half-lives have been utilized to reach a favorable equilibrium for the transgalactosylation reaction (27,37). However, competitive inhibition by glucose and/or galactose is another obstacle that remains which may be overcome by incorporating cells in the reaction (16,20,25,27,35).

[0009] The basidiomycete yeast *Sporobolomyces singularis* (formerly *Bullera singularis*) cannot utilize galactose to grow but proliferates on lactose due to the activity of its β-hexosyl-transferase (BHT, EC 3.2.1.21). Studies have shown that the BHT has transgalactosylation activity even at low lactose concentrations and very limited lactose hydrolysis. In addition, the enzyme does not appear to be inhibited by lactose concentrations above 20% and has the potential for conversions into GOS close the maximum theoretical of 75% (1,9,10,28). Unlike β-galactosidases, the BHT from *S. singularis* simultaneously carries out glycosyl-hydrolase and β-hexosyl-transferase activities, converting lactose to GOS without extracellular accumulation of galactose. Two molecules of lactose are required during the transgalactosylation event: one molecule is hydrolyzed and the second acts as galactose acceptor, generating the trisaccharide galactosyl-lactose (β-D-Gal(1-4)-β-D-Gal(1-4)-β-D-Glc) and residual glucose. Galactosyl-lactose can also act as acceptor of a new galactose to generate the tetrasaccharide galactosylgalactosyl-lactose (β-D-Gal(1-4)-β-D-Gal(1-4)-β-D-Gal(1-4)-β-D-Glc), and similarly for the tetrasaccharide and subsequent products. The tri, tetra, and penta saccharides accumulating in *S. singularis* have been collectively designated GOS (9,10).

[0010] For practical interests, a recombinant secreted BHT could have several advantages over the native enzyme, including improved large scale production and purification. Currently, purification of active enzyme from *S. singularis* requires cell lysis followed by multiple chromatography steps (1,4,16). Previous attempts to express recombinant β-hexosyl-transferase in *E. coli* BL21 have resulted in high levels of production, but the enzyme was inactive and insoluble (16).

3. SUMMARY ~~OF THE INVENTION~~

[0011] ~~In particular non-limiting embodiments, t~~The present ~~invention~~ disclosure provides an isolated DNA coding for a recombinant β-hexosyl-transferase (rBHT) protein having the amino acid sequence set forth in SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20. In one aspect, there is provided an isolated DNA coding for a recombinant β-hexosyl-transferase (rBHT) protein having the amino acid sequence set forth in SEQ ID NO. 4, 6, 8, 10, 12, 14, 16, 18 or 20. The isolated DNA coding for the recombinant β-hexosyl-transferase (rBHT) protein may have the nucleic acid sequence set forth in SEQ ID NO. 1, 3, 5, 7, 9, 11, 13, 15, 17 or 19.

[0012] The ~~invention~~ disclosure also provides, an enzymatically active recombinant β-hexosyl-transferase (rBHT) protein wherein the protein has the amino acid sequence set forth in SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20. In another aspect, there is provided an enzymatically active recombinant β-hexosyl-transferase (rBHT) protein wherein the protein has the amino acid sequence set forth in SEQ ID NO. 4, 6, 8, 10, 12, 14, 16, 18 or 20. The rBHT protein may be membrane bound or may be a soluble enzyme.

[0013] The enzymatically active rBHT producing GOS may, or may not be, inhibited by galactose.

[0014] The ~~invention~~ disclosure also provides a method for producing enzymatically active recombinant β-hexosyl-transferase (rBHT) protein in a eukaryotic host cell which comprises transforming the eukaryotic host cell with a plasmid under the control of a suitable promotor wherein the plasmid contains an isolated DNA coding for an rBHT protein having the amino acid sequence set forth in SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20. In another aspect, there is provided a method for producing enzymatically active recombinant β-hexosyl-transferase (rBHT) protein in a eukaryotic host cell which comprises transforming the eukaryotic host cell with a plasmid under the control of a suitable promotor wherein the plasmid contains an isolated DNA coding for a rBHT protein having the amino acid sequence set forth in SEQ ID NO. 4, 6, 8, 10, 12, 14, 16, 18 or 20.

[0015] In some embodiments, the isolated DNA is linked to a DNA coding for a signal peptide. The signal peptide may be an *S. cerevisiae* α-factor signal peptide and the suitable promotor may be an alcohol oxidase promotor.

[0016] In some embodiments, the enzymatically active rBHT protein has a specific activity of about 8 $U.mg^{-1}$ at 20°C.

[0017] The eukaryotic host cell may be a yeast cell such as *Pichia pastoris*.

[0018] The ~~invention~~ disclosure also provides a method for producing galacto-oligosaccharides (GOS) which comprises reacting lactose with an enzymatically active recombinant β-hexosyl-transferase (rBHT) protein having the amino acid sequence set forth in SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 under suitable conditions so as to produce GOS. In another aspect, there is provided a method for producing galacto-oligosaccharides (GOS) which comprises reacting lactose with an enzymatically active recombinant β-hexosyl-transferase (rBHT) protein having the amino acid sequence set forth in SEQ ID NO. 4, 6, 8, 10, 12, 14, 16, 18 or 20 under suitable conditions so as to produce GOS.

[0019] The enzymatically active rBHT protein may be immobilized on a solid support. The solid support may be in a batch or continuous stirred-tank reactor, a packed-bed reactor, or an ultrafiltration membrane reactor. Alternatively, the enzymatically active rBHT protein may be used directly in a batch or continuous stirred-tank reactor, a packed-bed reactor, or an ultrafiltration membrane reactor. The method may further comprise a glucose removal system to avoid competitive glucose inhibition such as a generally recognized as safe (GRAS) organism. The glucose removal system may be used simultaneously with the enzymatically active rBHT protein.

[0020] The ~~invention~~ disclosure is also directed to a modified lactose-containing foodstuff or food byproduct comprising a recombinant β-hexosyl-transferase (rBHT) protein having the amino acid sequence set forth in SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20. In another aspect, there is provided a modified lactose-containing foodstuff or food byproduct comprising a recombinant β-hexosyl-transferase (rBHT) protein having the amino acid sequence set forth in SEQ ID NO. 4, 6, 8, 10, 12, 14, 16, 18 or 20. The lactose-containing foodstuff or food byproduct may be a dairy product or byproduct such as whey. In

some embodiments, the foodstuff or food byproduct is a baby dessert, a baby juice, a baby snack, a baby yoghurt drink, an energy drink, a fitness water or thirst quencher, a frozen dairy dessert, a fruit drink (vitamin/mineral fortified), a fruit pie filling, a fruit preparation, an infant formula, an infant meal replacement drink, a jelly jam, a meal replacement drink, a milk, a milk-based drink, a milk substitute, a syrup flavoring for milk, a yoghurt, or a whey.

4. BRIEF DESCRIPTION OF THE FIGURES

[0021] **FIGURE 1A-1C**. Gel electrophoresis of purified rBHT expressed in *P. pastoris*. (1A) SDS-PAGE of purified rBHT silver stained: Lane 1, 0.5 µg rBHT; lane 2, 0.1 µg rBHT. (1B) Western blot analysis with anti-rBHT antiserum. Lane M indicates the molecular masses (kDa) of the marker proteins are shown to the right of Panel A. (1C) Zymogram of rBHT. Lane 1, purified rBHT-6XHIS expressed in *E. coli* BLR cultures; Lane 2, broth from untransformed methanol induced GS115; Lane 3, broth from methanol induced recombinant GS115/*rBHT*. Activity was visualized by the formation of a blue precipitate resulting from enzymatic cleavage of X-GAL.

[0022] **FIGURE 2A-2D**. rBHT relative activity dependence on (2A) pH. (2B) Temperatures from 20°C to 80°C. (2C) Concentration of galactose (solid circle) and glucose (solid square). (2D) Thermal stability at 20°C to 50°C. Samples were removed every 12 h and assayed for activity at 20°C. Enzyme activity assays were conducted in 50 mM sodium phosphate (pH 5.0) containing 1.3 mM ONP-Glu at 20°C, except for (A) which used sodium phosphate (pH 5-11) or citrate (pH 2-5) buffers. Enzyme activities were calculated relative to the value taken at time zero (100%). The initial concentration of tested enzyme was 0.2 $U.ml^{-1}$ assayed at 20°C (K_m=0.37 mM and V_{max}=0.09 $mM.min^{-1}$). Data represents the means of two experiments with a reliability of ±5%.

[0023] **FIGURE 3**. Synthesis of galactosyl-lactose from lactose (2%) using partially purified rBHT (0.5 $U.g^{-1}$ lactose) in 5 mM sodium phosphate buffer pH 5.0 incubated at 42°C. Concentrations of lactose, glucose, galactose and galactosyl-lactose are shown in $g.l^{-1}$. The residual non-quantified GOS species are shown as signal intensity readings from the refractive-index detector. Data represents the means of two experiments with a reliability of ±5%.

[0024] **FIGURE 4A-4B**. Synthesis of galacto-oligosaccharides from lactose (20%) using *P. pastoris* resting cells (harboring membrane-bound rBHT at 0.5 $U.g^{-1}$ lactose) in 5 mM sodium phosphate buffer pH 5.0 incubated at 42°C (4A) or 30°C (4B). Concentrations of lactose, glucose, galactose and galactosyl-lactose are shown in $g.l^{-1}$. The residual non-