FIG. 1 is a schematic illustration showing the synthetic pathway of the major neutral fucosyl-oligosaccharides found in human milk.

FIG. 2 is a schematic demonstrating metabolic pathways and the changes introduced into them to engineer 2'-fucosyllactose (2'-FL) synthesis in *Escherichia coli* (*E. coli*). Specifically, the lactose synthesis pathway and the GDP-fucose synthesis pathway are illustrated. In the GDP-fucose synthesis pathway: *manA* = phosphomannose isomerase (PMI), *manB* = phosphomannomutase (PMM), *manC* = mannose-1-phosphate guanylyltransferase (GMP), *gmd* = GDP-mannose-4,6-dehydratase, *fcl* = GDP-fucose synthase (GFS), and Δ*wcaJ* = mutated UDP-glucose lipid carrier transferase.

FIG. 3A and FIG. 3B show the sequence identity and a multiple sequence alignment of 4 previously known lactose-utilizing α(1,2)-fucosyltransferase protein sequences. FIG. 3A is a table showing the sequence identity between the 4 known lactose-utilizing α(1,2)-fucosyltransferases: *H. pylori* futC (SEQ ID NO: 1), *H. mustelae* FutL (SEQ ID NO: 2), *Bacteroides vulgatus* futN (SEQ ID NO: 3), and *E. coli 0126* wbgL (SEQ ID NO: 4). FIG. 3B shows multiple sequence alignment of the 4 known α(1,2)-fucosyltransferases. The ovals highlight regions of particularly high sequence conservation between the four enzymes in the alignment.

FIG. 4 shows the sequence alignment of the 12 identified α(1,2)-fucosyltransferase syngenes identified, along with the 4 previously known lactose-utilizing α(1,2)-fucosyltransferase protein sequences. The 4 known lactose-utilizing α(1,2)-fucosyltransferases are boxed and include *H. pylori* futC (SEQ ID NO: 293), *H. mustelae* FutL (SEQ ID NO: 2), *Bacteroides vulgatus* futN (SEQ ID NO: 3), and *E. coli 0126* wbgL (SEQ ID NO: 294). The 12 identified α(1,2)-fucosyltransferase are as follows: *Prevotella melaninogenica* FutO (SEQ ID NO: 10), *Clostridium bolteae* +13 FutP (SEQ ID NO: 292), *Lachnospiraceae sp.* FutQ (SEQ ID NO: 12), *Methanosphaerula palustris* FutR (SEQ ID NO: 13), *Tannerella sp.* FutS (SEQ ID NO: 14), *Bacteroides caccae* FutU (SEQ ID NO: 15), *Butyrivibrio* FutV (SEQ ID NO: 16), *Prevotella sp.* FutW (SEQ ID NO: 17), *Parabacteroides johnsonii* FutX (SEQ ID NO: 18), *Akkermansia muciniphilia* FutY (SEQ ID NO: 19), *Salmonella enterica* FutZ (SEQ ID NO: 20), *Bacteroides sp.* FutZA (SEQ ID NO: 21). The sequence for *Clostridium bolteae* FutP (without the 13 additional amino acids in the N-terminus) (SEQ ID NO: 11) is also shown in the alignment.

What is claimed is:

1. A method for producing a fucosylated oligosaccharide in a bacterium comprising providing bacterium comprising an exogenous lactose-utilizing α(1,2) fucosyltransferase enzyme, wherein the amino acid sequence of said enzyme comprises at least 22% identity to SEQ ID NO: 1 or at least 25% to SEQ ID NO:3 and

(i) at least 50% identity to SEQ ID NO: 11;

(ii) at least 50% identity to SEQ ID NO: 12;

(iii) at least 50% identity to SEQ ID NO: 13;

(iv) at least 50% identity to SEQ ID NO: 14; or

(v) at least 50% identity to SEQ ID NO: 19.

2. The method of claim 1, further comprising retrieving the fucosylated oligosaccharide from said bacterium or from a culture supernatant of said bacterium.

3. The method of claim 1 or 2, wherein said fucosylated oligosaccharide comprises 2'-fucosyllactose (2'-FL), lactodifucotetraose (LDFT), Lacto-N-fucopentaose I (LNF I), or lacto-N-difucohexaose I (LDFH I).

4. The method of any one of the preceding claims, wherein the bacterium further comprises an exogenous lactose-utilizing α(1,3) fucosyltransferase enzyme and/or an exogenous lactose-utilizing α(1,4) fucosyltransferase enzyme.

5. The method of claim 4, wherein the exogenous lactose-utilizing α(1,3) fucosyltransferase enzyme comprises (i) a *Helicobacter pylori* 26695 *futA* gene; or (ii) a Helicobacter pylori UA948 FucTa gene or a Helicobacter pylori strain DMS6709 FucT III gene.

6. The method of any one of the preceding claims, wherein said bacterium further comprises a reduced level of β-galactosidase activity, a defective colanic acid synthesis pathway, an inactivated ATP-dependent intracellular protease, an inactivated *lacA*, or a combination thereof.

7. The method of claim 6, wherein

(i) said method further comprises culturing said bacterium in the presence of tryptophan and in the absence of thymidine;

(ii) said reduced level of β-galactosidase activity comprises a deleted or inactivated endogenous lacZ gene and/or a deleted or inactivated endogenous lacI gene of said bacterium;

(iii) said reduced level of β-galactosidase activity comprises a deleted or inactivated endogenous lacZ gene and/or a deleted or inactivated endogenous lacI gene of said bacterium, wherein said reduced level of β-galactosidase activity further comprises an exogenous lacZ gene or variant thereof, wherein said exogenous lacZ gene or variant thereof comprises an β-galactosidase activity level less than wild-type bacterium;

(iv) said reduced level of β-galactosidase activity comprises an activity level less than wild-type bacterium;

(v) said reduced level of β-galactosidase activity comprises an activity level less than wild-type bacterium, wherein said reduced level of β-galactosidase activity comprises less than 6,000 units of β-galactosidase activity;

(vi) said reduced level of β-galactosidase activity comprises an activity level less than wild-type bacterium, wherein said reduced level of β-galactosidase activity comprises less than 1,000 units of β-galactosidase activity;

(vii) said bacterium comprises a lacIq gene promoter immediately upstream of a lacY gene;

(viii) said defective colonic acid synthesis pathway comprises the inactivation of the wcaJ gene of said bacterium;

(ix) said inactivated ATP-dependent intracellular protease is a null mutation, inactivating mutation, or deletion of an endogenous Ion gene;

(x) said inactivated ATP-dependent intracellular protease is an inactivating mutation of an endogenous lon gene, wherein said inactivating mutation of an endogenous lon gene comprises the insertion of a functional E. coli $lacZ^+$ gene;

(xi) said bacterium further comprises a functional lactose permease gene;

(xii) said bacterium further comprises a functional lactose permease gene, wherein said bacterium comprises E. coli lacY;

(xiii) said bacterium further comprises an exogenous E. coli rcsA or E. coli rcsB gene;

(xiv) said bacterium further comprises a mutation in the thyA gene;

(xv) said bacterium accumulates intracellular lactose in the presence of exogenous lactose;

(xvi) said bacterium accumulates intracellular GDP-fucose; or

(xvii) said bacterium comprises the genotype ΔampC::$P_{trp}{}^{B}$cI, Δ(lacI-lacZ)::FRT,P_{lacIq}lacY^{+}, ΔwcaJ::FRT, thyA::Tn10, Δlon:(npts, lacZ$^{+)}$.

8. The method of any one of the preceding claims, wherein said bacterium is a member of the *Bacillus, Pantoea, Lactobacillus, Lactococcus, Streptococcus, Proprionibacterium, Enterococcus, Bifidobacterium, Sporolactobacillus*, *Micromomospora*, *Micrococcus, Rhodococcus*, or *Pseudomonas* genus, preferably wherein said production strain is selected from the group consisting of *Bacillus licheniformis, Bacillus subtilis*, *Bacillus coagulans*, *Bacillus thermophilus*, *Bacillus laterosporus*, *Bacillus megaterium*, *Bacillus mycoides*, *Bacillus pumilus*, *Bacillus lentus*, *Bacillus cereus*, *and Bacillus circulans, Erwinia herbicola* (*Pantoea agglomerans*), *Citrobacter freundii*, *Pantoea citrea*, *Pectobacterium carotovorum*, *Xanthomonas campestris Lactobacillus acidophilus, Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus jensenii*, *Lactococcus lactis, Streptococcus thermophiles, Proprionibacterium freudenreichii, Enterococcus faecium, Enterococcus thermophiles*), *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium bifidum, Pseudomonas fluorescens* and *Pseudomonas aeruginosa.*

9. The method of any of the preceding claims, wherein said bacterium comprises a nucleic acid construct comprising an isolated nucleic acid encoding said α(1,2) fucosyltransferase enzyme.

10. A purified fucosylated oligosaccharide produced by any one of the preceding claims.

11. A nucleic acid construct comprising an isolated nucleic acid encoding a lactose-utilizing α(1,2) fucosyltransferase enzyme for the production of said enzyme in a host bacteria production strain, wherein the amino acid sequence of said enzyme comprises at least 22% identity to SEQ ID NO: 1 or at least 25% identity to SEQ ID No:3 and

(i) at least 50% identity to SEQ ID NO: 11;

(ii) at least 50% identity to SEQ ID NO: 12;

(iii) at least 50% identity to SEQ ID NO: 13;

(iv) at least 50% identity to SEQ ID NO: 14; or

(v) at least 50% identity to SEQ ID NO: 19.

12. The method or construct of claim 9 or 11, wherein said nucleic acid is operably linked to one or more heterologous control sequences that direct the production of the enzyme in the bacterium, preferably wherein said heterologous control sequence comprises a bacterial promoter and operator, a bacterial ribosome binding site, a bacterial transcriptional terminator, a plasmid selectable marker, and/or an origin of replication.

13. An isolated bacterium comprising an isolated nucleic acid encoding a lactose-accepting α (1,2) fucosyltransferase enzyme, wherein the amino acid sequence of said enzyme encoded by said nucleic acid comprises at least 22% identity to SEQ ID NO: 1 or at least 25% identity to SEQ ID No:3 and

(i) at least 50% identity to SEQ ID NO: 11;

(ii) at least 50% identity to SEQ ID NO: 12;

(iii) at least 50% identity to SEQ ID NO: 13;

(iv) at least 50% identity to SEQ ID NO: 14; or

(v) at least 50% identity to SEQ ID NO: 19.

14. The method, construct, or isolated bacterium of any one of claims 1 to 9 or 11 to 13, wherein said α(1,2) fucosyltransferase enzyme comprises any one selected from *Tannerella sp.* FutS, *Clostridium bolteae* +13 FutP, *Methanosphaerula palustries* FutR, *Akkermansia muciniphilia* FutY, *Clostridium bolteae* FutP, or a functional variant or fragment thereof.

15. The method, construct, or isolated bacterium of any one of claims 1 to 9 or 11 to 14, wherein said α(1,2) fucosyltransferase enzyme comprises any one of amino acid sequences SEQ ID NO: 10-21 and 292, or a functional variant or fragment thereof.

16. The isolated bacterium of any one of claims 13 to 15, further comprising a α(1,3) fucosyltransferase enzyme and/or an α(1,4) fucosyltransferase enzyme.

17. The method, construct, or isolated bacterium of any one of claims 1 to 9 or 11 to 16, wherein said bacterium is *Escherichia coli.*

18. The method, construct, or isolated bacterium of any one of claims 1 to 9 or 11 to 17, wherein said bacterium further comprises reduced level of β-galactosidase activity, a defective colanic acid synthesis pathway, an inactivated adenosine-5'-triphosphate (ATP)-dependent intracellular protease, an inactivated endogenous *lacA* gene, or any combination thereof, preferably wherein said bacterium comprises the genotype Δ*ampC*::P_{trp}^{B}*cI*, Δ(*lacI-lacZ*)::*FRT*, P_{laclq}*lacY*$^{+}$, Δ*wcaJ*::FRT, *thyA*::Tn*10*, Δ*lon*:(*npt3*, *lacZ*$^{+}$).

19. The purified fucosylated oligosaccharide of claim 10, wherein

(a) at least 99% of said purified fucosylated oligosaccharide comprises 2'-fucosyllactose (2'-FL), lactodifucotetraose (LDFT), Lacto-N-fucopentaose I (LNF 1), and/or lacto-N-difucohexaose I (LDFH I) by weight; and

(b) said purified fucosylated oligosaccharide lacks 3-fucosyllactose (3-FL) or contains an amount of 3-FL that is less than 0.5% the level of 2'-FL.

20. The purified fucosylated oligosaccharide of claim 19, wherein said purified fucosylated oligosaccharide does not comprises acetyl-lactose that is detectable by thin layer chromatography, or which is in the form of a crystalline powder.

21. The method, construct, or isolated bacterium of any one of claims 1 to 9, 11 to 18, wherein said enzyme is not a mutant enzyme.

22. The method, construct, or isolated bacterium of claim 21, wherein said enzyme naturally occurs in a bacterium that naturally exists in the gastrointestinal tract.

23. The method, construct, or isolated bacterium of any one of claims 1 to 9 or 11 to 18 or 21 or 22, wherein the enzyme comprises

(i) an amino acid sequence having at least 75% identity to SEQ ID NO: 11;

(ii) an amino acid sequence having at least 75% identity to SEQ ID NO: 12;

(iii) an amino acid sequence having at least 75% identity to SEQ ID NO: 13;

(iv) an amino acid sequence having at least 75% identity to SEQ ID NO: 14; or

(v) an amino acid sequence having at least 75% identity to SEQ ID NO: 19.

24. The method, construct, or isolated bacterium of claim 23, wherein the enzyme comprises

(i) the amino acid sequence of SEQ ID NO: 11;

(ii) the amino acid sequence of SEQ ID NO: 12;

(iii) the amino acid sequence of SEQ ID NO: 13;

(iv) the amino acid sequence of SEQ ID NO: 14; or

(v) the amino acid sequence of SEQ ID NO: 19.

25. The method, construct, or isolated bacterium of claim 23, wherein the enzyme comprises an amino acid sequence having at least 75% identity to SEQ ID NO: 13.

26. The method, construct, or isolated bacterium of claim 25, wherein the enzyme comprises the amino acid sequence of SEQ ID NO: 13.