active melibiose transporter was essential for the identification of the α -gal positive clones and hence the isolation of α -galactosidase encoding genes.

Moreover this strain is a *recA* mutant which minimises recombination of introduced DNA with host DNA, thus increasing the stability of inserts.

20 Genomic DNA Extraction from *Bifidobacterium bifidum*

Genomic DNA was isolated from the *Bifidobacterium bifidum* strain (NCIMB 41171) by using the method of Lawson *et al.* (1989).

According to this method, cells were harvested from plates in 0.5 ml of TES buffer in 1.5 ml ependorfs. 10 μ l of lysozyme/mutanolysin mixture (4:1, lysozyme 10 mg/ml; mutanolysin 1 mg/ml) were added and the mixture was then mixed and incubated for 30 minutes at 37°C. The cells were then treated with 10 μ l of proteinase K (at 20 mg/ml) and 10 μ l RNase (10 mg/ml), mixed and incubated for 1 hour at 65°C. After incubation, 100 μ l of 10% SDS was added and the cell lysates were gently mixed by inversion and incubated for a further 15 minutes at 65°C, followed by addition of 0.62 ml of phenol/chloroform and

mixed by inversion until an emulsion formed. The cell lysate was centrifuged at 6,500 rpm for 10 minutes and the upper aqueous layer was transferred to a clean ependorf using a flamed, wide bore blue pipette tip. The extraction (deproteinization step) was repeated until cell debris was removed completely. The DNA was precipitated by the addition of 1 ml ice-cold ethanol followed by incubation for at least 30 minutes on ice or stored overnight in a –20°C freezer. The genomic DNA was recovered by centrifugation at 13,000 rpm for 5 minutes and after drying it was re-suspended in 50 µl of sterile 10 mM Tris-Cl pH 8.

The extracted DNA was analysed by gel electrophoresis and the concentration measured at 260nm. It was stored in –20°C or –70°C for prolonged periods of time and multiple thawing and freezing was avoided in order to reduce the possibility of degradation.

Vector DNA Preparation

The vector used throughout this study was the pBluescript KS(+) (Stratagene, North Torrey Pines Road). This cloning vehicle was chosen because of the *lac* promoter which pBluescript KS (+) encodes which is necessary for the transcription initiation of genes which lack their own promoter.

The vector was digested with the following restriction enzymes: *Pst*I, *Bam*HI and *Eco*RI according to the manufacturer's instructions using a tenfold excess of enzyme over DNA (enzyme units:µgr DNA equal to ten units of enzyme per one µgr of plasmid DNA or ten enzyme units per 0.5 pmol of plasmid DNA). After enzyme heat inactivation (20 min at 65°C) the restriction patterns were analysed by horizontal gel electrophoresis analysis.

The vectors were further dephosphorylated with calf intestinal alkaline phosphatase CIAP (Promega, Southampton, UK) according to the manufacturer's instructions. The efficiency of the treatment was tested by self-ligation of the vector (with Bacteriophage T4 DNA ligase according to manufacturer's instructions) following transformation into DH5a cells.

The presence of a single fragment in the gel indicated the complete vector digestion and the single restriction digestion of it. The sufficient digestion of the vector was tested also by transforming unligated molecules into competent *E. coli* DH5a cells. The number of formed colonies on LB agar plates supplemented with
 5 ampicillin (100µgr/ml) was an indicator of the undigested molecules and the expected background during the subsequent experiments.

Genomic DNA Library Construction

Genomic DNA was partially digested with three restriction enzymes that recognise
 10 frequently occurring hexa-nucleotide sequences within prokaryotic DNA. *EcoRI*, *BamHI* and *PstI* are type II restriction endonucleases specifically recognizing the sequences 5'G/AATTC'3, 5'G/GATCC'3 and 5'CTGCA/G'3 respectively, and make double-strand breaks within these sequences generating 5'overhangs of four nucleotides, AATT, GATC for *EcoRI* and *BamHI* respectively, and 3'overhangs, ACGT for *PstI*.

15

All these enzymes were active and able to cleave DNA only in the presence of divalent magnesium ions. These ions were the only required cofactor.

Restriction Digestion of DNA.

20 All restriction digestions of the genomic DNA samples were incubated for 2 hours at 37°C and finally heat inactivated at 65°C for 20 minutes. The reactions were then cooled at room temperature and the appropriate amount of loading buffer was added, followed by gentle mixing with a sealed glass capillary. The solutions then were loaded into wells of a 0.8% agarose gel (power supply 4-5volts/cm for 14-16 hours) and the size of the digested
 25 DNA was estimated with that of 1kbp DNA standards (Promega, UK) (Sambrook J. Molecular Cloning: A Laboratory Manual (2002)).

Purification of the fragments generated after restriction digestion.

Fragment purification from the reaction mixtures and the agarose gels was done by
 30 using the QIAEX gel extraction kit from Qiagen (West Sussex, UK). Protocols are

described with details in the manufacturer's manual.

DNA Ligation and Transformation

After purification of the DNA fragments with the Qiaex gel extraction kit, they were
 5 ligated with CIAP-treated pBluescript KS (+) vector. For ligation, appropriate amounts of
 DNA were transferred to sterile 0.5 ml microfuge tubes as shown in Table 1.

Tube	DNA
A	Vector (15 fmoles [~ 29.7 ng])
B	Vector (15 fmoles ~ 29.7 ng DNA) plus insert (foreign 15 fmoles ~ 69.3 ng)
C	pUC control (0.056 fmoles [~ 100 pg])

The molar ratio of plasmid DNA vector to insert DNA fragment should be $\sim 1:1$ in the ligation reaction. The final DNA concentration should be ~ 10 ng/ μ l.

Table 1: Ligation mixtures. Tube A shows the number of self-ligated vector DNA which
 10 must be subtracted from the total number of transformants after transformation. Tube B
 shows the ligation of the vector with the DNA fragments and tube C shows the control in
 order that the transformation efficiency to be calculated.

Before each ligation the DNA fragments were warmed at 45°C for 5 minutes to
 melt any cohesive termini that reannealed during fragment preparation. A molar ratio of
 15 vector:insert DNA of 1:1 was chosen for all ligation reactions and the reaction assembly
 was done according to Promega's instructions.

To tubes A and B $1.0\ \mu\text{l}$ of 10x ligation buffer and 0.5 Weiss units of T4 DNA
 ligase (Promega, UK) were added and the ligation volume was adjusted to $10\ \mu\text{l}$ with
 20 molecular biology grade water. To tubes C $1.0\ \mu\text{l}$ of 10x ligation buffer were added and the
 ligation volume was adjusted to $10\ \mu\text{l}$ with molecular biology grade water.

DNA fragments were added to the tubes together with the water and then warmed to
 45°C for 5 minutes to melt any cohesive termini that were reannealed during preparation.
 25 The DNA was chilled to 0°C before the remainder of the ligation reagents were added and
 the reaction mixtures were incubated overnight at 16°C (Sambrook and Russell, 2001).

After ethanol precipitation and purification of the ligated fragments (in order to remove the ligation mixture which cause reduction of the transformation efficiency) transformations were performed according to Hanahan instructions. ~50ng of ligated DNA in 5µl solution was added to 100µl of competent *E. coli* RA11r cells. After heat treatment and expression of the ampicillin resistance gene the cells were spread over the surface of LB plates containing ampicillin (100µg/ml), X-α-Gal (40µl of 2% X-α-Gal) and IPTG (7µl of 20%IPTG).

The number of transformants from each ligation reaction was measured. The number of transformants commonly obtained from tube C was 2×10^5 - 1×10^6 cfu/µg whereas from tube A was 500-600 cfu/µg. The number of transformants in tube A was an indication of the efficient treatment of the vector DNA. The number of transformants in tube B was in a range from 2 - 4×10^4 cfu/µg.

15 Number of Transformants

Ligation mixtures with *Pst*I chromosomal DNA gave rise to two α-galactosidase positive clones (pMelA1 and pMelA2) out of approximately 2500 screened transformants, whereas with *Eco*RI and *Bam*HI -treated chromosomal DNA did not give any positive clone out of approximately 4000 total screened transformants.

20 Positive Clone Digestion

The two *Pst*I positive clones were digested with *Eco*RI, *Pst*I, *Bam*HI, *Hind*III, *Sma*I, and *Kpn*I restriction enzymes. Restriction enzymes *Eco*RI, *Pst*I and *Bam*HI showed similar restriction pattern, one fragment of ~5kbp (gene of interest) and one ~3kbp (plasmid DNA) indicating that these enzymes were cut at the same positions. *Hind*III gave a fragment at 6.5kbp and a fragment at 1.5kbp whereas enzymes *Sma*I and *Kpn*I gave one fragment with size ~8kbp indicating that they were cut at only one position. The similar restriction patterns for both plasmids were an indication that both contain the same DNA fragment insert.

DNA Sequencing

DNA sequencing was performed with the dideoxy chain-termination method of Sanger by using the BigDye Terminator v.3.0 cycle sequencing kit (Applied Biosystems, USA) and analysed with the ABI Prism 3100, a fluorescence-based DNA analysis system incorporating capillary electrophoresis.

The 5'- and 3'- ends of the inserted DNA fragments were sequenced with vector specific primers. The inserts were further sequenced by using the Genome Priming System (GPS-1) (New England Biolabs, UK). GPS-1 is a TN7 transposon-based in vitro system which uses TnsABC Transposase to insert Transposon randomly into the DNA target. The donor: target DNA mass ratio of 1:4 was used according to the manufacturer instructions. The number of isolated plasmids for sequencing after insertion of the Transprimer into the target plasmid was 25. This number was calculated according to the manufacturer instructions and it assumes a 5-fold depth of coverage.

Unique priming sites on both ends of the Transprimer element allowed the sequencing of both strands of the target DNA at the position of the insertion.

The sequencing reaction mix contained approximately 400-600ng plasmid DNA, 3.2pmol of primer solution and 4µl of BigDye Terminator solution.

Open Reading Frame Identification

The open reading frame (ORF) was located by using the ORF finder from NCBI. The bacterial genetic code was used and the frame length was determined to be 300bp. The nucleotide sequence was translated in all six possible frames and one open reading frame of 759 amino acids encoding a putative α -galactosidase was identified (The translation is shown in Figure 2). The start and stop codon was confirmed.

The *Bifidobacterium* α -galactosidase gene on plasmid pMelA1 was expressed in *E. coli* under growth conditions which would normally repress expression from the inducible *E. coli lacZ* promoter located in the flanking region of the cloning vector. This observation

indicated that endogenous, internal bifidobacterial sequences upstream of the α -galactosidase gene may serve as a transcription initiation signal in *E. coli*.

The transcription start is indicated with bold italic letter. The above results indicate
5 that the gene is controlled from its own promoter for transcription.

Example 2

Synthesis with the α -galactosidase cloned enzyme isolated from *Bifidobacterium bifidum* 10 NCIMB 41171 in *E. coli* host (strain RA11)

The following described synthesis, unless otherwise stated, was performed with the whole *E. coli* RA11 host cells after treatment of the *E. coli* biomass (collected by centrifugation at 10,000 g) with toluene at a concentration of 2000 ppm in order to increase cell permeability and also to render the cells non-viable by destroying their cytoplasmic
15 membrane. The *E. coli* biomass was prepared as described in Example 1 under "*E. coli* strains".

Synthesis with cloned enzyme

Synthesis with α -galactosidase was performed at a substrate concentration of 40%
20 (w/w) initial mellibiose concentration. The synthesis solution was prepared in 0.1 M phosphate buffer at pH 6.0. Synthesis was performed at 40°C in shaking waterbath at 150 rpm. The pH optimum for the specific enzyme was chosen based on activity measurements (using p-nitrophenyl- α -D-galactopyranoside as substrate) of a specific enzymatic preparation at varying pH values.

25

For α -galactosidase synthesis 2 ml of an *E. coli* RA11 cell suspension (with an activity of 0.3 U/ml) were centrifuged (at 10,000 g) to collect the biomass and the supernatant was discarded. This biomass was re-suspended with 1 g of 40% (w/w) mellibiose substrate solution in order to perform the synthesis.

30

The concentrations of the different sugars present in the mixture during synthesis are shown in Figure 4. High performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) chromatograms of galactooligosaccharide mixtures synthesized by the α -galactosidase cloned from *B. bifidum* NCIMB 41171 are shown in Figure 5. The galactooligosaccharide mixture sugar concentrations at the optimum synthesis time point are shown in table 1.

Table 1. Carbohydrate composition of α -galactooligosaccharide synthesis at 40 % (w/w) initial mellibiose concentration at the time point where maximum oligosaccharide concentration was observed.

Synthesis Init. Subst.	GOS DP $\geq$ 3	GOS DP=2	Mel	Glc	Gal
% (w/w)	Concentration (% of total sugars)				
40	13.93	6.61	38.06	24.1	17.29

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Mel: Mellibiose, Glc: glucose, Gal: galactose, DP: degree of polymerisation

Claims

1. A DNA sequence which
 - a) encodes a protein with an amino acid sequence as given in SEQ. ID. NO: 2, or
 - b) hybridises under stringent hybridization conditions to the sequence of a), or
 - c) is a degenerative of the sequence of a) or b), which is from 75% to 95% homologous.
2. The DNA sequence according to Claim 1, wherein the sequence is given in SEQ. ID NO: 1 or a fragment or degenerative thereof which is from 75% to 95% homologous.
3. The DNA sequence according to Claim 1 or Claim 2, wherein said sequence comprises nucleotide substitutions, additions or deletions which result in less than 25% change in the amino acid sequence according to SEQ. ID NO: 2.
4. A DNA sequence according to Claim 1 or Claim 2, wherein said sequence comprises nucleotide substitutions which result in conservative amino acid substitutions.
5. An enzyme encoded by a DNA sequence of any one of Claims 1 to 4.
6. An enzyme comprising an amino acid sequence according to SEQ. ID NO: 2 or a fragment thereof.
7. An α -galactosidase having the sequence as defined in SEQ. ID NO: 2.
8. A recombinant vector comprising a DNA sequence of any one of Claims 1 to 4.
9. The vector according to Claim 8, wherein said vector is an expression vector.
10. A host cell comprising a DNA sequence of any one of Claims 1 to 4.

11. A host cell comprising the vector of Claim 8 or Claim 9.
12. The host cell according to Claim 10 or Claim 11, wherein said cell is a bacterial cell, a yeast cell or a fungal cell.
- 5 13. The host cell according to Claim 12, wherein said cell is selected from the group consisting of Bifidobacterium, Lactococcus, Lactobacillus, Escherichia, Bacillus and Aspergillus.
- 10 14. The host cell according to Claim 13, wherein the cell is selected from the group consisting of Bifidobacterium bifidum, Bacillus subtilis, Bacillus circulans and Aspergillus niger.
- 15 15. Use of an enzyme of any one of Claims 5 to 7, or a cell of any one of Claims 10 to 14, for producing α -galactobiose disaccharides.
- 20 16. Use of an enzyme of any one of Claims 5 to 7, or a cell of any one of Claims 10 to 14, for producing α -galactobiose disaccharides to be part of a product selected from the group consisting of dairy products, such as liquid milk, dried milk powder, baby milks, baby formula, ice cream, yoghurt, cheese, fermented dairy products, beverages such as fruit juice, infant foods, cereals, bread, biscuits, confectionary, cakes, food supplements, dietary supplements, probiotic comestible products, prebiotic comestible products, animal feeds, poultry feeds and medicaments.
- 25 17. Use of a host cell of any one of Claims 10 to 14, for producing a product selected from the group consisting of dairy products, such as liquid milk, dried milk powder, baby milks, baby formula, ice cream, yoghurt, cheese, fermented dairy products, beverages such as fruit juice, infant foods, cereals, bread, biscuits, confectionary, cakes, food supplements,

dietary supplement, probiotic comestible product, prebiotic comestible product, animal feeds, poultry feeds and medicaments.

18. A process for producing an enzyme of any one of Claim 5 to 7 comprising culturing
5 a host cell of any one of Claims 10 to 14 in a suitable culture medium under conditions permitting expression of said enzyme and recovering the resulting enzyme from the culture.

19. A process for producing a disaccharide comprising contacting an enzyme of any one
of Claims 5 to 7 or a host cell of any one of Claims 10 to 14 with a solution of mellibiose.
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Sequence listing
SEQUENCE LISTING

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acgtcgagc	3129

<210> 2
 <211> 759
 <212> PRT

Sequence listing

<213> Bifidobacterium bifidum

<400> 2

Met Ser Leu Ile Glu Gln Phe His Gly Ala Ala Ala Asp Gly Thr Glu
1 5 10 15

Leu Thr Ala Ile Tyr Ala Glu Gln Pro Ala Ala Asp Val Ala Phe Ala
20 25 30

Leu Val Phe Ala Gly His Gly Leu Pro Arg Phe Val His Trp Gly Arg
35 40 45

Pro Leu Ala Ala Pro Gly Thr Val Leu Ala Ala Tyr Asp Ala Leu Arg
50 55 60

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

Met Pro Thr Gln Ser Glu Ser Trp Ile Gly Ala Pro Arg Leu Asp Ile
85 90 95

Arg Arg Ala Gly Val Thr Pro Phe Cys Ala Phe Thr Val Thr Gly Ile
100 105 110

Ala Ile Arg Gln Asp Glu Arg Gln Gly Val Asp Val Ser Asp Gly Val
115 120 125

Asp Gly Ala Ala His Thr Val Thr Gln Gln Val Pro Val Val Thr Val
130 135 140

Thr Ala Ser Asp Ala Glu Gln Gly Val Glu Leu Ser Trp Thr Ala Glu
145 150 155 160

Leu Leu Pro Gly Gly Leu Ile Arg Gln Arg Thr Thr Leu Arg Asn Leu
165 170 175

Pro Ala Gly Asn Leu Pro Thr Gly Asp Leu Glu Val Gly Lys Val Glu
180 185 190

Leu Gly Phe Pro Leu Pro Ala Leu Ala Thr Glu Ile Leu Thr Thr Thr
195 200 205

Gly His His Leu Arg Glu Arg Ser Pro Gln Arg Gln Pro Leu Thr Glu
210 215 220

Gly Arg Phe Glu Lys Val Ser Met Ala Gly Arg Pro Gly Phe Asp Ala
225 230 235 240

Ser Leu Leu Leu Ser Ala Gly Glu Pro Gly Phe Gly Phe Glu His Gly
245 250 255

Sequence listing

Glu Val Tyr Ser Val His Val Gly Trp Ser Gly Asn Ser Val Leu Ser
260 270

Ala Glu Arg Gln Pro Tyr Thr Thr Gly Leu Ile Gly Gly Gly Glu Val
275 280 285

Leu Leu Gly Gly Glu Ala Thr Leu Ala Arg Gly Glu Thr Tyr Thr Thr
290 295 300

Pro Trp Leu Tyr Gly Ser Tyr Gly Asp Gly Leu Asn Glu Val Ala Ala
305 310 315 320

Arg Phe His Asp Tyr Val Arg Ser Cys His Pro Asp Leu Ala Val Lys
325 330 335

Pro Arg Pro Val Ile Leu Asn Thr Trp Glu Ala Val Tyr Phe Asp His
340 345 350

Asp Tyr Asp Thr Leu Lys Ala Leu Ala Asp Lys Ala Gly Asp Ser Gly
355 360 365

Val Glu Arg Phe Val Val Asp Asp Gly Trp Phe Gly Ser Arg Arg Asp
370 375 380

Ser Thr Ser Gly Leu Gly Asp Trp Gln Ile Ala Gln Asp Val Trp Pro
385 390 395 400

Asp Gly Pro Lys Ser Leu Lys Ala Leu Ala Asp Tyr Val His Gly Lys
405 410 415

Gly Met Glu Phe Gly Leu Trp Phe Glu Pro Glu Met Val Asn Pro Asp
420 425 430

Ser Asp Val Ala Arg Ala His Pro Asp Trp Val Leu Arg Pro Thr Ala
435 440 445

Asn Arg Leu Pro Met Gln Gly Arg Ser Gln Gln Val Leu Asp Leu Thr
450 455 460

Asn Pro Asp Ala Tyr Arg Tyr Ile His Asp Ser Ile Asp Ala Leu Val
465 470 475 480

Gly Glu Leu Gly Ile Asp Tyr Ile Lys Trp Asp His Asn Lys Phe Val
485 490 495

Thr Glu Ala Val Ser Pro Arg Thr Gly Arg Pro Ala Val His Gly Gln
500 505 510

Thr Leu Ala Val Tyr Arg Met Phe Arg Asp Leu Glu Val Ala His Pro
515 520 525

Sequence listing

Gly Leu Glu Ile Glu Ser Cys Ala Ser Gly Gly Gly Arg Ile Asp Leu
530 535 540

Gly Ile Leu Glu Phe Ala Ser Arg Val Trp Thr Ser Asp Cys Val Asp
545 550 555 560

Pro Val Glu Arg Ala Asp Ile Gln Arg Tyr Thr Ser Leu Leu Val Pro
565 570 575

Pro Cys Met Met Gly Glu His Val Gly Ala Ser Pro Ala His Ser Thr
580 585 590

His Arg Ala Thr Ser Gln Glu Met Arg Met Ala Met Ala Phe Phe Gly
595 600 605

His Met Gly Val Glu Trp Asn Leu Leu Lys Glu Pro Asp Glu Ala Leu
610 615 620

Asn Lys Leu Gly Glu Trp Val Ala Glu Tyr Lys Arg His Arg Ala Trp
625 630 635 640

Phe Ala Ile Asp Thr Cys Val His Ala Asp Ile Ala Asp Pro Ala Val
645 650 655

Arg Val Asp Gly Met Val Lys Pro Asp Arg Ser Ala Ala Phe Tyr Arg
660 665 670

Phe Thr Gln Leu Thr Thr Ser Gln Thr Leu Pro Ala Ala Pro Ile Arg
675 680 685

Val Pro Gly Leu Asp Pro Asp Gly Thr Tyr Arg Ile Gln Pro Leu Trp
690 695 700

Leu Asp Leu Asp Leu Asp Gly Leu Gly Leu Gly Ser Gly Gln Ser Pro
705 710 715 720

Leu Gly Trp Trp Thr Lys Asp Gly Val Leu Met Thr Gly Arg Ala Leu
725 730 735

Met Thr Tyr Gly Leu Arg Pro Pro Ser Leu His Pro Ala Gln Ser Val
740 745 750

Leu Phe Thr Ala Ile Arg Gln
755