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

[0011] The present 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, 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 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, 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 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 yeast 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 as described herein.

[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 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 produced 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 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 produced as described herein. The lactose-containing foodstuff or food byproduct may be a dairy product or byproduct such as whey. In

Claims

What is claimed is:

1. 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 or 20.

2. The isolated DNA coding for a recombinant β-hexosyl-transferase (rBHT) protein of claim 1 having the amino acid sequence set forth in SEQ ID NO. 12 or 14.

3. The isolated DNA coding for the recombinant β-hexosyl-transferase (rBHT) protein of claim 1, wherein the DNA has at least 97% identity with the nucleic acid sequence set forth in SEQ ID NO. 3, 5, 7, 9, 11, 13 or 19.

4. The isolated DNA coding for the recombinant β-hexosyl-transferase (rBHT) protein of claim 3, wherein the DNA has the nucleic acid sequence set forth in SEQ ID NO. 3, 5, 7, 9, 11, 13 or 19.

5. The isolated DNA coding for the recombinant β-hexosyl-transferase (rBHT) protein of claims 4, wherein the DNA has the nucleic acid sequence set forth in SEQ ID NO. 11 or 13.

6. 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 or 20.

7. The recombinant β-hexosyl-transferase (rBHT) protein of claim 6 having the amino acid sequence set forth in SEQ ID NO. 12 or 14.

8. The enzymatically active rBHT protein of claim 6, wherein the protein is membrane bound or a soluble enzyme.

9. A method for producing enzymatically active recombinant β-hexosyl-transferase (rBHT) protein in a yeast host cell which comprises transforming the yeast host cell with a plasmid under the control of a suitable promotor wherein the plasmid contains an isolated DNA coding for a rBHT protein according to any of claims 1-5.

10. The method of claim 9, wherein the suitable promotor is an alcohol oxidase promotor.

11. The method of claim 9, wherein the enzymatically active rBHT protein has a specific activity of about 8 $U.mg^{-1}$ at 20°C.

12. The method of claim 9, wherein the yeast host cell is a *Pichia pastoris* yeast cell.

13. A method for producing galacto-oligosaccharides (GOS) which comprises reacting lactose with an enzymatically active recombinant β-hexosyl-transferase (rBHT) protein produced according to any one of claims 9-12 under suitable conditions so as to produce GOS.

14. The method of claim 13, wherein the enzymatically active rBHT protein is immobilized on a solid support.

15. The method of claim 13, wherein the GOS is produced in a batch or continuous stirred-tank reactor, a packed-bed reactor, or an ultrafiltration membrane reactor.

16. The method of claim 13, further comprising a generally recognized as safe (GRAS) organism as a glucose removal system to avoid competitive glucose inhibition, preferably the glucose removal system is used simultaneously with the enzymatically active rBHT protein.

17. A modified lactose-containing foodstuff or food byproduct comprising a recombinant β-hexosyl-transferase (rBHT) protein produced according to any one of claims 9-12.

18. The modified lactose-containing foodstuff of claim 17, wherein the foodstuff is a dairy product or byproduct.

19. The lactose-containing foodstuff or food byproduct of claim 17, wherein 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.