

Production of fucosyllactose in host cells

Field of the invention

The present invention is in the technical field of synthetic biology and metabolic engineering. More particularly, the present invention is in the technical field of fermentation of metabolically engineered host cells. The present invention describes a method of producing fucosyllactose by fermentation with a genetically modified cell, as well as to the genetically modified cell used in the method. The genetically modified cell comprises at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis, more specifically the cell comprises a nucleic acid sequence coding for a fucosyltransferase thereby synthesizing fucosyllactose and at least one nucleic acid expressing a membrane protein, more specifically a nucleic acid sequence expressing a membrane protein enabling fucosyllactose transport.

Background

Today, more than 80 compounds belonging to the family of Human Milk Oligosaccharides (HMOs), have been structurally characterized. These HMOs represent a class of complex oligosaccharides that function as prebiotics. Additionally, the structural homology of HMO to epithelial epitopes accounts for protective properties against bacterial pathogens. Within the infant gastrointestinal tract, HMOs selectively nourish the growth of selected bacterial strains and are, thus, priming the development of a unique gut microbiota in breast milk-fed infants.

Some of these Human Milk oligosaccharides require the presence of particular fucosylated structures which most likely exhibit a particular biological activity. Production of these fucosylated oligosaccharides requires the action of a fucosyltransferase. Such fucosyltransferases, which belong to enzyme family of glycosyltransferases, are widely expressed in vertebrates, invertebrates, plants, fungi, yeasts, and bacteria. They catalyze the transfer of a fucose residue from a donor, generally guanosine-diphosphate fucose (GDP-fucose) to an acceptor, which include oligosaccharides, (glyco)proteins and (glyco)lipids. The thus fucosylated acceptor substrates are involved in a variety of biological and pathological processes.

In microbial fermentative production of fucosyllactose (FL), the FL is in many cases produced intracellularly in the industrial production host. One problem identified in the art as the true difficulty in producing oligosaccharides in cells is the intracellular enrichment of the produced oligosaccharides and their extraction. The intracellular enrichment is deemed to be responsible for the product-inhibitory effect on the production of the desired oligosaccharide. Synthesis may become slow or the desired oligosaccharide may reach cytotoxic concentrations resulting in metabolic arrest or even cell lysis.

It is an object of the present invention to provide for tools and methods by means of which fucosyllactose can be produced in an efficient, time and cost-effective way and which yields high amounts of the desired product.

According to the invention, this and other objects are achieved by providing a method and a cell for the production of fucosyllactose wherein the cell is genetically modified for the production of fucosyllactose and comprises at least one nucleic acid sequence encoding an enzyme involved in fucosyllactose synthesis, more specifically the cell comprises a nucleic acid sequence coding for a fucosyltransferase thereby synthesizing fucosyllactose. The cell furthermore also expresses a membrane protein, more specifically the cell furthermore also expresses a membrane protein previously unknown to enable fucosyllactose transport, according to the present invention.

Description

Summary of the invention

Surprisingly it has now been found that the membrane proteins used in the present invention provide for newly identified membrane proteins, more specifically the present invention provide for newly identified membrane proteins previously unknown to enable fucosyllactose transport and having a positive effect on fermentative production of fucosyllactose, providing a better yield, productivity, specific productivity and/or growth speed when used to genetically engineer a host cell producing fucosyllactose.

The invention also provides methods for producing fucosyllactose. The fucosyllactose is obtained with a host cell comprising the membrane protein of the present invention.

Definitions

The words used in this specification to describe the invention and its various embodiments are to be understood not only in the sense of their commonly defined meanings, but to include by special definition in this specification structure, material or acts beyond the scope of the commonly defined meanings. Thus, if an element can be understood in the context of this specification as including more than one meaning, then its use in a claim must be understood as being generic to all possible meanings supported by the specification and by the word itself.

The various embodiments and aspects of embodiments of the invention disclosed herein are to be understood not only in the order and context specifically described in this specification, but to include any order and any combination thereof. Whenever the context requires, all words used in the singular number shall be deemed to include the plural and vice versa. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization described herein are those well-known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. Generally, enzymatic reactions and purification steps are performed according to the manufacturer's specifications.

In the drawings and specification, there have been disclosed embodiments of the invention, and although specific terms are employed, the terms are used in a descriptive sense only and not for purposes of limitation, the scope of the invention being set forth in the following claims. It must be understood that the illustrated embodiments have been set forth only for the purposes of example and that it should not be taken as limiting the invention. It will be apparent to those skilled in the art that alterations, other embodiments, improvements, details and uses can be made consistent with the letter and spirit of the disclosure herein and within the scope of this disclosure, which is limited only by the claims, construed in accordance with the patent law, including the doctrine of equivalents. In the claims which follow, reference characters used to designate claim steps are provided for convenience of description only, and are not intended to imply any particular order for performing the steps.

According to the present invention, the term "polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" according to the present invention. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, are to be understood to be covered by the term "polynucleotides". It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. The term "polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene

encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to the skilled person. The same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Furthermore, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

"Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Similarly, a "synthetic" sequence, as the term is used herein, means any sequence that has been generated synthetically and not directly isolated from a natural source. "Synthesized", as the term is used herein, means any synthetically generated sequence and not directly isolated from a natural source.

"Recombinant" means genetically engineered DNA prepared by transplanting or splicing genes from one species into the cells of a host organism of a different species. Such DNA becomes part of the host's genetic makeup and is replicated.

The term "endogenous," within the context of the present disclosure refers to any polynucleotide, polypeptide or protein sequence which is a natural part of a cell and is occurring at its natural location in the cell chromosome.

The term "heterologous" when used in reference to a polynucleotide, gene, nucleic acid, polypeptide, or enzyme refers to a polynucleotide, gene, nucleic acid, polypeptide, or enzyme that is from a source or derived from a source other than the host organism species. In contrast a "homologous" polynucleotide, gene, nucleic acid, polypeptide, or enzyme is used herein to denote

a polynucleotide, gene, nucleic acid, polypeptide, or enzyme that is derived from the host organism species. When referring to a gene regulatory sequence or to an auxiliary nucleic acid sequence used for maintaining or manipulating a gene sequence (e.g. a promoter, a 5' untranslated region, 3' untranslated region, poly A addition sequence, intron sequence, splice site, ribosome binding site, internal ribosome entry sequence, genome homology region, recombination site, etc.), "heterologous" means that the regulatory sequence or auxiliary sequence is not naturally associated with the gene with which the regulatory or auxiliary nucleic acid sequence is juxtaposed in a construct, genome, chromosome, or episome. Thus, a promoter operably linked to a gene to which it is not operably linked to in its natural state (i.e. in the genome of a non-genetically engineered organism) is referred to herein as a "heterologous promoter," even though the promoter may be derived from the same species (or, in some cases, the same organism) as the gene to which it is linked.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or an insertion sequence or editing) together with additional regions that also may contain coding and/or non-coding sequences.

The term "modified expression" of a gene relates to a change in expression compared to the wild type expression of said gene in any phase of the production process of the fucosyllactose. Said modified expression is either a lower or higher expression compared to the wild type, wherein the term "higher expression" is also defined as "overexpression" of said gene in the case of an endogenous gene or "expression" in the case of a heterologous gene that is not present in the wild type strain. Lower expression is obtained by means of common well-known technologies for a skilled person (such as the usage of siRNA, CrispR, CrispRi, recombineering, homologous recombination, ssDNA mutagenesis, RNAi, miRNA, asRNA, mutating genes, knocking-out genes, transposon mutagenesis, ...) which are used to change the genes in such a way that they are less-able (i.e. statistically significantly 'less-able' compared to a functional wild-type gene) or completely unable (such as knocked-out genes) to produce functional final products. Overexpression or expression is obtained by means of common well-known technologies for a skilled person, wherein said gene is part of an "expression cassette" which relates to any sequence in which a promoter sequence, untranslated region sequence (containing either a ribosome binding sequence or Kozak sequence), a coding sequence (for instance a membrane protein gene sequence) and optionally a transcription terminator is present, and leading to the expression of a functional active protein. Said expression is either constitutive or conditional or regulated.

The term "constitutive expression" is defined as expression that is not regulated by transcription factors other than the subunits of RNA polymerase (e.g. the bacterial sigma factors) under certain growth conditions. Non-limiting examples of such transcription factors are CRP, LacI, ArcA, Cra,

IclR in *E. coli*, or, Aft2p, Crz1p, Skn7 in *Saccharomyces cerevisiae*, or, DeoR, GntR, Fur in *B. subtilis*. These transcription factors bind on a specific sequence and may block or enhance expression in certain growth conditions. RNA polymerase binds a specific sequence to initiate transcription, for instance via a sigma factor in prokaryotic hosts.

- 5 The term "regulated expression" is defined as expression that is regulated by transcription factors other than the subunits of RNA polymerase (e.g. bacterial sigma factors) under certain growth conditions. Examples of such transcription factors are described above. Commonly expression regulation is obtained by means of an inducer, such as but not limited to IPTG, arabinose, rhamnose, fucose, allo-lactose or pH shifts, or temperature shifts or carbon depletion or
10 substrates or the produced product.

The term "wild type" refers to the commonly known genetic or phenotypical situation as it occurs in nature.

- "Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively but retains essential properties. A typical
15 variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino
20 acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide
25 or polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to the persons skilled in the art.

- In some embodiments, the present disclosure contemplates making functional variants by
30 modifying the structure of a membrane protein as used in the present invention. Variants can be produced by amino acid substitution, deletion, addition, or combinations thereof. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (e.g., conservative mutations) will not have a major effect
35 on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide of the disclosure results in a functional homolog can be readily determined by assessing the ability of the variant polypeptide to produce a response in

cells in a fashion similar to the wild-type polypeptide, an in case of the present invention to provide better yield, productivity, and/or growth speed than a cell without the variant.

The term "functional homolog" as used herein describes those molecules that have sequence similarity and also share at least one functional characteristic such as a biochemical activity. More specifically, the term "functional homolog" as used herein describes those proteins that have sequence similarity (in other words, homology) and at the same time have at least one functional similarity such as a biochemical activity (Altenhoff *et al.*, PLoS Comput. Biol. 8 (2012) e1002514). Functional homologs are sometimes referred to as orthologs, where "ortholog" refers to a homologous gene or protein that is the functional equivalent of the referenced gene or protein in another species. Functional homologs will typically give rise to the same characteristics to a similar, but not necessarily the same, degree. Functionally homologous proteins give the same characteristics where the quantitative measurement produced by one homolog is at least 10 percent of the other; more typically, at least 20 percent, between about 30 percent and about 40 percent; for example, between about 50 percent and about 60 percent; between about 70 percent and about 80 percent; or between about 90 percent and about 95 percent; between about 98 percent and about 100 percent, or greater than 100 percent of that produced by the original molecule. Thus, where the molecule has enzymatic activity the functional homolog will have the above-recited percent enzymatic activities compared to the original enzyme. Where the molecule is a DNA-binding molecule (e.g., a polypeptide) the homolog will have the above-recited percentage of binding affinity as measured by weight of bound molecule compared to the original molecule.

A functional homolog and the reference polypeptide may be naturally occurring polypeptides, and the sequence similarity may be due to convergent or divergent evolutionary events.

Functional homologs can be identified by analysis of nucleotide and polypeptide sequence alignments. For example, performing a query on a database of nucleotide or polypeptide sequences can identify homologs of biomass-modulating polypeptides. Sequence analysis can involve BLAST, Reciprocal BLAST, or PSI-BLAST analysis of non-redundant databases using amino acid sequence of a biomass-modulating polypeptide as the reference sequence. Amino acid sequence is, in some instances, deduced from the nucleotide sequence. Typically, those polypeptides in the database that have greater than 40 percent sequence identity are candidates for further evaluation for suitability as a biomass-modulating polypeptide. Amino acid sequence similarity allows for conservative amino acid substitutions, such as substitution of one hydrophobic residue for another or substitution of one polar residue for another. If desired, manual inspection of such candidates can be carried out in order to narrow the number of candidates to be further evaluated. Manual inspection can be performed by selecting those candidates that appear to have domains present in productivity-modulating polypeptides, e.g., conserved functional domains.

"Fragment", with respect to a polynucleotide, refers to a clone or any part of a polynucleotide molecule, particularly a part of a polynucleotide that retains a usable, functional characteristic. Useful fragments include oligonucleotides and polynucleotides that may be used in hybridization or amplification technologies or in the regulation of replication, transcription, or translation. A
5 "polynucleotide fragment" refers to any subsequence of a polynucleotide, typically, of at least about 9 consecutive nucleotides, for example at least about 30 nucleotides or at least about 50 nucleotides of any of the sequences provided herein. Exemplary fragments can additionally or alternatively include fragments that comprise, consist essentially of, or consist of a region that encodes a conserved family domain of a polypeptide. Exemplary fragments can additionally or
10 alternatively include fragments that comprise a conserved domain of a polypeptide.

Fragments may additionally or alternatively include subsequences of polypeptides and protein molecules, or a subsequence of the polypeptide. In some cases, the fragment or domain is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact
15 polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA-binding site or domain that binds to a DNA promoter region, an activation domain, or a domain for protein-protein interactions, and may initiate transcription. Fragments can vary in size from as few as 3 amino acid residues to the full length of the intact polypeptide, for example at least about 20 amino acid residues in length, for example at least
20 about 30 amino acid residues in length. Preferentially a fragment is a functional fragment that has at least one property or activity of the polypeptide from which it is derived, such as, for example, the fragment can include a functional domain or conserved domain of a polypeptide. A domain can be characterized, for example, by a Pfam (<https://pfam.xfam.org/>) (El-Gebali et al., Nucleic Acids Res. 47 (2019) D427-D432) or Conserved Domain Database (CDD) (<https://www.ncbi.nlm.nih.gov/cdd>) (Lu et al., Nucleic Acids Res. 48 (2020) D265-D268)
25 designation. The Pfam database as used herein refers to the Pfam database Pfam 32.0 as released in Sept 2018 and the CDD database as used herein refers to the CDD database v3.17 as released on 3rd April 2019.

The terms "fucosyllactose", "fucosyl lactose" and "FL" as used herein are used interchangeably and refer to an oligosaccharide comprising a fucose residue and a lactose residue. Such
30 fucosyllactose refers to 2'-fucosyllactose, 3-fucosyllactose, or difucosyllactose or any combination thereof; fucosyllactoses refers to a combination of at least any two of 2'-fucosyllactose, 3-fucosyllactose, or difucosyllactose.

The terms "alpha-1,2'-fucosyltransferase", "alpha 1,2' fucosyltransferase", "2'-fucosyltransferase",
35 "α-1,2'-fucosyltransferase", "α 1,2' fucosyltransferase", "2'fucosyltransferase", "2'-FT" or "2'FT" as used in the present invention, are used interchangeably and refer to a glycosyltransferase that catalyses the transfer of fucose from the donor substrate GDP-L-fucose, to the acceptor molecule lactose in an alpha-1,2-linkage. A polynucleotide encoding an "alpha-1,2-fucosyltransferase" or

any of the above terms, refers to a polynucleotide encoding such glycosyltransferase that catalyses the transfer of fucose from the donor substrate GDP-L-fucose, to the acceptor molecule lactose in an alpha-1,2-linkage.

The terms "2'-fucosyllactose", "alpha-1,2'-fucosyllactose", "alpha 1,2' fucosyllactose", "α-1,2'-fucosyllactose", "α 1,2' fucosyllactose", "Fucα1-2Galβ1-4Glc", 2'FL" or "2'-FL" as used in the present invention, are used interchangeably. In a preferred embodiment these terms refer to the product obtained by the catalysis of the alpha-1,2'-fucosyltransferase transferring the fucose residue from GDP-L-fucose to lactose in an alpha-1,2'-linkage.

The terms "alpha-1,3-fucosyltransferase", "alpha 1,3 fucosyltransferase", "3-fucosyltransferase", "α-1,3-fucosyltransferase", "α 1,3 fucosyltransferase", "3 fucosyltransferase", "3-FT" or "3FT" as used in the present invention, are used interchangeably and refer to a glycosyltransferase that catalyses the transfer of fucose from the donor substrate GDP-L-fucose, to the acceptor molecule lactose in an alpha-1,3-linkage. A polynucleotide encoding an "alpha-1,3-fucosyltransferase" or any of the above terms, refers to a polynucleotide encoding such glycosyltransferase that catalyses the transfer of fucose from the donor substrate GDP-L-fucose, to the acceptor molecule lactose in an alpha-1,3-linkage.

The terms "3-fucosyllactose", "alpha-1,3-fucosyllactose", "alpha 1,3 fucosyllactose", "α-1,3-fucosyllactose", "α 1,3 fucosyllactose", "Galβ1-4(Fucα1-3)Glc", 3FL" or "3-FL" as used in the present invention, are used interchangeably. In a preferred embodiment these terms refer to the product obtained by the catalysis of the alpha-1,3-fucosyltransferase transferring the fucose residue from GDP-L-fucose to lactose in an alpha-1,3-linkage.

The terms "difucosyllactose", "di-fucosyllactose", "lactodifucotetraose", "2',3-difucosyllactose", "2',3 difucosyllactose", "α-2',3-fucosyllactose", "α 2',3 fucosyllactose", "Fucα1-2Galβ 1-4(Fucα1-3)Glc", "DFLac", 2',3 diFL", "DFL" or "diFL" as used in the present invention, are used interchangeably. In a preferred embodiment these terms refer to the product obtained by the catalysis of the alpha-1,3-fucosyltransferase transferring the fucose residue to a 2'FL resulting in a 2',3-difucosyllactose or refer to the product obtained by the catalysis of the alpha-1,2-fucosyltransferase transferring the fucose residue to a 3FL resulting in 2', 3 difucosyllactose.

"Oligosaccharide" as the term is used herein and as generally understood in the state of the art, refers to a saccharide polymer containing a small number, typically three to ten, of simple sugars, i.e. monosaccharides.

"SET" or "Sugar Efflux Transporter" as used herein refers to membrane proteins of the SET family which are proteins with InterPro domain IPR001214 as defined by InterPro 75.0 (release date 4th July 2019) and/or are proteins that belong to the eggNOGv4.5 family ENOG410XTE9 as defined by the Eggnogdb 1.0.2 database (release date 3rd Nov 2017). Identification of the InterPro domain can be done by using the online tool on <https://www.ebi.ac.uk/interpro/> or a standalone version of InterProScan (<https://www.ebi.ac.uk/interpro/download.html>) using the default values.

Identification of the orthology family in eggNOGv4.5 can be done using the online version or a standalone version of eggNOG-mapperv1 (<http://eggnogdb.embl.de/#/app/home>).

The term “membrane proteins” as used herein refers to proteins that are part of or interact with the cells membrane and control the flow of molecules and information across the cell. The membrane proteins are thus involved in transport, be it import into or export out of the cell.

Such membrane proteins can be porters, P-P-bond-hydrolysis-driven transporters, β -Barrel Porins, auxiliary transport proteins, putative transport proteins or phosphotransfer-driven group translocators as defined by the Transporter Classification Database that is operated and curated by the Saier Lab Bioinformatics Group available via www.tcdb.org and providing a functional and phylogenetic classification of membrane transport proteins. This Transporter Classification Database details a comprehensive IUBMB approved classification system for membrane transport proteins known as the Transporter Classification (TC) system. The TCDB classification searches as described here are defined based on TCDB. org as released on 17th June 2019.

Porters is the collective name of uniporters, symporters, and antiporters that utilize a carrier-mediated process (Saier *et al.*, Nucleic Acids Res. 44 (2016) D372-D379). They belong to the electrochemical potential-driven transporters and are also known as secondary carrier-type facilitators. Membrane proteins are included in this class when they utilize a carrier-mediated process to catalyze uniport when a single species is transported either by facilitated diffusion or in a membrane potential-dependent process if the solute is charged; antiport when two or more species are transported in opposite directions in a tightly coupled process, not coupled to a direct form of energy other than chemiosmotic energy; and/or symport when two or more species are transported together in the same direction in a tightly coupled process, not coupled to a direct form of energy other than chemiosmotic energy, of secondary carriers (Forrest *et al.*, Biochim. Biophys. Acta 1807 (2011) 167-188). These systems are usually stereospecific. Solute:solute countertransport is a characteristic feature of secondary carriers. The dynamic association of porters and enzymes creates functional membrane transport metabolons that channel substrates typically obtained from the extracellular compartment directly into their cellular metabolism (Moraes and Reithmeier, Biochim. Biophys. Acta 1818 (2012), 2687-2706). Solutes that are transported via this porter system include but are not limited to cations, organic anions, inorganic anions, nucleosides, amino acids, polyols, phosphorylated glycolytic intermediates, osmolites, siderophores,

Membrane proteins are included in the class of P-P-bond hydrolysis-driven transporters if they hydrolyze the diphosphate bond of inorganic pyrophosphate, ATP, or another nucleoside triphosphate, to drive the active uptake and/or extrusion of a solute or solutes (Saier *et al.*, Nucleic Acids Res. 44 (2016) D372-D379). The membrane protein may or may not be transiently phosphorylated, but the substrate is not phosphorylated. Substrates that are transported via the class of P-P-bond hydrolysis-driven transporters include but are not limited to cations, heavy metals, beta-glucan, UDP-glucose, lipopolysaccharides, teichoic acid.

The β -Barrel porins membrane proteins form transmembrane pores that usually allow the energy independent passage of solutes across a membrane. The transmembrane portions of these proteins consist exclusively of β -strands which form a β -barrel (Saier *et al.*, Nucleic Acids Res. 44 (2016) D372-D379). These porin-type proteins are found in the outer membranes of Gram-negative bacteria, mitochondria, plastids, and possibly acid-fast Gram-positive bacteria. Solutes that are transported via these β -Barrel porins membrane proteins include but are not limited to nucleosides, raffinose, glucose, beta-glucosides, oligosaccharides.

The auxiliary transport proteins are defined to be proteins that facilitate transport across one or more biological membranes but do not themselves participate directly in transport. These membrane proteins always function in conjunction with one or more established transport systems such as but not limited to outer membrane factors (OMFs), polysaccharide (PST) porters, the ATP-binding cassette (ABC)-type transporters. They may provide a function connected with energy coupling to transport, play a structural role in complex formation, serve a biogenic or stability function or function in regulation (Saier *et al.*, Nucleic Acids Res. 44 (2016) D372-D379). Examples of auxiliary transport proteins include but are not limited to the polysaccharide copolymerase family involved in polysaccharide transport, the membrane fusion protein family involved in bacteriocin and chemical toxin transport.

Putative transport protein comprises families which will either be classified elsewhere when the transport function of a member becomes established or will be eliminated from the Transporter Classification system if the proposed transport function is disproven. These families include a member or members for which a transport function has been suggested, but evidence for such a function is not yet compelling (Saier *et al.*, Nucleic Acids Res. 44 (2016) D372-D379). Examples of putative transporters classified in this group under the TCDB system as released on 17th June 2019 include but are not limited to copper transporters.

The phosphotransfer-driven group translocators are also known as the PEP-dependent phosphoryl transfer-driven group translocators of the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS). The product of the reaction, derived from extracellular sugar, is a cytoplasmic sugar-phosphate. The enzymatic constituents, catalyzing sugar phosphorylation, are superimposed on the transport process in a tightly coupled process. The PTS system is involved in many different aspects comprising in regulation and chemotaxis, biofilm formation, and pathogenesis (Lengeler, J. Mol. Microbiol. Biotechnol. 25 (2015) 79-93; Saier, J. Mol. Microbiol. Biotechnol. 25 (2015) 73-78). Membrane protein families classified within the phosphotransfer-driven group translocators under the TCDB system as released on 17th June 2019 include PTS systems linked to transport of glucose-glucosides, fructose-mannitol, lactose-N,N'-Diacetylchitobiose-beta-glucoside, glucitol, galactitol, mannose-fructose-sorbose and ascorbate.

It should be understood for those skilled in the art that for the databases used herein, comprising Pfam 32.0 (released Sept 2018), CDD v3.17 (released 3rd April 2019), eggnoodb 1.0.2 (released

3rd Nov 2017), InterPro 75.0 (released 4th July 2019) and TCDB (released 17th June 2019), the content of each database is fixed at each release and is not to be changed. When the content of a specific database is changed, this specific database receives a new release version with a new release date. All release versions for each database with their corresponding release dates and specific content as annotated at these specific release dates are available and known to those skilled in the art.

The term "enabling transport" means to introduce the activity of transport of a solute over the cytoplasm membrane and/or the cell wall. Said transport may be enabled by introducing and/or increasing the expression of a transporter protein as described in the present invention. The term "enhancing transport" means to improve the activity of transport of a solute over the cytoplasm membrane and/or the cell wall. Said transport may be enhanced by introducing and/or increasing the expression of a transporter protein as described in the present invention. "Expression" of a transporter protein is defined as "overexpression" of the gene encoding said transporter protein in the case said gene is an endogenous gene or "expression" in the case the gene encoding said transporter protein is a heterologous gene that is not present in the wild type strain.

Hybridisation

The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to, for example, a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The term "stringency" refers to the conditions under which a hybridisation takes place. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration, ionic strength and hybridisation buffer composition. Generally, low stringency conditions are selected to be about 30 °C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Medium stringency conditions are when the temperature is 20 °C below T_m, and high stringency conditions are when the temperature is 10 °C below T_m. High stringency hybridisation conditions are typically used for isolating hybridising sequences that have high sequence similarity to the target nucleic acid sequence. However, nucleic acids may deviate in sequence and still encode a substantially identical polypeptide, due to the degeneracy of the

genetic code. Therefore, medium stringency hybridisation conditions may sometimes be needed to identify such nucleic acid molecules.

The T_m is the temperature under defined ionic strength and pH, at which 50 % of the target sequence hybridises to a perfectly matched probe. The T_m is dependent upon the solution conditions and the base composition and length of the probe. For example, longer sequences hybridise specifically at higher temperatures. The maximum rate of hybridisation is obtained from about 16 °C up to 32 °C below T_m . The presence of monovalent cations in the hybridisation solution reduce the electrostatic repulsion between the two nucleic acid strands thereby promoting hybrid formation; this effect is visible for sodium concentrations of up to 0.4M (for higher concentrations, this effect may be ignored). Formamide reduces the melting temperature of DNA-DNA and DNA-RNA duplexes with 0.6 to 0.7 °C for each % formamide, and addition of 50 % formamide allows hybridisation to be performed at 30 to 45 °C, though the rate of hybridisation will be lowered. Base pair mismatches reduce the hybridisation rate and the thermal stability of the duplexes. On average and for large probes, the T_m decreases about 1 °C per % base mismatch. The T_m may be calculated using the following equations, depending on the types of hybrids:

1) DNA-DNA hybrids (Meinkoth and Wahl, Anal. Biochem., 138: 267-284, 1984):

$$T_m = 81.5\text{ °C} + 16.6 \times (\log_{10}[\text{Na}^+]a) + 0.41 \times (\% [\text{G+Cb}] - 500 \times [\text{Lc}]^{-1} - 0.61 \times (\% \text{ formamide}))$$

2) DNA-RNA or RNA-RNA hybrids:

$$T_m = 79.8\text{ °C} + 18.5 \times (\log_{10}[\text{Na}^+]a) + 0.58 \times (\% [\text{G+Cb}]) + 11.8 \times (\% [\text{G+Cb}])^2 - 820 \times [\text{Lc}]^{-1}$$

3) oligo-DNA or oligo-RNA hybrids:

$$\text{For } < 20 \text{ nucleotides: } T_m = 2/n$$

$$\text{For } 20 - 35 \text{ nucleotides: } T_m = 22 + 1.46/n$$

Wherein:

a: or for other monovalent cation, but only accurate in the 0.01-0.4 M range.

b: only accurate for % GC in the 30 % to 75 % range.

c: L = length of duplex in base pairs,

d: oligo, oligonucleotide,

n: effective length of primer = $2 \times (\text{no. of G+C}) + (\text{no. of A+T})$.

Non-specific binding may be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein containing solutions, additions of heterologous RNA, DNA, and SDS to the hybridisation buffer, and treatment with Rnase. For non-homologous probes, a series of hybridizations may be performed by varying one of (i) progressively lowering the annealing temperature (for example from 68°C to 42°C) or (ii) progressively lowering the formamide concentration (for example from 50 % to 0 %). The skilled artisan is aware of various parameters which may be altered during hybridisation and which will either maintain or change the stringency conditions.

Besides the hybridisation conditions, specificity of hybridisation typically also depends on the function of post-hybridisation washes. To remove background resulting from nonspecific hybridisation, samples are washed with dilute salt solutions. Critical factors of such washes include the ionic strength and temperature of the final wash solution: the lower the salt concentration and the higher the wash temperature, the higher the stringency of the wash. Wash conditions are typically performed at or below hybridisation stringency. A positive hybridisation gives a signal that is at least twice of that of the background. Generally, suitable stringent conditions for nucleic acid hybridisation assays or gene amplification detection procedures are as set forth above. More or less stringent conditions may also be selected. The skilled artisan is aware of various parameters which may be altered during washing and which will either maintain or change the stringency conditions.

For example, typical high stringency hybridisation conditions for DNA hybrids longer than 50 nucleotides encompass hybridisation at 65 °C in 1x SSC or at 42 °C in 1x SSC and 50 % formamide, followed by washing at 65 °C in 0.3x SSC. Examples of medium stringency hybridisation conditions for DNA hybrids longer than 50 nucleotides encompass hybridisation at 50 °C in 4x SSC or at 40 °C in 6x SSC and 50 % formamide, followed by washing at 50 °C in 2x SSC. The length of the hybrid is the anticipated length for the hybridising nucleic acid. When nucleic acids of known sequence are hybridised, the hybrid length may be determined by aligning the sequences and identifying the conserved regions described herein. 1 xSSC is 0.15M NaCl and 15mM sodium citrate; the hybridisation solution and wash solutions may additionally include 5x Denhardt's reagent, 0.5-1.0 % SDS, 100 micro g/ml denatured, fragmented salmon sperm DNA, 0.5 % sodium pyrophosphate.

For the purposes of defining the level of stringency, reference can be made to Sambrook et al. (2001) *Molecular Cloning: a laboratory manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, CSH, New York or to *Current Protocols in Molecular Biology*, John Wiley and Sons, N.Y. (1989 and yearly updates).

The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 15 °C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50 % of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Exemplary stringent hybridization conditions can be as following: 50 % formamide, 5xSSC, and 1 % SDS, incubating at 42 °C, or, 5xSSC, 1 % SDS, incubating at 65 °C, with wash in 0.2xSSC, and 0.1 % SDS at 65 °C.

The term "purified" refers to material that is substantially or essentially free from components which interfere with the activity of the biological molecule. For cells, saccharides, nucleic acids,

and polypeptides, the term "purified" refers to material that is substantially or essentially free from components which normally accompany the material as found in its native state. Typically, purified saccharides, oligosaccharides, proteins or nucleic acids of the invention are at least about 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 % or 85 % pure, usually at least about 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, or 99 % pure as measured by band intensity on a silver stained gel or other method for determining purity. Purity or homogeneity can be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein or nucleic acid sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized. For oligosaccharides, e.g., 3-fucosyllactose, purity can be determined using methods such as but not limited to thin layer chromatography, gas chromatography, NMR, HPLC, capillary electrophoresis or mass spectroscopy.

The terms "identical" or percent "identity" or % "identity" in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using sequence comparison algorithms or by visual inspection. For sequence comparison, one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are inputted into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Percent identity can be determined using BLAST and PSI-BLAST (Altschul et al., 1990, J Mol Biol 215:3, 403- 410; Altschul et al., 1997, Nucleic Acids Res 25: 17, 3389-402). For the purposes of this invention, percent identity is determined using MatGAT2.01 (Campanella et al., 2003, BMC Bioinformatics 4:29). The following default parameters for protein are employed: (1) Gap cost Existence: 12 and Extension: 2; (2) The Matrix employed was BLOSUM50.

The term "control sequences" refers to sequences recognized by the host cells transcriptional and translational systems, allowing transcription and translation of a polynucleotide sequence to a polypeptide. Such DNA sequences are thus necessary for the expression of an operably linked coding sequence in a particular host cell or organism. Such control sequences can be, but are not limited to, promoter sequences, ribosome binding sequences, Shine Dalgarno sequences, Kozak sequences, transcription terminator sequences. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers. DNA for a presequence or secretory leader may be operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of

the sequence; or a ribosome binding site is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Said control sequences can furthermore be controlled with external chemicals, such as, but not limited to, IPTG, arabinose, lactose, allo-

5 lactose, rhamnose or fucose via an inducible promoter or via a genetic circuit that either induces or represses the transcription or translation of said polynucleotide to a polypeptide.

Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous.

10 As used herein, the term "cell productivity index (CPI)" refers to the mass of the product produced by the recombinant cells divided by the mass of the recombinant cells produced in the culture.

Detailed description of the invention

In a first embodiment, the present invention provides a method for the production of fucosyllactose by a genetically modified cell. The method comprises the steps of:

15

- providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis, more specifically said cell comprising at least one nucleic acid sequence coding for a fucosyltransferase that transfers a fucose residue from a guanosine-diphosphate fucose (GDP-fucose) donor to a lactose acceptor thereby synthesizing fucosyllactose
- 20
- said cell further comprising i) a modified expression of an endogenous membrane protein, more specifically an endogenous membrane protein involved in fucosyllactose transport, even more specifically an endogenous membrane protein enabling and/or enhancing fucosyllactose transport, and/or ii) an expression of an heterologous membrane protein, more specifically an heterologous membrane protein involved in fucosyllactose transport, even more specifically an heterologous membrane protein enabling and/or enhancing fucosyllactose transport, and wherein said membrane protein is i) selected from the group of membrane proteins comprising any one of the PFAM domains found by searching the genomic neighbourhood of GT10 and GT11 fucosyltransferase families with interpro number
- 25
- 30 IPR001503 and IPR002516 as defined on InterPro 75.0 as released 4th July 2019 respectively, wherein said genomic neighbourhood window size is 14 genes before and 14 genes after the respective fucosyltransferase and wherein said membrane protein is not belonging to the SET family, or ii) selected from the group of membrane proteins comprising SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218, or functional homolog or functional fragment of any one of the membrane proteins comprising SEQ ID NOs 204, 206, 208, 210,
- 35 212, 214, 216, 218 or a sequence having at least 80% sequence identity to any one of said membrane proteins with SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218, and cultivating the cell in a medium under conditions permissive for the production of the desired

fucosyllactose. Preferably the fucosyllactose is separated from the cultivation as explained herein.

In a preferred embodiment of the present invention the host cell comprises a membrane protein

5 selected from the group of:

- a) porters;
- b) P-P-bond-hydrolysis-driven transporters;
- c) β -Barrel Porins;
- d) Auxiliary transport proteins;
- 10 e) Putative transport proteins; and
- f) Phosphotransfer-driven group translocators.

Another embodiment provides a method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:

- providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic
15 acid sequence coding for an enzyme involved in fucosyllactose synthesis, more specifically a fucosyltransferase that transfers a fucose residue from a GDP-fucose donor to a lactose acceptor thereby synthesizing fucosyllactose,
- said cell further comprising i) a modified expression of an endogenous membrane protein involved in fucosyllactose transport, more specifically enabling and/or enhancing
20 fucosyllactose transport and/or ii) an expression of a heterologous membrane protein involved in fucosyllactose transport, more specifically enabling and/or enhancing fucosyllactose, and wherein said membrane protein is selected from the group of
 - a) porters excluding SET;
 - b) P-P-bond-hydrolysis-driven transporters;
 - 25 c) β -Barrel Porins;
 - d) Auxiliary transport proteins;
 - e) Putative transport proteins; and
 - f) Phosphotransfer-driven group translocators
- cultivating the cell in a medium under conditions permissive for the production of the desired
30 fucosyllactose. Preferably the produced fucosyllactose is separated from the cultivation as explained herein.

In the method of the invention described herein the membrane protein is either an endogenous protein with a modified expression, preferably said endogenous protein is overexpressed; or the
35 membrane protein is a heterologous protein, which can be heterologously expressed by the cell. The heterologously expressed membrane protein will then be introduced and expressed, preferably overexpressed. In another embodiment, the endogenous protein can have a modified expression in the cell which also expresses a heterologous membrane protein.

The host cell used herein is preferably genetically modified for the production of fucosyllactose. In a further preferred embodiment, the cell used herein comprises a recombinant fucosyltransferase capable of modifying lactose or an intermediate into fucosyllactose.

The host cell used herein is optionally genetically modified for the production of fucosyllactose, wherein said host cell is modified to express the de novo synthesis of GDP-fucose. Said de novo synthesis of GDP-fucose is catalyzed by the enzymes mannose-6-phosphate isomerase, phosphomannomutase encoding gene, mannose-1-phosphate guanosyltransferase, GDP-mannose-4,6-dehydratase and GDP-L-fucose synthase. Preferably, said host cell is further modified to express one or more genes encoding for the enzymes of the de novo synthesis of GDP-fucose.

The host cell used herein is optionally genetically modified to import lactose in the cell, by the introduction and/or overexpression of a lactose permease. Said lactose permease is for example encoded by the lacY gene or the lac12 gene.

According to a further aspect of the invention, the polynucleotide encoding the membrane protein is adapted to the codon usage of the respective cell or expression system.

In a preferred aspect of the above embodiments, the porter is selected from the group of TCDB classes 2.A.1.1, 2.A.1.12, 2.A.1.15, 2.A.1.2, 2.A.1.3, 2.A.1.36, 2.A.1.38, 2.A.1.46, 2.A.1.68, 2.A.1.7, 2.A.1.81, 2.A.123, 2.A.2, 2.A.21, 2.A.58, 2.A.6.3, 2.A.66 and 2.A.7.1; the P-P-bond-hydrolysis-driven transporter is selected from the group of TCDB classes 3.A.1.1, 3.A.1.2, 3.A.1.10, 3.A.1.11 and 3.A.1.5; the β -Barrel Porin is selected from TCDB classes 1.B.18 and 1.B.3.1; the Auxiliary transport protein is selected from TCDB class 8.A.3; the Putative transport protein is selected from the group of TCDB classes 9.B.14 and 9.B.158; or the Phosphotransfer-driven group translocator is selected from the group of TCDB classes 4.A.1.1 and 4.A.4.1. Said TCDB classes are classified as defined on TCDB.org as released on 17th June 2019.

In another preferred aspect of the above embodiments, the porter is selected from the group of egglog families 05BZS, 05C0R, 05C2C, 05CT4, 05CXP, 05CZQ, 05D94, 05DXI, 05E5M, 05E5W, 05E8G, 05EAM, 05EDR, 05EGZ, 05F9N, 05JHE, 05PSV, 05W2Y, 05W3H, 05XJ5, 070Q9, 07CWC, 07QF7, 07QNK, 07RBJ, 07RJ1, 07T5E, 07VQ3, 0814C, 088QT, 08H15, 08N8A, 08SC4, 08Z4Q; the P-P-bond-hydrolysis-driven transporter is selected from the group of egglog families 05BZ1, 05CJ1, 05DMK, 05DFW, 05EY8, 05HAC, 05MFV, 07FKK, 07R5U, 07V1T, 08IJ9, 08JQ7, 172T7; the β -Barrel Porin is selected from the group of egglog families 05DAY, 08KDD; the Auxiliary transport protein is selected from the group of egglog family 07SYR; the Putative transport protein is selected from the group of egglog families 05CRE, 05GWF, 06N3A; or the Phosphotransfer-driven group translocator protein is selected from the group of egglog families 05C11, 05VI0. Said egglog families are classified as defined on egglogdb 1.0.2 as released on 3rd Nov 2017.

In another preferred aspect of the above embodiments, the porter is chosen from the PFAM list of PF00083, PF00474, PF00873, PF00893, PF01895, PF01943, PF02690, PF03083, PF04193,

PF05977, PF07690, PF07690, PF13347, PF13440, PF14667; the P-P-bond-hydrolysis-driven transporter is chosen from PFAM list PF00005, PF00532, PF00664, PF01061, PF08352, PF14524, PF13407, PF13416, PF17912; the β -Barrel Porin is chosen from PFAM list PF02264, PF02563, PF10531, PF18412; the Auxiliary transport protein is chosen from PFAM list PF13807, PF02706 the Putative transport protein is chosen from PFAM list PF01578, PF03932, PF05140, PF11045 and/or the Phosphotransfer-driven group translocator protein is chosen from PFAM list PF00367, PF00358, PF02378, PF03829. Said PFAM list is classified as defined on Pfam 32.0 as released on Sept 2018.

In another preferred aspect of the above embodiments, the porter is chosen from the interpro list IPR000390, IPR001036, IPR001411, IPR001734, IPR001927, IPR002797, IPR003663, IPR003841, IPR004316, IPR004633, IPR004638, IPR004734, IPR004812, IPR005275, IPR005828, IPR005829, IPR006603, IPR010290, IPR011701, IPR020846, IPR023008, IPR023721, IPR023722, IPR026022, IPR027417, IPR027463, IPR029303, IPR032896, IPR036259, IPR038078, IPR038377, IPR039672; the P-P-bond-hydrolysis-driven transporter is chosen from interpro list IPR000412, IPR001734, IPR001761, IPR003439, IPR003593, IPR005829, IPR005978, IPR005981, IPR006059, IPR006060, IPR006061, IPR008995, IPR011527, IPR011701, IPR013456, IPR013525, IPR013563, IPR015851, IPR015855, IPR017871, IPR019554, IPR020846, IPR025997, IPR026266, IPR027417, IPR028082, IPR029439, IPR033893, IPR036259, IPR036640, IPR038377, IPR039421, IPR040582; the β -Barrel Porin is chosen from interpro list IPR003192, IPR003715, IPR019554, IPR023738, IPR036998, IPR040716; the Auxiliary transport protein is chosen from interpro list IPR003856, IPR020846, IPR027417, IPR032807, IPR036259; the Putative transport protein is chosen from interpro list IPR002541, IPR003439, IPR003593, IPR004316, IPR005627, IPR006603, IPR007816, IPR017871, IPR020368, IPR020846, IPR023648, IPR027417, IPR036259, IPR036822; or the Phosphotransfer-driven group translocator protein is chosen from interpro list IPR001127, IPR001996, IPR003352, IPR004716, IPR010974, IPR011055, IPR013013, IPR018113, IPR018454, IPR036665, IPR036878. Said interpro list is classified as defined on InterPro 75.0 as released on 4th July 2019.

In a preferred aspect of the present invention, the method as described herein uses a host cell expressing a porter membrane protein selected from MdfA from *Escherichia coli* K12 MG1655 with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, Blon_2331 from *B. longum* subsp. *Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *B. longum* subsp. *Infantis* (strain ATCC 15697) with SEQ ID NO 42, wzx-like protein of *Chitinophaga* sp. CF118 with SEQ ID NO 58, *Prevotella ruminicola* (AR32) with SEQ ID NO 66, *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, or *Dyadobacter soli* DSM 25329 with SEQ ID NO 62, or functional homolog or functional fragment of any of the above porter membrane proteins or a sequence having at least 80% sequence identity to any one of said MdfA, IceT, Blon_2331, Blon_2332 membrane proteins or wzx-like membrane protein with SEQ ID NOs 02, 06, 40, 42,

- 58, 66, 64 or 62, respectively. In another preferred aspect, the method described herein uses a host cell expressing a P-P-bond-hydrolysis driven transporter membrane protein selected from ImrA from *Lactococcus lactis* SRCM103457 with SEQ ID NO 28, LpsE membrane protein from *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 and 74 or from *Flavobacterium*
- 5 *spartansii* with SEQ ID NOs 68 and 72, TolC from *Candidatus Planktophila sulfonica* with SEQ ID NO 76, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82, *Verrucomicrobia bacterium* CG1_02_43_26 with SEQ ID NO 84, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO
- 10 216, or ytfQ from *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any of the above P-P-bond-hydrolysis driven transporter membrane proteins or a sequence having at least 80% sequence identity to any one of said ImrA, LpsE, TolC, MsbA, malE, malK, araF, xylF or ytfQ membrane protein with SEQ ID NOs 28, 70 74, 68, 72, 76, 82, 84, 206, 208, 214, 216 or 218 respectively.
- 15 In yet another preferred aspect, the method described herein uses a host cell expressing a β -barrel porin selected from Wza from *Escherichia coli* K12 MG1655 with SEQ ID NO 34 or lamB from *Escherichia coli* K-12 MG1655 with SEQ ID NO 204 or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wza or lamB membrane protein with SEQ ID NOs 34 or 204, respectively.
- 20 In an alternative preferred aspect, the method described herein uses a host cell expressing an auxiliary transport protein selected from Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099) with SEQ ID NO 88, or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wzc membrane protein with SEQ ID NO 88.
- 25 In another alternative preferred aspect, the method described herein uses a host cell expressing a putative transport protein selected from CutC from *Clostridium* sp. CAG: 1013 with SEQ ID NO 90, *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, *Mitsuaria* sp. PDC51 with SEQ ID NO 94 or *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, or functional homolog or functional fragment of any one of said CutC membrane proteins or a sequence having
- 30 at least 80% sequence identity to any one of said CutC membrane protein with SEQ ID NOs 90, 92, 94 or 96, respectively.
- In another alternative preferred aspect, the method described herein uses a host cell expressing a phosphotransfer-driven group translocator selected from nagE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 210 or srlB from *Escherichia coli* K-12 MG1655 with SEQ ID NO 212
- 35 or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said nagE or srlB membrane protein with SEQ ID NOs 210 or 212, respectively.
- In yet a further alternative preferred aspect, the method described herein uses a host cell expressing a porter membrane protein selected from MdfA from *Escherichia coli* K12 MG1655

with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, YnfM from *Escherichia coli* K12 MG1655 with SEQ ID NO 04, Yhhs from *Escherichia coli* K12 MG1655 with SEQ ID NO 08, EmrD from *Escherichia coli* K12 MG1655 with SEQ ID NO 10, YdhC from *Escherichia coli* K12 MG1655 with SEQ ID NO 12, YbdA from *Escherichia coli* K12 MG1655 with

5 SEQ ID NO 14, YdeE from *Escherichia coli* K12 MG1655 with SEQ ID NO 16, MhpT from *Escherichia coli* K12 MG1655 with SEQ ID NO 18, YebQ from *Escherichia coli* K12 MG1655 with SEQ ID NO 20, YjhB from *Escherichia coli* K12 MG1655 with SEQ ID NO 22, Bcr from *Escherichia coli* K12 MG1655 with SEQ ID NO 24, FucP from *Escherichia coli* K12 MG1655 with SEQ ID NO 26, Wzx from *Escherichia coli* K12 MG1655 with SEQ ID NO 32, EmrE from *Escherichia coli*

10 K12 MG1655 with SEQ ID NO 38, Blon_2331 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 42, Blon_0247 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 46, Blon_0245 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 48, Blon_0345 from *Bifidobacterium longum subsp. Infantis* (strain

15 ATCC 15697) with SEQ ID NO 50, CDT2 from *Neurospora crassa* OR74A with SEQ ID NO 52, CDT2 from *Aspergillus oryzae* RIB40 with SEQ ID NO 54, Wzx from *Chitinophaga* sp. CF118 with SEQ ID NO 58, Wzx from *Eubacterium* sp. CAG:581 with SEQ ID NO 60, Wzx from *Dyadobacter soli* (DSM 25329) with SEQ ID NO 62, Wzx from *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, Wzx from *Prevotella ruminicola* (AR32) with SEQ ID NO 66, NAPO

20 from *Brachyspira hampsonii* P280/1 with SEQ ID NO 86, NAm from *Actinobaculum suis* (DSM 20639) with SEQ ID NO 98, NAm from *Ruminococcus gnavus* with SEQ ID NO 100, NAm from *Curtobacterium* sp. 314Chir4.1 with SEQ ID NO 102, , Nap from *Niabella drilacis* (DSM25811) with SEQ ID NO 104, Nap from *Saccharicrinis fermentans* (DSM 9555) with SEQ ID NO 106, mdtD from *Citrobacter freundii* MGH152 with SEQ ID NO 108, mdtD from *Citrobacter werkmanii*

25 NBRC 105721 with SEQ ID NO 110, mdtD from *Citrobacter amalonaticus* with SEQ ID NO 112, mdtD from *Klebsiella oxytoca* with SEQ ID NO 114, mdtD from *Escherichia albertii* B156 with SEQ ID NO 116, yegB from *Salmonella enterica subsp. Salamae* with SEQ ID NO 118, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2 with SEQ ID NO 120, Tcr_1_D38215 from *Klebsiella pneumoniae* with SEQ ID NO 122, mdtD from *Pseudocitrobacter faecalis* with SEQ ID NO 124,

30 Cmr from *Yokenella regensburgei* (ATCC43003) with SEQ ID NO 126, MdfA from *Cronobacter muytjensii* with SEQ ID NO 128, MdfA from *Klebsiella oxytoca* with SEQ ID NO 130, MFS from *Citrobacter koseri* with SEQ ID NO 132, MdfA from *Escherichia marmotae* with SEQ ID NO 134, Cmr from *Shigella flexneri* with SEQ ID NO 136, MdfA from *Salmonella enterica subsp. Salamae* with SEQ ID NO 138, Cmr from *Citrobacter youngae* (ATCC 29220) with SEQ ID NO 140, MdfA

35 from *Citrobacter freundii* with SEQ ID NO 142, MdfA from *Enterobacter kobei* with SEQ ID NO 144, MdfA from *Enterobacter* sp. with SEQ ID NO 146, MdfA from *Lelliottia* sp. WB101 with SEQ ID NO 148, MdfA from *Enterobacter ludwigii* EcWSU1 with SEQ ID NO 150, Sweet-like protein from *Actinoplanes utahensis* with SEQ ID NO 152, Sweet-like protein from *Chitinophagaceae*

bacterium PMG_246 with SEQ ID NO 154, Sweet-like protein from *Rhizobium* sp. PDC82 with SEQ ID NO 156, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711) with SEQ ID NO 158, Sweet-like protein from *Morganella morganii* IS15 with SEQ ID NO 160, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078) with SEQ ID NO 162, Sweet-like protein from *Bradyrhizobium* sp. BTAi1 with SEQ ID NO 164, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NO 166, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10 with SEQ ID NO 168, Sweet-like protein from *Herbaspirillum aquaticum* with SEQ ID NO 170, Sweet-like protein from *Flavobacterium bacterium* MS024-2A with SEQ ID NO 172, rmd-like from *Sinorhizobium medicae* WSM419 with SEQ ID NO 182, arabinose efflux from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 184 or functional homolog or functional fragment of any of the above porter membrane proteins or a protein having an amino acid sequence having at least 80% sequence identity to any one of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, EmrE, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rmd, Sweet-like or arabinose efflux membrane proteins with SEQ ID NOs 02, 06, 04, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 32, 38, 40, 42, 46, 48, 50, 52, 54, 58, 60, 62, 64, 66, 86, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 182 or 184 respectively; a P-P-bond-hydrolysis-driven transporter is selected from LmrA from *Lactococcus lactis* strain SRCM103457 with SEQ ID NO 28, OppF from *Escherichia coli* strain K12 MG1655 with SEQ ID NO 30, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695) with SEQ ID NO 36, Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697) with SEQ ID NO 44, LpsE from *Flavobacterium spartansii* with SEQ ID NOs 68 or 72, LpsE from *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 or 74, TolC from *Candidatus Planktophila sulfonica* with SEQ ID NO 76, TolC from *Butyrivibrio hungatei* XBD2006 with SEQ ID NO 78, MsbA from *Roseburia intestinalis* CAG:13 with SEQ ID NO 80, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26 with SEQ ID NO 84, Wzm from *Rhizobium* sp. Root149 with SEQ ID NO 174, Wzm from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 176, Wzm from *Escherichia coli* 113303 with SEQ ID NO 196, Wzt from *Rhizobium* sp. Root149 with SEQ ID NO 178, Wzt from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 180, Wzt from *Escherichia coli* 113303 with SEQ ID NO 194, Nodj from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NOs 188 or 190, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 216, or ytfQ from *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any one of said LmrA, OppF, Wzk,

Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt or Nodj membrane proteins with SEQ ID NOs 28, 30, 36, 44, 68, 72, 70, 74, 76, 78, 80, 82, 84, 174, 176, 196, 178, 180, 194, 188, 190, 206, 208, 214, 216 or 218 respectively; a putative transport protein is selected from Cytochrome C biogenesis protein from *Helicobacter pylori* with SEQ ID NO 56, CutC from *Clostridium* sp. CAG: 1013 with
5 SEQ ID NO 90, CutC from *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, CutC from *Mitsuaria* sp. PDC51 with SEQ ID NO 94, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, ybjM from *Escherichia coli* K12 MG1655 with SEQ ID NO 190, ybjM from *Enterobacteriaceae bacterium* ENNIH1 with SEQ ID NO 192 or functional homolog or functional fragment of a polynucleotide encoding any one of the above putative transport proteins;
10 or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein with SEQ ID NOs 56, 90, 92, 94, 96, 190 or 192, respectively.

As used herein, a protein having an amino acid sequence having at least 80% sequence identity to any of the enlisted membrane proteins, is to be understood as that the sequence has 80%,
15 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% sequence identity to the full length of the amino acid sequence of the respective membrane protein.

The amino acid sequence of such membrane protein can be a sequence chosen from SEQ ID NO 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52,
20 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 204, 206, 208, 210, 212, 214, 216 or 218 of the attached sequence listing, or an amino acid sequence that has at least 80% sequence identity, 80%, 81%,
25 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5% sequence identity to the full length amino acid sequence of any one of SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128,
30 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 204, 206, 208, 210, 212, 214, 216 or 218.

In a further aspect of the present invention, the method as described herein uses a host cell expressing a membrane protein that is a transporter protein involved in transport of compounds
35 across the outer membrane of the cell wall.

In a further preferred aspect the method for the production of fucosyllactose as described herein further comprises at least one of the following steps:

- i) Adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per litre of initial reactor volume wherein the total reactor volume ranges from 250 mL (millilitre) to 10.000 m³ (cubic meter), preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said lactose feed;
- ii) Adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution;
- iii) Adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/L, preferably 75 g/L, more preferably 100 g/L, more preferably 125 g/L, more preferably 150 g/L, more preferably 175 g/L, more preferably 200 g/L, more preferably 225 g/L, more preferably 250 g/L, more preferably 275 g/L, more preferably 300 g/L, more preferably 325 g/L, more preferably 350 g/L, more preferably 375 g/L, more preferably, 400 g/L, more preferably 450 g/L, more preferably 500 g/L, even more preferably, 550 g/L, most preferably 600 g/L; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;
- said method resulting in a fucosyllactose concentration of at least 50 g/L, preferably at least 75 g/L, more preferably at least 90 g/L, more preferably at least 100 g/L, more preferably at least 125 g/L, more preferably at least 150 g/L, more preferably at least 175 g/L, more preferably at least 200 g/L in the final volume of said culture medium.
- Preferably the lactose feed is accomplished by adding lactose from the beginning of the cultivating in a concentration of at least 5mM, preferably in a concentration of 30, 40, 50, 60, 70, 80, 90, 100, 150 mM, more preferably in a concentration > 300 mM.
- In another aspect the lactose feed is accomplished by adding lactose to the cultivation medium in a concentration, such that throughout the production phase of the cultivation a lactose concentration of at least 5 mM, preferably 10 mM or 30 mM is obtained.
- In a further embodiment of the methods described herein the host cells are cultivated for at least about 60, 80, 100, or about 120 hours or in a continuous manner.
- In another embodiment of the methods described herein a carbon and energy source, preferably sucrose, glucose, fructose, glycerol, maltose, maltodextrine, trehalose, polyols, starch, succinate, malate, pyruvate, lactate, ethanol, citrate, and/or lactose, is also added, preferably continuously to the culture medium, preferably with the lactose.
- In a preferred embodiment, a carbon-based substrate is provided, preferably sucrose, in the culture medium for 3 or more days, preferably up to 7 days; and/or provided, in the culture

medium, at least 100, advantageously at least 105, more advantageously at least 110, even more advantageously at least 120 grams of sucrose per liter of initial culture volume in a continuous manner, so that the final volume of the culture medium is not more than three-fold, advantageously not more than two-fold, more advantageously less than two-fold of the volume of the culturing medium before the culturing.

Preferably, when performing the method as described herein, a first phase of exponential cell growth is provided by adding a carbon-based substrate, preferably glucose or sucrose, to the culture medium before the lactose is added to the culture medium in a second phase.

In an alternative preferable embodiment, in the method as described herein, the lactose is added already in the first phase of exponential growth together with the carbon-based substrate.

In another embodiment, the method as described herein produces only one fucosyllactose of the group consisting of 2'-fucosyllactose, 3-fucosyllactose and difucosyllactose.

In an alternative embodiment the method as described herein is producing a mixture of fucosyllactoses.

Such mixture can comprise at least two of the group consisting of 2'-fucosyllactose, 3-fucosyllactose and difucosyllactose.

In the method described herein the genetically modified cell is selected from the group consisting of microorganism, plant, or animal cells, preferably said microorganism is a bacterium, fungus or a yeast, preferably said plant is a rice, cotton, rapeseed, soy, maize or corn plant, preferably said animal is an insect, fish, bird or non-human mammal, all as described herein.

In a specific exemplary embodiment, the method of the invention provides the production of fucosyllactose in high yield. The method comprises the step of culturing or fermenting, in an aqueous culture or fermentation medium containing lactose, a genetically modified cell, preferably an *E. coli*, more preferably an *E. coli* cell modified by knocking out the genes *lacZ*, *lacY*, *lacA*, *glgC*, *agp*, *pfkA*, *pfkB*, *pgi*, *arcA*, *iclR*, *wcaJ*, *lon* and *thyA*. Even more preferably, additionally the *E. coli lacY* gene, a fructose kinase gene (*frk*) originating from *Zymomonas mobilis* and a sucrose phosphorylase (*SP*) originating from *Bifidobacterium adolescentis* can be knocked in into the genome and expressed constitutively. The constitutive promoters originate from the promoter library described by De Mey et al. (BMC Biotechnology, 2007). These genetic modifications are also described in WO2016075243 and WO2012007481. Additionally, the modified *E. coli* cell has a recombinant gene which encodes a single fucosyltransferase, in an exemplary embodiment this can be an α -1,3-fucosyltransferase, that is capable of modifying lactose to produce 3-fucosyllactose (3-FL). The cell furthermore comprises a recombinant gene which encodes the expression of any one of the membrane proteins as described herein.

Another aspect of the present invention provides a host cell genetically modified for the production of a fucosyllactose, wherein the host cell comprises at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis, more specifically a fucosyltransferase that transfers a fucose residue from a GDP-fucose donor to a lactose acceptor thereby synthesizing

fucosyllactose, and wherein the cell further comprises i) a modified expression of an endogenous membrane protein involved in fucosyllactose transport, more specifically enabling and/or enhancing fucosyllactose transport and/or ii) an expression of a heterologous membrane protein involved in fucosyllactose transport, more specifically enabling and/or enhancing fucosyllactose transport. The membrane protein is i) selected from the group of membrane proteins comprising any one of the PFAM domains found by searching the genomic neighbourhood of GT10 and GT11 fucosyltransferase families with interpro number IPR001503 and IPR002516 respectively, as defined on InterPro 75.0 as released on 4th July 2019, wherein said genomic neighbourhood window size is 14 genes before and 14 genes after the respective fucosyltransferase and wherein said membrane protein is not belonging to the SET family, or ii) selected from the group of membrane proteins comprising SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218, or functional homolog or functional fragment of any one of the membrane proteins comprising SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218 or a sequence having at least 80% sequence identity to any one of said membrane proteins with SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218.

Alternatively or preferably the membrane protein is selected from the group of

- a) porters excluding SET;
- b) P-P-bond-hydrolysis-driven transporters;
- c) β -Barrel Porins;
- d) Auxiliary transport proteins;
- e) Putative transport proteins; and
- f) Phosphotransfer-driven group translocators.

Alternatively or preferably, the membrane protein is selected from the group of i) porter membrane proteins selected from MdfA from *Escherichia coli* K12 MG1655 with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, YnfM from *Escherichia coli* K12 MG1655 with SEQ ID NO 04, Yhhs from *Escherichia coli* K12 MG1655 with SEQ ID NO 08, EmrD from *Escherichia coli* K12 MG1655 with SEQ ID NO 10, YdhC from *Escherichia coli* K12 MG1655 with SEQ ID NO 12, YbdA from *Escherichia coli* K12 MG1655 with SEQ ID NO 14, YdeE from *Escherichia coli* K12 MG1655 with SEQ ID NO 16, MhpT from *Escherichia coli* K12 MG1655 with SEQ ID NO 18, YebQ from *Escherichia coli* K12 MG1655 with SEQ ID NO 20, YjhB from *Escherichia coli* K12 MG1655 with SEQ ID NO 22, Bcr from *Escherichia coli* K12 MG1655 with SEQ ID NO 24, FucP from *Escherichia coli* K12 MG1655 with SEQ ID NO 26, WzxE from *Escherichia coli* K12 MG1655 with SEQ ID NO 32, EmrE from *Escherichia coli* K12 MG1655 with SEQ ID NO 38, Blon_2331 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 42, Blon_0247 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 46, Blon_0245 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with

SEQ ID NO 48, Blon_0345 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 50, CDT2 from *Neurospora crassa* OR74A with SEQ ID NO 52, CDT2 from *Aspergillus oryzae* RIB40 with SEQ ID NO 54, Wzx from *Chitinophaga* sp. CF118 with SEQ ID NO 58, Wzx from *Eubacterium* sp. CAG:581 with SEQ ID NO 60, Wzx from *Dyadobacter soli* (DSM 25329) with SEQ ID NO 62, Wzx from *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, Wzx from *Prevotella ruminicola* (AR32) with SEQ ID NO 66, NAPO from *Brachyspira hampsonii* P280/1 with SEQ ID NO 86, NAm from *Actinobaculum suis* (DSM 20639) with SEQ ID NO 98, NAm from *Ruminococcus gnavus* with SEQ ID NO 100, NAm from *Curtobacterium* sp. 314Chir4.1 with SEQ ID NO 102, Nap from *Niabella drilacis* (DSM25811) with SEQ ID NO 104, Nap from *Saccharicrinis fermentans* (DSM 9555) with SEQ ID NO 106, mdtD from *Citrobacter freundii* MGH152 with SEQ ID NO 108, mdtD from *Citrobacter werkmanii* NBRC 105721 with SEQ ID NO 110, mdtD from *Citrobacter amalonaticus* with SEQ ID NO 112, mdtD from *Klebsiella oxytoca* with SEQ ID NO 114, mdtD from *Escherichia albertii* B156 with SEQ ID NO 116, yegB from *Salmonella enterica subsp. Salamae* with SEQ ID NO 118, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2 with SEQ ID NO 120, Tcr_1_D38215 from *Klebsiella pneumoniae* with SEQ ID NO 122, mdtD from *Pseudocitrobacter faecalis* with SEQ ID NO 124, Cmr from *Yokenella regensburgei* (ATCC43003) with SEQ ID NO 126, MdfA from *Cronobacter muytjensii* with SEQ ID NO 128, MdfA from *Klebsiella oxytoca* with SEQ ID NO 130, MFS from *Citrobacter koseri* with SEQ ID NO 132, MdfA from *Escherichia marmotae* with SEQ ID NO 134, Cmr from *Shigella flexneri* with SEQ ID NO 136, MdfA from *Salmonella enterica subsp. Salamae* with SEQ ID NO 138, Cmr from *Citrobacter youngae* (ATCC 29220) with SEQ ID NO 140, MdfA from *Citrobacter freundii* with SEQ ID NO 142, MdfA from *Enterobacter kobei* with SEQ ID NO 144, MdfA from *Enterobacter* sp. with SEQ ID NO 146, MdfA from *Lelliottia* sp. WB101 with SEQ ID NO 148, MdfA from *Enterobacter ludwigii* EcWSU1 with SEQ ID NO 150, Sweet-like protein from *Actinoplanes utahensis* with SEQ ID NO 152, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246 with SEQ ID NO 154, Sweet-like protein from *Rhizobium* sp. PDC82 with SEQ ID NO 156, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711) with SEQ ID NO 158, Sweet-like protein from *Morganella morganii* IS15 with SEQ ID NO 160, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078) with SEQ ID NO 162, Sweet-like protein from *Bradyrhizobium* sp. BTAi1 with SEQ ID NO 164, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NO 166, Sweet-like protein from *Xanthomonas campestris pv. vesicatoria* str. 85-10 with SEQ ID NO 168, Sweet-like protein from *Herbaspirillum aquaticum* with SEQ ID NO 170, Sweet-like protein from *Flavobacterium bacterium* MS024-2A with SEQ ID NO 172, rnd-like from *Sinorhizobium medicae* WSM419 with SEQ ID NO 182, arabinose efflux from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 184 or functional homolog or functional fragment of any of the above porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, Wzx, Blon_2331,

Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins with SEQ ID NOs 02, 06, 04, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 32, 38, 40, 42, 46, 48, 50, 52, 54, 58, 60, 62, 64, 66, 86, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 182 or 184 respectively; ii) P-P-bond-hydrolysis-driven transporters selected from LmrA from *Lactococcus lactis* strain SRCM103457 with SEQ ID NO 28, OppF from *Escherichia coli* strain K12 MG1655 with SEQ ID NO 30, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695) with SEQ ID NO 36, Blon_2475 from *Bifidobacterium longum* subsp. Infantis (strain ATCC 15697) with SEQ ID NO 44, LpsE from *Flavobacterium spartansii* with SEQ ID NOs 68 or 72, LpsE from *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 or 74, TolC from *Candidatus Planktophilia sulfonica* with SEQ ID NO 76, TolC from *Butyrivibrio hungatei* XBD2006 with SEQ ID NO 78, MsbA from *Roseburia intestinalis* CAG:13 with SEQ ID NO 80, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26 with SEQ ID NO 84, Wzm from *Rhizobium* sp. Root149 with SEQ ID NO 174, Wzm from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 176, Wzm from *Escherichia coli* 113303 with SEQ ID NO 196, Wzt from *Rhizobium* sp. Root149 with SEQ ID NO 178, Wzt from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 180, Wzt from *Escherichia coli* 113303 with SEQ ID NO 194, Nodj from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NOs 186 or 188, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 216, or ytfQ from *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt, Nodj, malE, malK, araF, xylF or ytfQ membrane proteins with SEQ ID NOs 28, 30, 36, 44, 68, 72, 70, 74, 76, 78, 80, 82, 84, 174, 176, 196, 178, 180, 194, 188, 190, 206, 208, 214, 216 or 218 respectively; iii) putative transport proteins selected from Cytochrome C biogenesis protein from *Helicobacter pylori* with SEQ ID NO 56, CutC from *Clostridium* sp. CAG: 1013 with SEQ ID NO 90, CutC from *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, CutC from *Mitsuaria* sp. PDC51 with SEQ ID NO 94, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, ybjM from *Escherichia coli* K12 MG1655 with SEQ ID NO 190, ybjM from *Enterobacteriaceae bacterium* ENNIH1 with SEQ ID NO 192 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein with SEQ ID NOs 56, 90, 92, 94, 96, 190 or 192, respectively; iv) β -barrel porins selected from Wza from *Escherichia coli* K12 MG1655 with SEQ ID NO 34 or lamB from *Escherichia coli* K12 MG1655 with SEQ ID NO 204 or functional

homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wza or lamB membrane protein with SEQ ID NOs 34 or 204, respectively; v) auxiliary transport protein is selected from Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099) with SEQ ID NO 88, or functional homolog or functional fragment thereof
5 or a sequence having at least 80% sequence identity to said Wzc membrane protein with SEQ ID NO 88; vi).phosphotransfer-driven group translocators selected from nagE from *Escherichia coli* K12 MG1655 with SEQ ID NO 210 or srlB from *Escherichia coli* K12 MG1655 with SEQ ID NO 212, or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said nagE or srlB membrane protein with SEQ ID NOs 210 or 212,
10 respectively.

In a further aspect of the present invention, the cell as described herein expresses a membrane protein that is a transporter protein involved in transport of compounds across the outer membrane of the cell wall.

Another aspect provides for a cell to be stably cultured in a medium, wherein said medium can
15 be any type of growth medium comprising minimal medium, complex medium or growth medium enriched in certain compounds like for example but not limited to vitamins, trace elements, amino acids.

Preferably the cell is transformed to comprise at least one nucleic acid sequence encoding a protein selected from the group consisting of lactose transporter, fucose transporter, transporter
20 for a nucleotide-activated sugar

In the methods described herein the cell can be a cell of any organism. The term 'organism' or 'cell' as used herein refers to a microorganism chosen from the list consisting of a bacterium, a yeast or a fungus, or, refers to a plant cell, animal cell, a mammalian cell, an insect cell and a protozoal cell. The latter bacterium preferably belongs to the phylum of the Proteobacteria or the
25 phylum of the Firmicutes or the phylum of the Cyanobacteria or the phylum Deinococcus-Thermus. The latter bacterium belonging to the phylum Proteobacteria belongs preferably to the family *Enterobacteriaceae*, preferably to the species *Escherichia coli*. The latter bacterium preferably relates to any strain belonging to the species *Escherichia coli* such as but not limited to *Escherichia coli* B, *Escherichia coli* C, *Escherichia coli* W, *Escherichia coli* K12, *Escherichia coli* Nissle. More specifically, the latter term relates to cultivated *Escherichia coli* strains –
30 designated as *E. coli* K12 strains – which are well-adapted to the laboratory environment, and, unlike wild type strains, have lost their ability to thrive in the intestine. Well-known examples of the *E. coli* K12 strains are K12 Wild type, W3110, MG1655, M182, MC1000, MC1060, MC1061, MC4100, JM101, NZN111 and AA200. Hence, preferably the present invention specifically relates
35 to a mutated and/or transformed *Escherichia coli* strain as indicated above wherein said *E. coli* strain is a K12 strain. More specifically, the present invention relates to a mutated and/or transformed *Escherichia coli* strain as indicated above wherein said K12 strain is *E. coli* MG1655. The latter bacterium belonging to the phylum Firmicutes belongs preferably to the Bacilli,

preferably from the species *Bacillus*. The latter yeast preferably belongs to the phylum of the Ascomycota or the phylum of the Basidiomycota or the phylum of the Deuteromycota or the phylum of the Zygomycetes. The latter yeast belongs preferably to the genus *Saccharomyces*, *Pichia*, *Hansunella*, *Kluyveromyces*, *Yarrowia*, *Eremothecium*, *Zygosaccharomyces* or
5 *Debaromyces*. The latter fungus belongs preferably to the genus *Rhizopus*, *Dictyostelium* or *Aspergillus*. "Plant cells" includes cells of flowering and non-flowering plants, as well as algal cells, for example *Chlamydomonas*, *Chlorella*, etc. Preferably, said plant cell is a tobacco, alfalfa, rice, tomato, corn, maize or soybean cell; said mammalian cell is a CHO cell or a HEK cell; said insect cell is an *S. frugiperda* cell and said protozoal cell is a *L. tarentolae* cell.

10 In a preferred embodiment the cell is a cell of a microorganism, wherein more preferably said microorganism is a bacterium or a yeast. In a more preferred embodiment, the microorganism is a bacterium, most preferably *Escherichia coli*. Examples using such *E. coli* are described herein. In another more preferred embodiment, the bacterium is yeast. Examples using yeast for the production of fucosyllactose and useable in the present invention are e.g. described by Hollands
15 et al. (*Metabolic Engineering* 52 (2019) 232-242).

It is generally preferred that the cell's catabolic pathway for selected mono-, di- or oligosaccharides is at least partially inactivated, the mono-, di-, or oligosaccharides being involved in and/or required for the synthesis of fucosyllactose.

In a further embodiment the present invention provides a method for the production of
20 fucosyllactose, wherein a cell as described herein is used for culturing in a medium under conditions permissive for the production of said fucosyllactose. The fucosyllactose is then separated from the cultivation. As used herein, conditions permissive for the production are to be understood to be conditions relating to physical or chemical parameters enabling growth of and living cells, including but not limited to temperature, pH, pressure, osmotic pressure and
25 product/educt concentration. Preferably, such permissive conditions may include temperature range of 30+/-20°C, a pH range of 7+/-3.

The cell according to the invention produces fucosyllactose. The fucosyllactose is chosen from the group consisting of 2'-fucosyllactose, 3-fucosyllactose and difucosyllactose.

Another aspect of the present invention provides for the use of a membrane protein selected from
30 the group of membrane proteins as defined herein in the fermentative production of fucosyllactose. The fucosyllactose is chosen from the group consisting of 2'-fucosyllactose, 3-fucosyllactose and difucosyllactose.

In a further aspect, the present invention provides for the use of a cell as defined herein, in a method for the production of fucosyllactose.

35 In yet another aspect, the present invention provides for the use of a cell as defined herein wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose and/or difucosyllactose.

Furthermore, the invention also relates to the fucosyllactose obtained by the methods according to the invention, as well as to the use of a polynucleotide, the vector, host cells, microorganisms

or the polypeptide as described above for the production of fucosyllactose. The fucosyllactose may be used as food additive, prebiotic, symbiotic, for the supplementation of baby food, adult food or feed, or as either therapeutically or pharmaceutically active compound. With the novel methods, fucosyllactose can easily and effectively be provided, without the need for complicated, time and cost consuming synthetic processes.

As used herein, the term "separating" means harvesting, collecting or retrieving the fucosyllactose from the host cell and/or the medium of its growth as explained herein.

Fucosyllactose can be separated in a conventional manner from the cultivation or aqueous culture medium, in which the mixture was made. In case the fucosyllactose is still present in the cells producing the fucosyllactose, conventional manners to free or to extract the fucosyllactose out of the cells can be used, such as cell destruction using high pH, heat shock, sonication, French press, homogenisation, enzymatic hydrolysis, chemical hydrolysis, solvent hydrolysis, detergent, hydrolysis,... The culture medium, reaction mixture and/or cell extract, together and separately called fucosyllactose containing mixture or cultivation, can then be further used for separating the fucosyllactose.

Typically oligosaccharides, and fucosyllactose being an oligosaccharide, are purified by first removing macro components, i.e. first the cells and cell debris, then the smaller components, i.e. proteins, endotoxins and other components between 1000 Da and 1000 kDa and then the oligosaccharide is desalted by means of retaining the oligosaccharide with a nanofiltration membrane or with electrodialysis in a first step and ion exchange also known as ion exchange chromatography in a second step, which consists of a cation exchange resin and anion exchange resin, wherein most preferably the cation exchange chromatography is performed before the anion exchange chromatography. These steps do not separate sugars from each other with a small difference in degree of polymerization. Said separation is done for instance by chromatographical separation.

This preferably involves clarifying the fucosyllactose containing mixtures to remove suspended particulates and contaminants, particularly cells, cell components, insoluble metabolites and debris produced by culturing the genetically modified cell and/or performing the enzymatic reaction. In this step, the fucosyllactose containing mixture can be clarified in a conventional manner. Preferably, the fucosyllactose containing mixture is clarified by centrifugation, flocculation, decantation and/or filtration. A second step of separating the fucosyllactose from the fucosyllactose containing mixture preferably involves removing substantially all the proteins, as well as peptides, amino acids, RNA and DNA and any endotoxins and glycolipids that could interfere with the subsequent separation step, from the fucosyllactose containing mixture, preferably after it has been clarified. In this step, proteins and related impurities can be removed from the fucosyllactose containing mixture in a conventional manner. Preferably, proteins, salts, byproducts, colour and other related impurities are removed from the fucosyllactose containing mixture by ultrafiltration, nanofiltration, reverse osmosis, microfiltration, activated charcoal or

carbon treatment, tangential flow high-performance filtration, tangential flow ultrafiltration, affinity chromatography, ion exchange chromatography (such as but not limited to cation exchange, anion exchange, mixed bed ion exchange), hydrophobic interaction chromatography and/or gel filtration (i.e., size exclusion chromatography), particularly by chromatography, more particularly by ion exchange chromatography or hydrophobic interaction chromatography or ligand exchange chromatography. With the exception of size exclusion chromatography, proteins and related impurities are retained by a chromatography medium or a selected membrane, while fucosyllactose remains in the fucosyllactose containing mixture.

Contaminating compounds with a molecular weight above 1000 Da (dalton) are removed by means of ultrafiltration membranes with a cut-off above 1000 Da to approximately 1000 kDa. The membrane retains the contaminant and the oligosaccharide goes to the filtrate. Typical ultrafiltration principles are well known in the art and are based on Tubular modules, Hollow fiber, spiral-wound or plates; These are used in cross flow conditions or as a dead-end filtration. The membrane composition is well known and available from several vendors, and are composed of PES (Polyethylene sulfone), polyvinylpyrrolidone, PAN (Polyacrylonitrile), PA (Poly-amide), Polyvinylidene difluoride (PVDF), NC (Nitrocellulose), ceramic materials or combinations thereof. Components smaller than the oligosaccharide, for instance monosaccharides, salts, disaccharides, acids, bases, medium constituents are separated by means of a nano-filtration or/and electrodialysis. Such membranes have molecular weight cut-offs between 100 Da and 1000 Da. For an oligosaccharide such as 2'-fucosyllactose the optimal cut-off is between 300 Da and 500 Da, minimizing losses in the filtrate. Typical membrane compositions are well known and are for example polyamide (PA), TFC, PA-TFC, Polypiperazine-amide, PES, Cellulose Acetate or combinations thereof.

Fucosyllactose is further isolated from the culture medium and/or cell with or without further purification steps by evaporation, lyophilization, crystallization, precipitation, and/or drying, spray drying. Said further purification steps allow the formulation of fucosyllactose in combination with other oligosaccharides and/or products, for instance but not limited to the co-formulation by means of spray-drying, drying or lyophilization or concentration by means of evaporation in liquid form.

In an even further aspect, the present invention also provides for a further purification of the fucosyllactose. A further purification of said fucosyllactose may be accomplished, for example, by use of (activated) charcoal or carbon, nanofiltration, ultrafiltration or ion exchange to remove any remaining DNA, protein, LPS, endotoxins, or other impurity. Alcohols, such as ethanol, and aqueous alcohol mixtures can also be used. Another purification step is accomplished by crystallization or precipitation of the product. Another purification step is to spray dry or lyophilize fucosyllactose.

The separated and preferably also purified fucosyllactose can be used as a supplement in infant formulas and for treating various diseases in newborn infants.

As will be shown in the examples herein, the method and the cell of the invention provide at least one of the following surprising advantages when using the membrane proteins as defined herein:

- Better fucosyllactose titers (enhanced) (g/L),
- Better production rate r (g fucosyllactose / L/h),
- 5 - Better cell performance index CPI (g fucosyllactose/ g X),
- Better specific productivity Q_p (g fucosyllactose /g X /h),
- Better yield on sucrose Y_s (g fucosyllactose / g sucrose),
- Better sucrose uptake/conversion rate Q_s (g sucrose / g X /h),
- Better lactose conversion/consumption rate r_s (g lactose/h),
- 10 - Enhanced fucosyllactose secretion, and/or
- Enhanced growth speed of the production host,

when compared to a fucosyllactose production host with an identical genetic background but lacking the expression of the heterologous membrane protein or modulated expression of the endogenous membrane protein.

15

Moreover, the present invention relates to the following specific embodiments:

1. Method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:

- providing a cell capable of producing fucosyllactose, said cell comprising at least one
- 20 - nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis
- said cell further comprising i) a modified expression of an endogenous membrane protein and/or ii) an expression of a heterologous membrane protein, and wherein said membrane protein is selected from the group of membrane proteins comprising any one
- 25 - of the PFAM domains found by searching the genomic neighbourhood of GT10 and GT11 fucosyltransferase families with interpro number IPR001503 and IPR002516 respectively, wherein said genomic neighbourhood window size is 14 genes before and 14 genes after the respective fucosyltransferase and wherein said membrane protein is not belonging to the SET family
- cultivating the cell in a medium under conditions permissive for the production of the
- 30 - desired fucosyllactose
- preferably separating the fucosyllactose from the cultivation.

2. Method according to embodiment 1 wherein said membrane protein is selected from the group of

- a) porters;
- 35 b) P-P-bond-hydrolysis-driven transporters;
- c) β -Barrel Porins;
- d) Auxiliary transport proteins; and
- e) Putative transport proteins

3. Method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:
 - providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis
 - 5 - said cell further comprising i) a modified expression of an endogenous membrane protein and/or ii) an expression of a heterologous membrane protein, and wherein said membrane protein is selected from the group of
 - a) porters and wherein said membrane protein is not belonging to the SET family;
 - b) P-P-bond-hydrolysis-driven transporters;
 - 10 c) β -Barrel Porins;
 - d) Auxiliary transport proteins; and
 - e) Putative transport proteins
 - cultivating the cell in a medium under conditions permissive for the production of the desired fucosyllactose
 - 15 - preferably separating the fucosyllactose from the cultivation.
4. Method according to any one of embodiments 2 or 3, wherein said porter is selected from the group of TCDB classes 2.A.1.1, 2.A.1.12, 2.A.1.15, 2.A.1.2, 2.A.1.3, 2.A.1.36, 2.A.1.38, 2.A.1.46, 2.A.1.68, 2.A.1.7, 2.A.1.81, 2.A.123, 2.A.2, 2.A.21, 2.A.58, 2.A.6.3, 2.A.66 and 2.A.7.1.
- 20 5. Method according to any one of embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is selected from the group of TCDB classes 3.A.1.1, 3.A.1.10, 3.A.1.11 and 3.A.1.5.
6. Method according to any one of embodiments 2 or 3, wherein said β -Barrel Porin is selected from TCDB class 1.B.18.
- 25 7. Method according to any one of embodiments 2 or 3, wherein said Auxiliary transport protein is selected from TCDB class 8.A.3.
8. Method according to any one of embodiments 2 or 3, wherein said Putative transport protein is selected from the group of TCDB classes 9.B.14 and 9.B.158.
9. Method according to any one of embodiments 2 or 3, wherein said porter is selected from the group of eggnoG families 05BZS, 05C0R, 05C2C, 05CT4, 05CXP, 05CZQ, 05D94, 05DXI, 05E5M, 05E5W, 05E8G, 05EAM, 05EDR, 05EGZ, 05F9N, 05JHE, 05PSV, 05W2Y, 05W3H, 05XJ5, 070Q9, 07CWC, 07QF7, 07QNK, 07RBJ, 07RJ1, 07T5E, 07VQ3, 0814C, 088QT, 08H15, 08N8A, 08SC4, 08Z4Q.
- 30 10. Method according to any one of embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is selected from the group of eggnoG families 05BZ1, 05CJ1, 05EY8, 05HAC, 05MFV, 07V1T, 08IJ9, 08JQ7.
11. Method according to any one of embodiments 2 or 3, wherein said β -Barrel Porin is selected from the group of eggnoG family 05DAY.

12. Method according to any one of embodiments 2 or 3, wherein said Auxiliary transport protein is selected from the group of eggnog family 07SYR.
13. Method according to any one of embodiments 2 or 3, wherein said Putative transport protein is selected from the group of eggnog families 05CRE, 05GWF, 06N3A.
- 5 14. Method according to any one of embodiments 2 or 3, wherein said porter is chosen from the PFAM list of PF00083, PF00474, PF00873, PF00893, PF01895, PF01943, PF02690, PF03083, PF04193, PF05977, PF07690, PF07690, PF13347, PF13440 and PF14667.
15. Method according to any one of embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is chosen from PFAM list PF00005, PF00664, PF01061, PF08352,
10 PF14524 and PF17912.
16. Method according to any one of embodiments 2 or 3, wherein said β -Barrel Porin is chosen from PFAM list PF02563, PF10531 and PF18412.
17. Method according to any one of embodiments 2 or 3, wherein said Auxiliary transport protein is chosen from PFAM list PF13807 and PF02706.
- 15 18. Method according to any one of embodiments 2 or 3, wherein said Putative transport protein is chosen from PFAM list PF01578, PF03932, PF05140 and PF11045.
19. Method according to any one of embodiments 2 or 3, wherein said porter is chosen from the interpro list IPR000390, IPR001036, IPR001411, IPR001734, IPR001927, IPR002797,
20 IPR003663, IPR003841, IPR004316, IPR004633, IPR004638, IPR004734, IPR004812, IPR005275, IPR005828, IPR005829, IPR006603, IPR010290, IPR011701, IPR020846, IPR023008, IPR023721, IPR023722, IPR026022, IPR027417, IPR027463, IPR029303, IPR032896, IPR036259, IPR038078, IPR038377, IPR039672
20. Method according to any one of embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is chosen from interpro list IPR000412, IPR001734, IPR003439,
25 IPR003593, IPR005829, IPR005978, IPR005981, IPR008995, IPR011527, IPR011701, IPR013525, IPR013563, IPR015851, IPR017871, IPR019554, IPR020846, IPR027417, IPR029439, IPR036259, IPR036640, IPR038377, IPR039421 and IPR040582.
21. Method according to any one of embodiments 2 or 3, wherein said β -Barrel Porin is chosen from interpro list IPR003715, IPR019554 and IPR040716.
- 30 22. Method according to embodiment 2, wherein said Auxiliary transport protein is chosen from interpro list IPR003856, IPR020846, IPR027417, IPR032807 and IPR036259.
23. Method according to any one of embodiments 2 or 3, wherein said Putative transport protein is chosen from interpro list IPR002541, IPR003439, IPR003593, IPR004316, IPR005627,
35 IPR006603, IPR007816, IPR017871, IPR020368, IPR020846, IPR023648, IPR027417, IPR036259 and IPR036822.
24. Method according to any one of embodiments 2 or 3, wherein said porter membrane protein is selected from MdfA from *Escherichia coli* K12 MG1655, IceT from *Escherichia coli* K12 MG1655, Blon_2331 from *B. longum* subsp. *Infantis* (strain ATCC 15697), Blon_2332 from

- B. longum* subsp. *Infantis* (strain ATCC 15697), wzx-like protein of *Chitinophaga* sp. CF118, *Prevotella ruminicola* (AR32), *Lactococcus raffinolactis* (ATCC 43920), or *Dyadobacter soli* DSM 25329, or functional homolog or functional fragment of any one of the above porter membrane protein or a sequence having at least 80% sequence identity to any one of said
- 5 MdfA, IceT, Blon_2331, Blon_2332 membrane proteins or wzx-like membrane protein.
25. Method according to any one of embodiments 2 or 3, wherein said P-P-bond-hydrolysis driven transporter membrane protein is selected from ImrA from *Lactococcus lactis* subsp. *lactis* bv. *Diacetylactis*, LpsE membrane protein from *Sporomusa sphaeroides* DSM 2875, *Flavobacterium spartansii*, TolC from *Candidatus Planktophila sulfonica*, MsbA from
- 10 *Pedobacter ginsengisoli* or *Verrucomicrobia bacterium* CG1_02_43_26, or functional homolog or functional fragment of any of the above P-P-bond-hydrolysis driven transporter membrane protein or a sequence having at least 80% sequence identity to any of said ImrA, LpsE, TolC or MsbA membrane protein.
26. Method according to any one of embodiments 2 or 3, wherein said β -barrel porin is selected
- 15 from Wza from *Escherichia coli* K12 MG1655 or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wza membrane protein.
27. Method according to any one of embodiments 2 or 3, wherein said auxiliary transport protein is selected from Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099), or functional homolog or functional fragment thereof or a sequence having at
- 20 least 80% sequence identity to said Wzc membrane protein.
28. Method according to any one of embodiments 2 or 3, wherein said putative transport protein is selected from CutC from *Clostridium* sp. CAG: 1013, *Odoribacter splanchnicus* DSM 20712, *Mitsuaria* sp. PDC51 or *Prevotella intermedia* ATCC 25611 (DSM 20706), or functional homolog or functional fragment of any of said CutC membrane proteins or a
- 25 sequence having at least 80% sequence identity to any of said CutC membrane protein.
29. Method according to any one of embodiments 2 or 3, wherein said porter membrane protein is selected from MdfA from *Escherichia coli* K12 MG1655, IceT from *Escherichia coli* K12 MG1655, YnfM from *Escherichia coli* K12 MG1655, Yhhs from *Escherichia coli* K12 MG1655, EmrD from *Escherichia coli* K12 MG1655, YdhC from *Escherichia coli* K12 MG1655, YbdA
- 30 from *Escherichia coli* K12 MG1655, YdeE from *Escherichia coli* K12 MG1655, MhpT from *Escherichia coli* K12 MG1655, YebQ from *Escherichia coli* K12 MG1655, YjhB from *Escherichia coli* K12 MG1655, Bcr from *Escherichia coli* K12 MG1655, FucP from *Escherichia coli* K12 MG1655, WzxE from *Escherichia coli* K12 MG1655, EmrE from *Escherichia coli* K12 MG1655, Blon_2331 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697),
- 35 Blon_2332 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_0247 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_0245 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_0345 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), CDT2 from *Neurospora crassa*

- OR74A, CDT2 from *Aspergillus oryzae* RIB40, Wzx from *Chitinophaga* sp. CF118, Wzx from *Eubacterium* sp. CAG:581, Wzx from *Dyadobacter soli* (DSM 25329), Wzx from *Lactococcus raffinolactis* (ATCC 43920), Wzx from *Prevotella ruminicola* (AR32), NAPO from *Brachyspira hampsonii* P280/1, NAm from *Actinobaculum suis* (DSM 20639), NAm from *Ruminococcus gnavus*, NAm from *Curtobacterium* sp. 314Chir4.1, NAm from *Planctomycetes bacterium* GWF2_42_9, Nap from *Niabella drilacis* (DSM25811), Nap from *Saccharicrinis fermentans* (DSM 9555), mdtD from *Citrobacter freundii* MGH152, mdtD from *Citrobacter werkmanii* NBRC 105721, mdtD from *Citrobacter amalonaticus*, mdtD from *Klebsiella oxytoca*, mdtD from *Escherichia albertii* B156, yegB from *Salmonella enterica* subsp. *Salamae*, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2, Tcr_1_D38215 from *Klebsiella pneumoniae*, mdtD from *Pseudocitrobacter faecalis*, Cmr from *Yokenella regensburgei* (ATCC43003), MdfA from *Cronobacter muytjensii*, MdfA from *Klebsiella oxytoca*, MFS from *Citrobacter koseri*, MdfA from *Escherichia marmotae*, Cmr from *Shigella flexneri*, MdfA from *Salmonella enterica* subsp. *Salamae*, Cmr from *Citrobacter youngae* (ATCC 29220), MdfA from *Citrobacter freundii*, MdfA from *Enterobacter kobei*, MdfA from *Enterobacter* sp., MdfA from *Lelliottia* sp. WB101, MdfA from *Enterobacter ludwigii* EcWSU1, Sweet-like protein from *Actinoplanes utahensis*, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246, Sweet-like protein from *Rhizobium* sp. PDC82, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711), Sweet-like protein from *Morganella morganii* IS15, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078), Sweet-like protein from *Bradyrhizobium* sp. BTAi1, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10, Sweet-like protein from *Herbaspirillum aquaticum*, Sweet-like protein from *Flavobacterium bacterium* MS024-2A, rnd-like from *Sinorhizobium medicae* WSM419, arabinose efflux from *Azospirillum brasiliense* LMG 04375 or functional homolog or functional fragment of any of the above porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, EmrE, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins.
30. Method according to any one of embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is selected from LmrA from *Lactococcus lactis* strain SRCM103457, OppF from *Escherichia coli* strain K12 MG1655, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695), Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), LpsE from *Flavobacterium spartansii*, LpsE from *Sporomusa sphaeroides* DSM 2875, TolC from *Candidatus Planktophila sulfonica*, TolC from *Butyrivibrio hungatei* XBD2006, MsbA from *Roseburia intestinalis* CAG:13, MsbA from *Pedobacter ginsengisoli*, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26, Wzm from *Rhizobium* sp. Root149, Wzm from

Azospirillum brasiliense LMG 04375, Wzm from *Escherichia coli* 113303, Wzt from *Rhizobium* sp. Root149, Wzt from *Azospirillum brasiliense* LMG 04375, Wzt from *Escherichia coli* 113303, Nodj from *Bradyrhizobium japonicum* USDA 110 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt or Nodj membrane proteins.

31. Method according to any one of embodiments 2 or 3, wherein said putative transport protein is selected from Cytochrome C biogenesis protein from *Helicobacter pylori*, CutC from *Clostridium* sp. CAG: 1013, CutC from *Odoribacter splanchnicus* DSM 20712, CutC from *Mitsuaria* sp. PDC51, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706), ybjM from *Escherichia coli* K12 MG1655, ybjM from *Enterobacteriaceae bacterium* ENNIH1 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein.

32. Method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:

- providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis
- said cell further comprising i) a modified expression of an endogenous membrane protein and/or ii) an expression of a heterologous membrane protein, and wherein said membrane protein is selected from the group of membrane proteins consisting of the porter membrane proteins MdfA from *Escherichia coli* K12 MG1655, IceT from *Escherichia coli* K12 MG1655, YnfM from *Escherichia coli* K12 MG1655, Yhhs from *Escherichia coli* K12 MG1655, EmrD from *Escherichia coli* K12 MG1655, YdhC from *Escherichia coli* K12 MG1655, YbdA from *Escherichia coli* K12 MG1655, YdeE from *Escherichia coli* K12 MG1655, MhpT from *Escherichia coli* K12 MG1655, YebQ from *Escherichia coli* K12 MG1655, YjhB from *Escherichia coli* K12 MG1655, Bcr from *Escherichia coli* K12 MG1655, FucP from *Escherichia coli* K12 MG1655, Wzx from *Escherichia coli* K12 MG1655, EmrE from *Escherichia coli* K12 MG1655, Blon_2331 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_2332 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_0247 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_0245 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), Blon_0345 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), CDT2 from *Neurospora crassa* OR74A, CDT2 from *Aspergillus oryzae* RIB40, Wzx from *Chitinophaga* sp. CF118, Wzx from *Eubacterium* sp. CAG:581, Wzx from *Dyadobacter soli* (DSM 25329), Wzx from *Lactococcus raffinolactis* (ATCC 43920), Wzx from *Prevotella ruminicola*

(AR32), NAPO from *Brachyspira hampsonii* P280/1, NAm from *Actinobaculum suis* (DSM 20639), NAm from *Ruminococcus gnavus*, NAm from *Curtobacterium* sp. 314Chir4.1, NAm from *Planctomycetes bacterium* GWF2_42_9, Nap from *Niabella drilacis* (DSM25811), Nap from *Saccharicrinis fermentans* (DSM 9555), mdtD from *Citrobacter freundii* MGH152, mdtD from *Citrobacter werkmanii* NBRC 105721, mdtD from *Citrobacter amalonaticus*, mdtD from *Klebsiella oxytoca*, mdtD from *Escherichia albertii* B156, yegB from *Salmonella enterica* subsp. *Salamae*, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2, Tcr_1_D38215 from *Klebsiella pneumoniae*, mdtD from *Pseudocitrobacter faecalis*, Cmr from *Yokenella regensburgei* (ATCC43003), MdfA from *Cronobacter muytjensii*, MdfA from *Klebsiella oxytoca*, MFS from *Citrobacter koseri*, MdfA from *Escherichia marmotae*, Cmr from *Shigella flexneri*, MdfA from *Salmonella enterica* subsp. *Salamae*, Cmr from *Citrobacter youngae* (ATCC 29220), MdfA from *Citrobacter freundii*, MdfA from *Enterobacter kobei*, MdfA from *Enterobacter* sp., MdfA from *Lelliottia* sp. WB101, MdfA from *Enterobacter ludwigii* EcWSU1, Sweet-like protein from *Actinoplanes utahensis*, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246, Sweet-like protein from *Rhizobium* sp. PDC82, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711), Sweet-like protein from *Morganella morganii* IS15, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078), Sweet-like protein from *Bradyrhizobium* sp. BTAi1, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10, Sweet-like protein from *Herbaspirillum aquaticum*, Sweet-like protein from *Flavobacteria bacterium* MS024-2A, rnd-like from *Sinorhizobium medicae* WSM419, arabinose efflux from *Azospirillum brasiliense* LMG 04375 or functional homolog or functional fragment of any of the above porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, EmrE, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins; and the P-P-bond-hydrolysis-driven transporters LmrA from *Lactococcus lactis* strain SRCM103457, OppF from *Escherichia coli* strain K12 MG1655, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695), Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), LpsE from *Flavobacterium spartansii*, LpsE from *Sporomusa sphaeroides* DSM 2875, TolC from *Candidatus Planktrophila sulfonica*, TolC from *Butyrivibrio hungatei* XBD2006, MsbA from *Roseburia intestinalis* CAG:13, MsbA from *Pedobacter ginsengisoli*, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26, Wzm from *Rhizobium* sp. Root149, Wzm from *Azospirillum brasiliense* LMG 04375, Wzm from *Escherichia coli* 113303, Wzt from *Rhizobium* sp. Root149, Wzt from *Azospirillum brasiliense* LMG 04375, Wzt from *Escherichia coli*

113303, Nodj from *Bradyrhizobium japonicum* USDA 110 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt or Nodj membrane proteins; and a β -barrel porin membrane protein Wza from *Escherichia coli* K12 MG1655 or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wza membrane protein; and auxiliary transport protein Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099), or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wzc membrane protein; and putative transport proteins Cytochrome C biogenesis protein from *Helicobacter pylori*, CutC from *Clostridium* sp. CAG: 1013, CutC from *Odoribacter splanchnicus* DSM 20712, CutC from *Mitsuaria* sp. PDC51, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706), ybjM from *Escherichia coli* K12 MG1655, ybjM from *Enterobacteriaceae* bacterium ENNIH1 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein.

33. Method for the production of fucosyllactose according to any one of the previous embodiments, the method further comprising at least one of the following steps:

- i) Adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per initial reactor volume, preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said lactose feed;
- ii) Adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution;
- iii) Adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/l, preferably 75 g/l, more preferably 100 g/l, more preferably 125 g/l, more preferably 150 g/l, more preferably 175 g/l, more preferably 200 g/l, more preferably 225 g/l, more preferably 250 g/l, more preferably 275 g/l, more preferably 300 g/l, more preferably 325 g/l, more preferably 350 g/l, more preferably 375 g/l, more preferably, 400 g/l, more preferably 450 g/l, more preferably 500 g/l, even more preferably, 550 g/l, most preferably 600 g/l; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;

- iv) Said method resulting in a fucosyllactose concentration of at least 50 g/l, preferably at least 75 g/l, more preferably at least 90 g/l, more preferably at least 100 g/l, more preferably at least 125 g/l, more preferably at least 150 g/l, more preferably at least 175 g/l, more preferably at least 200 g/l in the final volume of said culture medium.
- 5 34. The method of embodiment 33, wherein the lactose feed is accomplished by adding lactose from the beginning of the cultivating in a concentration of at least 5mM, preferably in a concentration of 30, 40, 50, 60, 70, 80, 90, 100, 150 mM, more preferably in a concentration > 300 mM.
- 10 35. The method of any one of the embodiments 33 or 34, wherein said lactose feed is accomplished by adding lactose to the cultivation medium in a concentration, such, that throughout the production phase of the cultivation a lactose concentration of at least 5 mM, preferably 10 mM or 30 mM is obtained.
36. The method of any of the embodiments 33, 34 or 35, wherein the host cells are cultivated for at least about 60, 80, 100, or about 120 hours or in a continuous manner.
- 15 37. The method of any one of embodiments 33 to 36, wherein a carbon and energy source, preferably sucrose, glucose, fructose, glycerol, maltose, maltodextrines, trehalose, polyols, starch, succinate, malate, pyruvate, lactate, ethanol, citrate, lactose, is also added, preferably continuously to the culture medium, preferably with the lactose.
- 20 38. The method of any one of embodiments 33 to 37, wherein a first phase of exponential cell growth is provided by adding a carbon-based substrate, preferably glucose or sucrose, to the culture medium before the lactose is added to the culture medium in a second phase.
39. Method according to any one of the embodiments 1 to 38, wherein the method is producing a mixture of fucosyllactoses.
- 25 40. Method according to any one of embodiments 1 to 39, wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose and/or difucosyllactose.
- 30 41. Method according to any one of embodiment 1 to 40, wherein said genetically modified cell is selected from the group consisting of microorganism, plant, or animal cells, preferably said microorganism is a bacterium, fungus or a yeast, preferably said plant is a rice, cotton, rapeseed, soy, maize or corn plant, preferably said animal is an insect, fish, bird or non-human mammal.
42. Method according to embodiment 41, wherein the cell is an *Escherichia coli* cell.
43. Host cell genetically modified for the production of a fucosyllactose, wherein the host cell comprises at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis
- 35 - said cell further comprising i) a modified expression of an endogenous membrane protein and/or ii) an expression of a heterologous membrane protein, and wherein said membrane protein is selected from the group of membrane proteins comprising any one of the PFAM domains found by searching the genomic neighbourhood of GT10 and GT11

fucosyltransferase families with interpro number IPR001503 and IPR002516 respectively, wherein said genomic neighbourhood window size is 14 genes before and 14 genes after the respective fucosyltransferase and wherein said membrane protein is not belonging to the SET family.

- 5 44. Host cell according to embodiment 43, wherein said membrane protein is selected from the group of
- a) porters;
 - b) P-P-bond-hydrolysis-driven transporters;
 - c) β -Barrel Porins;
 - 10 d) Auxiliary transport proteins; and
 - e) Putative transport proteins.
45. Host cell genetically modified for the production of a fucosyllactose, wherein the host cell comprises at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis
- 15 - said cell further comprising i) a modified expression of an endogenous membrane protein and/or ii) an expression of a heterologous membrane protein, and wherein said membrane protein is selected from the group of
- a) porters and wherein said membrane protein is not belonging to the SET family;
 - b) P-P-bond-hydrolysis-driven transporters;
 - 20 c) β -Barrel Porins;
 - d) Auxiliary transport proteins; and
 - e) Putative transport proteins.
46. Host cell according to any one of the embodiments 44 or 45, wherein said membrane protein is chosen from the group of membrane proteins as defined in any one of the embodiments 4
- 25 to 32.
47. Host cell genetically modified for the production of a fucosyllactose according to any one of the embodiments 44 or 45, wherein porter membrane protein is selected from MdfA from *Escherichia coli* K12 MG1655, IceT from *Escherichia coli* K12 MG1655, YnfM from *Escherichia coli* K12 MG1655, Yhhs from *Escherichia coli* K12 MG1655, EmrD from *Escherichia coli* K12 MG1655, YdhC from *Escherichia coli* K12 MG1655, YbdA from *Escherichia coli* K12 MG1655, YdeE from *Escherichia coli* K12 MG1655, MhpT from *Escherichia coli* K12 MG1655, YebQ from *Escherichia coli* K12 MG1655, YjhB from *Escherichia coli* K12 MG1655, Bcr from *Escherichia coli* K12 MG1655, FucP from *Escherichia coli* K12 MG1655, Wzx from *Escherichia coli* K12 MG1655, EmrE from *Escherichia coli* K12 MG1655, Blon_2331 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697), Blon_2332 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697), Blon_0247 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697), Blon_0245 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697), Blon_0345 from
- 30
- 35

- Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), CDT2 from *Neurospora crassa* OR74A, CDT2 from *Aspergillus oryzae* RIB40, Wzx from *Chitinophaga* sp. CF118, Wzx from *Eubacterium* sp. CAG:581, Wzx from *Dyadobacter soli* (DSM 25329), Wzx from *Lactococcus raffinolactis* (ATCC 43920), Wzx from *Prevotella ruminicola* (AR32), NAPO from *Brachyspira hampsonii* P280/1, NAm from *Actinobaculum suis* (DSM 20639), NAm from *Ruminococcus gnavus*, NAm from *Curtobacterium* sp. 314Chir4.1, NAm from *Planctomycetes bacterium* GWF2_42_9, Nap from *Niabella drilacis* (DSM25811), Nap from *Saccharicrinis fermentans* (DSM 9555), mdtD from *Citrobacter freundii* MGH152, mdtD from *Citrobacter werkmanii* NBRC 105721, mdtD from *Citrobacter amalonaticus*, mdtD from *Klebsiella oxytoca*, mdtD from *Escherichia albertii* B156, yegB from *Salmonella enterica* subsp. *Salamae*, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2, Tcr_1_D38215 from *Klebsiella pneumoniae*, mdtD from *Pseudocitrobacter faecalis*, Cmr from *Yokenella regensburgei* (ATCC43003), MdfA from *Cronobacter muytjensii*, MdfA from *Klebsiella oxytoca*, MFS from *Citrobacter koseri*, MdfA from *Escherichia marmotae*, Cmr from *Shigella flexneri*, MdfA from *Salmonella enterica* subsp. *Salamae*, Cmr from *Citrobacter youngae* (ATCC 29220), MdfA from *Citrobacter freundii*, MdfA from *Enterobacter kobei*, MdfA from *Enterobacter* sp., MdfA from *Lelliottia* sp. WB101, MdfA from *Enterobacter ludwigii* EcWSU1, Sweet-like protein from *Actinoplanes utahensis*, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246, Sweet-like protein from *Rhizobium* sp. PDC82, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711), Sweet-like protein from *Morganella morganii* IS15, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078), Sweet-like protein from *Bradyrhizobium* sp. BTAi1, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10, Sweet-like protein from *Herbaspirillum aquaticum*, Sweet-like protein from *Flavobacterium bacterium* MS024-2A, rnd-like from *Sinorhizobium medicae* WSM419, arabinose efflux from *Azospirillum brasiliense* LMG 04375 or functional homolog or functional fragment of any of the above porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins.
48. Host cell according to any one of the embodiments 44 or 45, wherein said P-P-bond-hydrolysis-driven transporter is selected from LmrA from *Lactococcus lactis* strain SRCM103457, OppF from *Escherichia coli* strain K12 MG1655, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695), Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697), LpsE from *Flavobacterium spartansii*, LpsE from *Sporomusa sphaeroides* DSM 2875, TolC from *Candidatus Planktophilia sulfonica*, TolC from *Butyrivibrio hungatei* XBD2006, MsbA from *Roseburia intestinalis* CAG:13, MsbA from *Pedobacter ginsengisoli*,

- MsbA from *Verrucomicrobia bacterium* CG1_02_43_26, Wzm from *Rhizobium* sp. Root149, Wzm from *Azospirillum brasiliense* LMG 04375, Wzm from *Escherichia coli* 113303, Wzt from *Rhizobium* sp. Root149, Wzt from *Azospirillum brasiliense* LMG 04375, Wzt from *Escherichia coli* 113303, Nodj from *Bradyrhizobium japonicum* USDA 110 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt or Nodj membrane proteins.
49. Host cell according to any one of the embodiments 44 or 45, wherein said putative transport protein is selected from Cytochrome C biogenesis protein from *Helicobacter pylori*, CutC from *Clostridium* sp. CAG: 1013, CutC from *Odoribacter splanchnicus* DSM 20712, CutC from *Mitsuaria* sp. PDC51, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706), ybjM from *Escherichia coli* K12 MG1655, ybjM from *Enterobacteriaceae bacterium* ENNIH1 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein.
50. Host cell according to any one of the embodiments 44 or 45, wherein said β -barrel porin is selected from Wza from *Escherichia coli* K12 MG1655 or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wza membrane protein.
51. Host cell according to any one of the embodiments 44 or 45, wherein said auxiliary transport protein is selected from Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099), or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wzc membrane protein.
52. Cell to be stably cultured in a medium, which cell is adjusted for the production of fucosyllactose, the cell being transformed to comprise at least one nucleic acid sequence coding for an enzyme involved in fucosyllactose synthesis, characterized in that the cell in addition comprises i) a modified expression of an endogenous membrane protein and/or ii) an expression of a heterologous membrane protein and wherein said membrane protein is as defined in any one of the embodiments 1 to 33.
53. Cell according to any one of the embodiments 43 to 52, wherein said cell is selected from the group consisting of microorganism, plant, or animal cells, preferably said microorganism is a bacterium, fungus or a yeast, preferably said plant is a rice, cotton, rapeseed, soy, maize or corn plant, preferably said animal is an insect, fish, bird or non-human mammal.
54. Host cell according to embodiment 53, wherein the cell is an *Escherichia coli* cell.
55. Cell according to any one of the embodiments 43 to 54 wherein the cell comprises a catabolic pathway for selected mono-, di- or oligosaccharides which is at least partially inactivated, the

mono-, di-, or oligosaccharides being involved in and/or required for the synthesis of fucosyllactose.

56. Cell according to any one of the embodiments 43 to 55 wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose and/or difucosyllactose.

5 57. Method for the production of fucosyllactose, comprising the steps of:

- a) providing a cell according to any one of the embodiments 43 to 56,
- b) culturing the cell in a medium under conditions permissive for the production of said fucosyllactose,
- c) separating said fucosyllactose from the cultivation.

10 58. Use of a membrane protein selected from the group membrane proteins as defined in any one of the embodiments 1 to 31 in the fermentative production of fucosyllactose.

59. Use of a cell according to any one of the embodiments 43 to 56, in a method for the production of fucosyllactose.

15 60. Use of a cell according to embodiment 59 wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose and/or difucosyllactose.

Moreover, the present invention relates to the following preferred specific embodiments:

1. Method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:

- 20 - providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic acid sequence coding for a fucosyltransferase that transfers a fucose residue from a guanosine-diphosphate fucose (GDP-fucose) donor to a lactose acceptor thereby synthesizing fucosyllactose
- said cell further comprising i) a modified expression of an endogenous membrane protein enabling and/or enhancing fucosyllactose transport and/or ii) an expression of a
25 heterologous membrane protein enabling and/or enhancing fucosyllactose transport, and wherein said membrane protein is i) selected from the group of membrane proteins comprising any one of the PFAM domains found by searching the genomic neighbourhood of GT10 and GT11 fucosyltransferase families with interpro number
30 IPR001503 and IPR002516 as defined on InterPro 75.0 released on 4th July 2019 respectively, wherein said genomic neighbourhood window size is 14 genes before and 14 genes after the respective fucosyltransferase and wherein said membrane protein is not belonging to the SET family, or ii) selected from the group of membrane proteins comprising SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218, or functional homolog
35 or functional fragment of any one of the membrane proteins comprising SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218 or a sequence having at least 80% sequence identity to any one of said membrane proteins with SEQ ID NOs 204, 206, 208, 210, 212, 214, 216,
218

- cultivating the cell in a medium under conditions permissive for the production of the desired fucosyllactose
- preferably separating the fucosyllactose from the cultivation.
2. Method according to specific embodiment 1 wherein said membrane protein is selected from the group of
- a) porters;
 - b) P-P-bond-hydrolysis-driven transporters;
 - c) β -Barrel Porins;
 - d) Auxiliary transport proteins;
 - e) Putative transport proteins; and
 - f) Phosphotransfer-driven group translocators.
3. Method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:
- providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic acid sequence coding for a fucosyltransferase that transfers a fucose residue from a GDP-fucose donor to a lactose acceptor thereby synthesizing fucosyllactose
 - said cell further comprising i) a modified expression of an endogenous membrane protein enabling and/or enhancing fucosyllactose transport and/or ii) an expression of a heterologous membrane protein enabling and/or enhancing fucosyllactose transport, and wherein said membrane protein is selected from the group of
 - a) porters and wherein said membrane protein is not belonging to the SET family;
 - b) P-P-bond-hydrolysis-driven transporters;
 - c) β -Barrel Porins;
 - d) Auxiliary transport proteins;
 - e) Putative transport proteins; and
 - f) Phosphotransfer-driven group translocators
 - cultivating the cell in a medium under conditions permissive for the production of the desired fucosyllactose
 - preferably separating the fucosyllactose from the cultivation.
4. Method according to any one of specific embodiments 2 or 3, wherein said porter is selected from the group of TCDB classes 2.A.1.1, 2.A.1.12, 2.A.1.15, 2.A.1.2, 2.A.1.3, 2.A.1.36, 2.A.1.38, 2.A.1.46, 2.A.1.68, 2.A.1.7, 2.A.1.81, 2.A.123, 2.A.2, 2.A.21, 2.A.58, 2.A.6.3, 2.A.66 and 2.A.7.1, as defined by TCDB.org as released on 17th June 2019.
5. Method according to any one of specific embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is selected from the group of TCDB classes 3.A.1.1, 3.A.1.2, 3.A.1.10, 3.A.1.11 and 3.A.1.5, as defined by TCDB.org as released on 17th June 2019.

6. Method according to any one of specific embodiments 2 or 3, wherein said β -Barrel Porin is selected from TCDB classes 1.B.3.1 and 1.B.18, as defined by TCDB.org as released on 17th June 2019.
7. Method according to any one of specific embodiments 2 or 3, wherein said Auxiliary transport protein is selected from TCDB class 8.A.3, as defined by TCDB.org as released on 17th June 2019.
8. Method according to any one of specific embodiments 2 or 3, wherein said Putative transport protein is selected from the group of TCDB classes 9.B.14 and 9.B.158, as defined by TCDB.org as released on 17th June 2019.
9. Method according to any one of specific embodiments 2 or 3, wherein said phosphotransfer-driven group translocators is selected from the group of TCDB classes 4.A.1.1 and 4.A.4.1, as defined by TCDB.org as released on 17th June 2019.
10. Method according to any one of specific embodiments 2 or 3, wherein said porter is selected from the group of eggnoG families 05BZS, 05C0R, 05C2C, 05CT4, 05CXP, 05CZQ, 05D94, 05DXI, 05E5M, 05E5W, 05E8G, 05EAM, 05EDR, 05EGZ, 05F9N, 05JHE, 05PSV, 05W2Y, 05W3H, 05XJ5, 070Q9, 07CWC, 07QF7, 07QNK, 07RBJ, 07RJ1, 07T5E, 07VQ3, 0814C, 088QT, 08H15, 08N8A, 08SC4, 08Z4Q, as defined by eggnoGdb 1.0.2 as released on 3rd Nov 2017.
11. Method according to any one of specific embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is selected from the group of eggnoG families 05BZ1, 05CJ1, 05EY8, 05HAC, 05DMK, 05DFW, 05MFV, 07FKK, 07R5U, 07V1T, 08IJ9, 08JQ7, 172T7, as defined by eggnoGdb 1.0.2 as released on 3rd Nov 2017.
12. Method according to any one of specific embodiments 2 or 3, wherein said β -Barrel Porin is selected from the group of eggnoG family 05DAY, 08KDD, as defined by eggnoGdb 1.0.2 as released on 3rd Nov 2017.
13. Method according to any one of specific embodiments 2 or 3, wherein said Auxiliary transport protein is selected from the group of eggnoG family 07SYR, as defined by eggnoGdb 1.0.2 as released on 3rd Nov 2017.
14. Method according to any one of specific embodiments 2 or 3, wherein said Putative transport protein is selected from the group of eggnoG families 05CRE, 05GWF, 06N3A, as defined by eggnoGdb 1.0.2 as released on 3rd Nov 2017.
15. Method according to any one of specific embodiments 2 or 3, wherein said phosphotransfer-driven group translocators is selected from the group of eggnoG families 05CI1 and 05VI0, as defined by eggnoGdb 1.0.2 as released on 3rd Nov 2017.
16. Method according to any one of specific embodiments 2 or 3, wherein said porter is chosen from the PFAM list of PF00083, PF00474, PF00873, PF00893, PF01895, PF01943, PF02690, PF03083, PF04193, PF05977, PF07690, PF07690, PF13347, PF13440 and PF14667, as defined by Pfam 32.0 as released on Sept 2018.

17. Method according to any one of specific embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is chosen from PFAM list PF00005, PF00532, PF00664, PF01061, PF08352, PF14524, PF13407, PF13416 and PF17912, as defined by Pfam 32.0 as released on Sept 2018.
- 5 18. Method according to any one of specific embodiments 2 or 3, wherein said β -Barrel Porin is chosen from PFAM list PF02264, PF02563, PF10531 and PF18412, as defined by Pfam 32.0 as released on Sept 2018.
19. Method according to any one of specific embodiments 2 or 3, wherein said Auxiliary transport protein is chosen from PFAM list PF13807 and PF02706, as defined by Pfam 32.0 as released on Sept 2018.
- 10 20. Method according to any one of specific embodiments 2 or 3, wherein said Putative transport protein is chosen from PFAM list PF01578, PF03932, PF05140 and PF11045, as defined by Pfam 32.0 as released on Sept 2018.
21. Method according to any one of specific embodiments 2 or 3, wherein said phosphotransfer-driven group translocators is chosen from PFAM list PF00367, PF00358, PF02378, PF03829, as defined by Pfam 32.0 as released on Sept 2018.
- 15 22. Method according to any one of specific embodiments 2 or 3, wherein said porter is chosen from the interpro list IPR000390, IPR001036, IPR001411, IPR001734, IPR001927, IPR002797, IPR003663, IPR003841, IPR004316, IPR004633, IPR004638, IPR004734, IPR004812, IPR005275, IPR005828, IPR005829, IPR006603, IPR010290, IPR011701, IPR020846, IPR023008, IPR023721, IPR023722, IPR026022, IPR027417, IPR027463, IPR029303, IPR032896, IPR036259, IPR038078, IPR038377, IPR039672, as defined by InterPro 75.0 as released on 4th July 2019.
- 20 23. Method according to any one of specific embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is chosen from interpro list IPR000412, IPR001734, IPR001761, IPR003439, IPR003593, IPR005829, IPR005978, IPR005981, IPR006059, IPR006060, IPR006061, IPR008995, IPR011527, IPR011701, IPR013456, IPR013525, IPR013563, IPR015851, IPR015855, IPR017871, IPR019554, IPR020846, IPR025997, IPR026266, IPR027417, IPR028082, IPR029439, IPR033893, IPR036259, IPR036640, IPR038377, IPR039421 and IPR040582, as defined by InterPro 75.0 as released on 4th July 2019.
- 25 30 24. Method according to any one of specific embodiments 2 or 3, wherein said β -Barrel Porin is chosen from interpro list IPR003192, IPR003715, IPR019554, IPR023738, IPR036998 and IPR040716, as defined by InterPro 75.0 as released on 4th July 2019.
25. Method according to any one of specific embodiments 2 or 3, wherein said Auxiliary transport protein is chosen from interpro list IPR003856, IPR020846, IPR027417, IPR032807 and IPR036259, as defined by InterPro 75.0 as released on 4th July 2019.
- 35 26. Method according to any one of specific embodiments 2 or 3, wherein said Putative transport protein is chosen from interpro list IPR002541, IPR003439, IPR003593, IPR004316,

IPR005627, IPR006603, IPR007816, IPR017871, IPR020368, IPR020846, IPR023648, IPR027417, IPR036259 and IPR036822, as defined by InterPro 75.0 as released on 4th July 2019.

27. Method according to any one of specific embodiments 2 or 3, wherein said phosphotransfer-driven group translocators is chosen from interpro list IPR001127, IPR001996, IPR003352, IPR004716, IPR010974, IPR011055, IPR013013, IPR018113, IPR018454, IPR036665 and IPR036878, as defined by InterPro 75.0 as released on 4th July 2019.
28. Method according to any one of specific embodiments 2 or 3, wherein said porter membrane protein is selected from MdfA from *Escherichia coli* K12 MG1655 with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, Blon_2331 from *B. longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *B. longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 42, wzx-like protein of *Chitinophaga sp. CF118* with SEQ ID NO 58, *Prevotella ruminicola* (AR32) with SEQ ID NO 66, *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, or *Dyadobacter soli* DSM 25329 with SEQ ID NO 62, or functional homolog or functional fragment of any one of the above porter membrane protein or a sequence having at least 80% sequence identity to any one of said MdfA, IceT, Blon_2331, Blon_2332 membrane proteins or wzx-like membrane protein with SEQ ID NOs 02, 06, 40, 42, 58, 66, 64 or 62, respectively.
29. Method according to any one of specific embodiments 2 or 3, wherein said P-P-bond-hydrolysis driven transporter membrane protein is selected from ImrA from *Lactococcus lactis* strain SRCM103457 with SEQ ID NO 28, LpsE membrane protein from *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 or 74, *Flavobacterium spartansii* with SEQ ID NOs 68 or 72, TolC from *Candidatus Planktophila sulfonica* with SEQ ID NO 76, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82 or *Verrucomicrobia bacterium CG1_02_43_26* with SEQ ID NO 84, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 216, or ytfQ from *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any of the above P-P-bond-hydrolysis driven transporter membrane protein or a sequence having at least 80% sequence identity to any of said ImrA, LpsE, TolC, MsbA, malE, malK, araF, xylF or ytfQ membrane protein with SEQ ID NOs 28, 70, 74, 68, 72, 76, 82, 84, 206, 208, 214, 216, or 218, respectively.
30. Method according to any one of specific embodiments 2 or 3, wherein said β -barrel porin is selected from Wza from *Escherichia coli* K12 MG1655 with SEQ ID NO 34 or lamB from *Escherichia coli* K12 MG1655 with SEQ ID NO 204 or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wza or lamB membrane proteins with SEQ ID NOs 34 or 204, respectively.

31. Method according to any one of specific embodiments 2 or 3, wherein said auxiliary transport protein is selected from Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099) with SEQ ID NO 88, or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wzc membrane protein with
5 SEQ ID NO 88.
32. Method according to any one of specific embodiments 2 or 3, wherein said putative transport protein is selected from CutC from *Clostridium* sp. CAG: 1013 with SEQ ID NO 90, *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, *Mitsuaria* sp. PDC51 with SEQ ID NO 94 or *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, or
10 functional homolog or functional fragment of any of said CutC membrane proteins or a sequence having at least 80% sequence identity to any of said CutC membrane protein with SEQ ID NOs 90, 92, 94 or 96, respectively.
33. Method according to any one of specific embodiments 2 or 3, wherein said phosphotransfer-driven group translocator is selected from nagE from *Escherichia coli* K12 MG1655 with SEQ
15 ID NO 210, srlB from *Escherichia coli* K12 MG1655 with SEQ ID NO 212 or functional homolog or functional fragment of any of said nagE or srlB membrane protein or a sequence having at least 80% sequence identity to any of said nagE or srlB membrane protein with SEQ ID NOs 210 or 212, respectively.
34. Method according to any one of specific embodiments 2 or 3, wherein said porter membrane
20 protein is selected from MdfA from *Escherichia coli* K12 MG1655 with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, YnfM from *Escherichia coli* K12 MG1655 with SEQ ID NO 04, Yhhs from *Escherichia coli* K12 MG1655 with SEQ ID NO 08, EmrD from *Escherichia coli* K12 MG1655 with SEQ ID NO 10, YdhC from *Escherichia coli* K12 MG1655 with SEQ ID NO 12, YbdA from *Escherichia coli* K12 MG1655 with SEQ ID NO
25 14, YdeE from *Escherichia coli* K12 MG1655 with SEQ ID NO 16, MhpT from *Escherichia coli* K12 MG1655 with SEQ ID NO 18, YebQ from *Escherichia coli* K12 MG1655 with SEQ ID NO 20, YjhB from *Escherichia coli* K12 MG1655 with SEQ ID NO 22, Bcr from *Escherichia coli* K12 MG1655 with SEQ ID NO 24, FucP from *Escherichia coli* K12 MG1655 with SEQ ID NO 26, Wzx from *Escherichia coli* K12 MG1655 with SEQ ID NO 32, EmrE from *Escherichia coli* K12 MG1655 with SEQ ID NO 38, Blon_2331 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 42, Blon_0247 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 46, Blon_0245 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 48, Blon_0345
30 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 50, CDT2 from *Neurospora crassa* OR74A with SEQ ID NO 52, CDT2 from *Aspergillus oryzae* RIB40 with SEQ ID NO 54, Wzx from *Chitinophaga* sp. CF118 with SEQ ID NO 58, Wzx from *Eubacterium* sp. CAG:581 with SEQ ID NO 60, Wzx from *Dyadobacter soli* (DSM 25329) with

SEQ ID NO 62, Wzx from *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, Wzx from *Prevotella ruminicola* (AR32) with SEQ ID NO 66, NAPO from *Brachyspira hamptonii* P280/1 with SEQ ID NO 86, NAm from *Actinobaculum suis* (DSM 20639) with SEQ ID NO 98, NAm from *Ruminococcus gnavus* with SEQ ID NO 100, NAm from *Curtobacterium* sp. 314Chir4.1 with SEQ ID NO 102, Nap from *Niabella drilacis* (DSM25811) with SEQ ID NO 104, Nap from *Saccharicrinis fermentans* (DSM 9555) with SEQ ID NO 106, mdtD from *Citrobacter freundii* MGH152 with SEQ ID NO 108, mdtD from *Citrobacter werkmanii* NBRC 105721 with SEQ ID NO 110, mdtD from *Citrobacter amalonaticus* with SEQ ID NO 112, mdtD from *Klebsiella oxytoca* with SEQ ID NO 114, mdtD from *Escherichia albertii* B156 with SEQ ID NO 116, yegB from *Salmonella enterica* subsp. *Salamae* with SEQ ID NO 118, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2 with SEQ ID NO 120, Tcr_1_D38215 from *Klebsiella pneumoniae* with SEQ ID NO 122, mdtD from *Pseudocitrobacter faecalis* with SEQ ID NO 124, Cmr from *Yokenella regensburgei* (ATCC43003) with SEQ ID NO 126, MdfA from *Cronobacter muytjensii* with SEQ ID NO 128, MdfA from *Klebsiella oxytoca* with SEQ ID NO130, MFS from *Citrobacter koseri* with SEQ ID NO 132, MdfA from *Escherichia marmotae* with SEQ ID NO 134, Cmr from *Shigella flexneri* with SEQ ID NO 136, MdfA from *Salmonella enterica* subsp. *Salamae* with SEQ ID NO 138, Cmr from *Citrobacter youngae* (ATCC 29220) with SEQ ID NO 140, MdfA from *Citrobacter freundii* with SEQ ID NO 142, MdfA from *Enterobacter kobei* with SEQ ID NO 144, MdfA from *Enterobacter* sp. with SEQ ID NO 146, MdfA from *Lelliottia* sp. WB101 with SEQ ID NO 148, MdfA from *Enterobacter ludwigii* EcWSU1 with SEQ ID NO 150, Sweet-like protein from *Actinoplanes utahensis* with SEQ ID NO 152, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246 with SEQ ID NO 154, Sweet-like protein from *Rhizobium* sp. PDC82 with SEQ ID NO 156, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711) with SEQ ID NO 158, Sweet-like protein from *Morganella morganii* IS15 with SEQ ID NO 160, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078) with SEQ ID NO 162, Sweet-like protein from *Bradyrhizobium* sp. BTAi1 with SEQ ID NO 164, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NO 166, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10 with SEQ ID NO 168, Sweet-like protein from *Herbaspirillum aquaticum* with SEQ ID NO 170, Sweet-like protein from *Flavobacteria bacterium* MS024-2A with SEQ ID NO 172, rnd-like from *Sinorhizobium medicae* WSM419 with SEQ ID NO 182, arabinose efflux from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 184 or functional homolog or functional fragment of any of the above porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, EmrE, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins with SEQ ID NOs 02, 06, 04, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 32, 38, 40, 42, 46, 48, 50, 52,

54, 58, 60, 62, 64, 66, 86, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 182 or 184 respectively.

35. Method according to any one of specific embodiments 2 or 3, wherein said P-P-bond-hydrolysis-driven transporter is selected from LmrA from *Lactococcus lactis* strain SRCM103457 with SEQ ID NO 28, OppF from *Escherichia coli* strain K12 MG1655 with SEQ ID NO 30, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695) with SEQ ID NO 36, Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697) with SEQ ID NO 44, LpsE from *Flavobacterium spartansii* with SEQ ID NOs 68 or 72, LpsE from *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 or 74, TolC from *Candidatus Planktophila sulfonica* with SEQ ID NO 76, TolC from *Butyrivibrio hungatei* XBD2006 with SEQ ID NO 78, MsbA from *Roseburia intestinalis* CAG:13 with SEQ ID NO 80, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26 with SEQ ID NO 84, Wzm from *Rhizobium* sp. Root149 with SEQ ID NO 174, Wzm from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 176, Wzm from *Escherichia coli* 113303 with SEQ ID NO 196, Wzt from *Rhizobium* sp. Root149 with SEQ ID NO 178, Wzt from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 180, Wzt from *Escherichia coli* 113303 with SEQ ID NO 194, Nodj from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NOs 188 or 190, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 216, or ytfQ from *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt, Nodj, malE, malK, araF, xylF or ytfQ membrane proteins with SEQ ID NOs 28, 30, 36, 44, 68, 72, 70, 74, 76, 78, 80, 82, 84, 174, 176, 196, 178, 180, 194, 188, 190, 206, 208, 214, 216 or 218, respectively.
36. Method according to any one of specific embodiments 2 or 3, wherein said putative transport protein is selected from Cytochrome C biogenesis protein from *Helicobacter pylori* with SEQ ID NO 56, CutC from *Clostridium* sp. CAG: 1013 with SEQ ID NO 90, CutC from *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, CutC from *Mitsuaria* sp. PDC51 with SEQ ID NO 94, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, ybjM from *Escherichia coli* K12 MG1655 with SEQ ID NO 190, ybjM from *Enterobacteriaceae bacterium* ENNIH1 with SEQ ID NO 192 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein with SEQ ID NOs 56, 90, 92, 94, 96, 190 or 192, respectively.

37. Method for the production of fucosyllactose by a genetically modified cell, comprising the steps of:

- providing a cell capable of producing fucosyllactose, said cell comprising at least one nucleic acid sequence coding for a fucosyltransferase that transfers a fucose residue from a GDP-fucose donor to a lactose acceptor thereby synthesizing fucosyllactose
- said cell further comprising i) a modified expression of an endogenous membrane protein enabling and/or enhancing fucosyllactose transport and/or ii) an expression of a heterologous membrane protein enabling and/or enhancing fucosyllactose transport, and wherein said membrane protein is selected from the group of membrane proteins consisting of the porter membrane proteins MdfA from *Escherichia coli* K12 MG1655 with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, YnfM from *Escherichia coli* K12 MG1655 with SEQ ID NO 04, Yhhs from *Escherichia coli* K12 MG1655 with SEQ ID NO 08, EmrD from *Escherichia coli* K12 MG1655 with SEQ ID NO 10, YdhC from *Escherichia coli* K12 MG1655 with SEQ ID NO 12, YbdA from *Escherichia coli* K12 MG1655 with SEQ ID NO 14, YdeE from *Escherichia coli* K12 MG1655 with SEQ ID NO 16, MhpT from *Escherichia coli* K12 MG1655 with SEQ ID NO 18, YebQ from *Escherichia coli* K12 MG1655 with SEQ ID NO 20, YjhB from *Escherichia coli* K12 MG1655 with SEQ ID NO 22, Bcr from *Escherichia coli* K12 MG1655 with SEQ ID NO 24, FucP from *Escherichia coli* K12 MG1655 with SEQ ID NO 26, Wzx from *Escherichia coli* K12 MG1655 with SEQ ID NO 32, EmrE from *Escherichia coli* K12 MG1655 with SEQ ID NO 38, Blon_2331 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 42, Blon_0247 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 46, Blon_0245 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 48, Blon_0345 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 50, CDT2 from *Neurospora crassa* OR74A with SEQ ID NO 52, CDT2 from *Aspergillus oryzae* RIB40 with SEQ ID NO 54, Wzx from *Chitinophaga* sp. CF118 with SEQ ID NO 58, Wzx from *Eubacterium* sp. CAG:581 with SEQ ID NO 60, Wzx from *Dyadobacter soli* (DSM 25329) with SEQ ID NO 62, Wzx from *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, Wzx from *Prevotella ruminicola* (AR32) with SEQ ID NO 66, NAPO from *Brachyspira hamptonii* P280/1 with SEQ ID NO 86, NAm from *Actinobaculum suis* (DSM 20639) with SEQ ID NO 98, NAm from *Ruminococcus gnavus* with SEQ ID NO 100, NAm from *Curtobacterium* sp. 314Chir4.1 with SEQ ID NO 102, Nap from *Niabella drilacis* (DSM25811) with SEQ ID NO 104, Nap from *Saccharicrinis fermentans* (DSM 9555) with SEQ ID NO 106, mdtD from *Citrobacter freundii* MGH152 with SEQ ID NO 108, mdtD from *Citrobacter werkmanii* NBRC 105721 with SEQ ID NO 110, mdtD from *Citrobacter amalonaticus* with SEQ ID NO 112, mdtD from *Klebsiella oxytoca* with SEQ ID NO 114,

mdtD from *Escherichia albertii* B156 with SEQ ID NO 116, yegB from *Salmonella enterica* subsp. *Salamae* with SEQ ID NO 118, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2 with SEQ ID NO 120, Tcr_1_D38215 from *Klebsiella pneumoniae* with SEQ ID NO 122, mdtD from *Pseudocitrobacter faecalis* with SEQ ID NO 124, Cmr from *Yokenella regensburgei* (ATCC43003) with SEQ ID NO 126, MdfA from *Cronobacter muytjensii* with SEQ ID NO 128, MdfA from *Klebsiella oxytoca* with SEQ ID NO 130, MFS from *Citrobacter koseri* with SEQ ID NO 132, MdfA from *Escherichia marmotae* with SEQ ID NO 134, Cmr from *Shigella flexneri* with SEQ ID NO 136, MdfA from *Salmonella enterica* subsp. *Salamae* with SEQ ID NO 138, Cmr from *Citrobacter youngae* (ATCC 29220) with SEQ ID NO 140, MdfA from *Citrobacter freundii* with SEQ ID NO 142, MdfA from *Enterobacter kobei* with SEQ ID NO 144, MdfA from *Enterobacter* sp. with SEQ ID NO 146, MdfA from *Lelliottia* sp. WB101 with SEQ ID NO 148, MdfA from *Enterobacter ludwigii* EcWSU1 with SEQ ID NO 150, Sweet-like protein from *Actinoplanes utahensis* with SEQ ID NO 152, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246 with SEQ ID NO 154, Sweet-like protein from *Rhizobium* sp. PDC82 with SEQ ID NO 156, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711) with SEQ ID NO 158, Sweet-like protein from *Morganella morganii* IS15 with SEQ ID NO 160, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078) with SEQ ID NO 162, Sweet-like protein from *Bradyrhizobium* sp. BTai1 with SEQ ID NO 164, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NO 166, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10 with SEQ ID NO 168, Sweet-like protein from *Herbaspirillum aquaticum* with SEQ ID NO 170, Sweet-like protein from *Flavobacterium bacterium* MS024-2A with SEQ ID NO 172, rnd-like from *Sinorhizobium medicae* WSM419 with SEQ ID NO 182, arabinose efflux from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 184 or functional homolog or functional fragment of any of the above porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, Wzx, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins with SEQ ID NOs 02, 06, 04, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 32, 38, 40, 42, 46, 48, 50, 52, 54, 58, 60, 62, 64, 66, 86, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 182 or 184 respectively.; and the P-P-bond-hydrolysis-driven transporters LmrA from *Lactococcus lactis* strain SRCM103457 with SEQ ID NO 28, OppF from *Escherichia coli* strain K12 MG1655 with SEQ ID NO 30, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695) with SEQ ID NO 36, Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC

15697) with SEQ ID NO 44, LpsE from *Flavobacterium spartansii* with SEQ ID NOs 68 or 72, LpsE from *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 or 74, TolC from *Candidatus Planktophila sulfonica* with SEQ ID NO 76, TolC from *Butyrivibrio hungatei* XBD2006 with SEQ ID NO 78, MsbA from *Roseburia intestinalis* CAG:13 with
5 SEQ ID NO 80, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26 with SEQ ID NO 84, Wzm from *Rhizobium* sp. Root149 with SEQ ID NO 174, Wzm from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 176, Wzm from *Escherichia coli* 113303 with SEQ ID NO 196, Wzt from *Rhizobium* sp. Root149 with SEQ ID NO 178, Wzt from *Azospirillum brasiliense* LMG
10 04375 with SEQ ID NO 180, Wzt from *Escherichia coli* 113303 with SEQ ID NO 194, NodJ from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NOs 188 or 190, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 216, or ytfQ from
15 *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt, NodJ, malE, malK, araF, xylF or ytfQ membrane proteins with SEQ ID NOs 28, 30, 36, 44, 68, 72, 70,
20 74, 76, 78, 80, 82, 84, 174, 176, 196, 178, 180, 194, 188, 190, 206, 208, 214, 216, or 218, respectively.; and a β -barrel porin membrane protein Wza from *Escherichia coli* K12 MG1655 with SEQ ID NO 34 or lamB from *Escherichia coli* K12 MG1655 with SEQ ID NO 204 or functional homolog or functional fragment of any one of said Wza or lamB protein or a sequence having at least 80% sequence identity to any one of said Wza or
25 lamB membrane protein with SEQ ID NO 34 or 204, respectively; and auxiliary transport protein Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099) with SEQ ID NO 88, or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wzc membrane protein with SEQ ID NO 88; putative transport proteins Cytochrome C biogenesis protein from *Helicobacter pylori* with SEQ ID NO 56, CutC from *Clostridium* sp. CAG: 1013 with SEQ ID NO 90, CutC from *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, CutC from *Mitsuaria* sp. PDC51 with SEQ ID NO 94, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, ybjM from *Escherichia coli* K12 MG1655 with SEQ ID
30 NO 190, ybjM from *Enterobacteriaceae bacterium* ENNIH1 with SEQ ID NO 192 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein with SEQ ID NOs 56, 90, 92, 94, 96, 190 or 192, respectively; and phosphotransfer-driven group translocators nagE

from *Escherichia coli* K12 MG1655 with SEQ ID NO 210, srlB from *Escherichia coli* K12 MG1655 with SEQ ID NO 212 or functional homolog or functional fragment of any of said nagE or srlB membrane protein or a sequence having at least 80% sequence identity to any one of said nagE or srlB membrane protein with SEQ ID NOs 210 or 212, respectively.

38. Method for the production of fucosyllactose according to any one of the previous specific embodiments, wherein said membrane protein is a transporter protein involved in transport of compounds across the outer membrane of the cell wall.

39. Method for the production of fucosyllactose according to any one of the previous specific embodiments, the method further comprising at least one of the following steps:

i) adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per litre of initial reactor volume wherein the reactor volume ranges from 250 mL to 10.000 m³, preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said lactose feed;

ii) adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution;

iii) adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/L, preferably 75 g/L, more preferably 100 g/L, more preferably 125 g/L, more preferably 150 g/L, more preferably 175 g/L, more preferably 200 g/L, more preferably 225 g/L, more preferably 250 g/L, more preferably 275 g/L, more preferably 300 g/L, more preferably 325 g/L, more preferably 350 g/L, more preferably 375 g/L, more preferably, 400 g/L, more preferably 450 g/L, more preferably 500 g/L, even more preferably, 550 g/L, most preferably 600 g/L; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;

said method resulting in a fucosyllactose concentration of at least 50 g/L, preferably at least 75 g/L, more preferably at least 90 g/L, more preferably at least 100 g/L, more preferably at least 125 g/L, more preferably at least 150 g/L, more preferably at least 175 g/L, more preferably at least 200 g/L in the final volume of said culture medium.

40. The method of specific embodiment 39, wherein the lactose feed is accomplished by adding lactose from the beginning of the cultivating in a concentration of at least 5 mM, preferably in a concentration of 30, 40, 50, 60, 70, 80, 90, 100, 150 mM, more preferably in a concentration > 300 mM.

41. The method of any one of the specific embodiments 39 or 40, wherein said lactose feed is accomplished by adding lactose to the cultivation medium in a concentration, such, that throughout the production phase of the cultivation a lactose concentration of at least 5 mM, preferably 10 mM or 30 mM is obtained.
- 5 42. The method of any of the specific embodiments 39, 40 or 41, wherein the host cells are cultivated for at least about 60, 80, 100, or about 120 hours or in a continuous manner.
43. The method of any one of specific embodiments 39 to 42, wherein a carbon and energy source, preferably sucrose, glucose, fructose, glycerol, maltose, maltodextrines, trehalose, polyols, starch, succinate, malate, pyruvate, lactate, ethanol, citrate, lactose, is also added, preferably continuously to the culture medium, preferably with the lactose.
- 10 44. The method of any one of specific embodiments 39 to 43, wherein a first phase of exponential cell growth is provided by adding a carbon-based substrate, preferably glucose or sucrose, to the culture medium before the lactose is added to the culture medium in a second phase.
45. Method according to any one of the specific embodiments 1 to 44, wherein the method is producing a mixture of fucosyllactoses.
- 15 46. Method according to any one of specific embodiments 1 to 45, wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose and/or difucosyllactose.
47. Method according to any one of specific embodiment 1 to 46, wherein said genetically modified cell is selected from the group consisting of microorganism, plant, or animal cells, preferably said microorganism is a bacterium, fungus or a yeast, preferably said plant is a rice, cotton, rapeseed, soy, maize or corn plant, preferably said animal is an insect, fish, bird or non-human mammal.
- 20 48. Method according to specific embodiment 47, wherein the cell is an *Escherichia coli* cell.
49. Host cell genetically modified for the production of a fucosyllactose, wherein the host cell comprises at least one nucleic acid sequence coding for a fucosyltransferase that transfers a fucose residue from a GDP-fucose donor to a lactose acceptor thereby synthesizing fucosyllactose - said cell further comprising i) a modified expression of an endogenous membrane protein enabling and/or enhancing fucosyllactose transport and/or ii) an expression of a heterologous membrane protein enabling and/or enhancing fucosyllactose transport, and wherein said membrane protein is i) selected from the group of membrane proteins comprising any one of the PFAM domains found by searching the genomic neighbourhood of GT10 and GT11 fucosyltransferase families with interpro number IPR001503 and IPR002516 as defined on InterPro 75.0 released on 4th July 2019 respectively, wherein said genomic neighbourhood window size is 14 genes before and 14 genes after the respective fucosyltransferase and wherein said membrane protein is not belonging to the SET family, or ii) selected from the group of membrane proteins comprising SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218, or functional homolog or functional fragment of any one of the membrane proteins comprising SEQ ID NOs 204, 206, 208, 210,
- 25 30 35

212, 214, 216, 218 or a sequence having at least 80% sequence identity to any one of said membrane proteins with SEQ ID NOs 204, 206, 208, 210, 212, 214, 216, 218.

50. Host cell according to specific embodiment 49, wherein said membrane protein is selected from the group of

- 5 a) porters;
- b) P-P-bond-hydrolysis-driven transporters;
- c) β -Barrel Porins;
- d) Auxiliary transport proteins;
- e) Putative transport proteins; and
- 10 f) Phosphotransfer-driven group translocators.

51. Host cell genetically modified for the production of a fucosyllactose, wherein the host cell comprises at least one nucleic acid sequence coding for a fucosyltransferase that transfers a fucose residue from a GDP-fucose donor to a lactose acceptor thereby synthesizing fucosyllactose

- 15 - said cell further comprising i) a modified expression of an endogenous membrane protein enabling and/or enhancing fucosyllactose transport and/or ii) an expression of a heterologous membrane protein enabling and/or enhancing fucosyllactose transport, and wherein said membrane protein is selected from the group of
 - a) porters and wherein said membrane protein is not belonging to the SET family;
 - 20 b) P-P-bond-hydrolysis-driven transporters;
 - c) β -Barrel Porins;
 - d) Auxiliary transport proteins;
 - e) Putative transport proteins; and
 - f) Phosphotransfer-driven group translocators.

25 52. Host cell according to any one of the specific embodiments 50 or 51, wherein said membrane protein is chosen from the group of membrane proteins as defined in any one of the specific embodiments 4 to 38.

53. Host cell genetically modified for the production of a fucosyllactose according to any one of the specific embodiments 50 or 51, wherein porter membrane protein is selected from MdfA from *Escherichia coli* K12 MG1655 with SEQ ID NO 02, IceT from *Escherichia coli* K12 MG1655 with SEQ ID NO 06, YnfM from *Escherichia coli* K12 MG1655 with SEQ ID NO 04, Yhhs from *Escherichia coli* K12 MG1655 with SEQ ID NO 08, EmrD from *Escherichia coli* K12 MG1655 with SEQ ID NO 10, YdhC from *Escherichia coli* K12 MG1655 with SEQ ID NO 12, YbdA from *Escherichia coli* K12 MG1655 with SEQ ID NO 14, YdeE from *Escherichia coli* K12 MG1655 with SEQ ID NO 16, MhpT from *Escherichia coli* K12 MG1655 with SEQ ID NO 18, YebQ from *Escherichia coli* K12 MG1655 with SEQ ID NO 20, YjhB from *Escherichia coli* K12 MG1655 with SEQ ID NO 22, Bcr from *Escherichia coli* K12 MG1655 with SEQ ID NO 24, FucP from *Escherichia coli* K12 MG1655 with SEQ ID NO 26, Wzx from *Escherichia coli*

K12 MG1655 with SEQ ID NO 32, EmrE from *Escherichia coli* K12 MG1655 with SEQ ID NO 38, Blon_2331 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 40, Blon_2332 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 42, Blon_0247 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 46, Blon_0245 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 48, Blon_0345 from *Bifidobacterium longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 50, CDT2 from *Neurospora crassa* OR74A with SEQ ID NO 52, CDT2 from *Aspergillus oryzae* RIB40 with SEQ ID NO 54, Wzx from *Chitinophaga* sp. CF118 with SEQ ID NO 58, Wzx from *Eubacterium* sp. CAG:581 with SEQ ID NO 60, Wzx from *Dyadobacter soli* (DSM 25329) with SEQ ID NO 62, Wzx from *Lactococcus raffinolactis* (ATCC 43920) with SEQ ID NO 64, Wzx from *Prevotella ruminicola* (AR32) with SEQ ID NO 66, NAPO from *Brachyspira hampsonii* P280/1 with SEQ ID NO 86, NAm from *Actinobaculum suis* (DSM 20639) with SEQ ID NO 98, NAm from *Ruminococcus gnavus* with SEQ ID NO 100, NAm from *Curtobacterium* sp. 314Chir4.1 with SEQ ID NO 102, Nap from *Niabella drilacis* (DSM25811) with SEQ ID NO 104, Nap from *Saccharicrinis fermentans* (DSM 9555) with SEQ ID NO 106, mdtD from *Citrobacter freundii* MGH152 with SEQ ID NO 108, mdtD from *Citrobacter werkmanii* NBRC 105721 with SEQ ID NO 110, mdtD from *Citrobacter amalonaticus* with SEQ ID NO 112, mdtD from *Klebsiella oxytoca* with SEQ ID NO 114, mdtD from *Escherichia albertii* B156 with SEQ ID NO 116, yegB from *Salmonella enterica subsp. Salamae* with SEQ ID NO 118, mdtD from *Klebsiella pneumoniae* 30684/NJST258_2 with SEQ ID NO 120, Tcr_1_D38215 from *Klebsiella pneumoniae* with SEQ ID NO 122, mdtD from *Pseudocitrobacter faecalis* with SEQ ID NO 124, Cmr from *Yokenella regensburgei* (ATCC43003) with SEQ ID NO 126, MdfA from *Cronobacter muytjensii* with SEQ ID NO 128, MdfA from *Klebsiella oxytoca* with SEQ ID NO130, MFS from *Citrobacter koseri* with SEQ ID NO 132, MdfA from *Escherichia marmotae* with SEQ ID NO 134, Cmr from *Shigella flexneri* with SEQ ID NO 136, MdfA from *Salmonella enterica subsp. Salamae* with SEQ ID NO 138, Cmr from *Citrobacter youngae* (ATCC 29220) with SEQ ID NO 140, MdfA from *Citrobacter freundii* with SEQ ID NO 142, MdfA from *Enterobacter kobei* with SEQ ID NO 144, MdfA from *Enterobacter* sp. with SEQ ID NO 146, MdfA from *Lelliottia* sp. WB101 with SEQ ID NO 148, MdfA from *Enterobacter ludwigii* EcWSU1 with SEQ ID NO 150, Sweet-like protein from *Actinoplanes utahensis* with SEQ ID NO 152, Sweet-like protein from *Chitinophagaceae bacterium* PMG_246 with SEQ ID NO 154, Sweet-like protein from *Rhizobium* sp. PDC82 with SEQ ID NO 156, Sweet-like protein from *Kineococcus rhizosphaerae* (DSM 19711) with SEQ ID NO 158, Sweet-like protein from *Morganella morganii* IS15 with SEQ ID NO 160, Sweet-like protein from *Geodermatophilus obscurus* (strain ATCC 25078) with SEQ ID NO 162, Sweet-like protein from *Bradyrhizobium* sp. BTAi1 with SEQ ID NO 164, Sweet-like protein from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NO 166, Sweet-like protein from *Xanthomonas campestris* pv. *vesicatoria* str. 85-10 with SEQ ID NO 168, Sweet-like

- protein from *Herbaspirillum aquaticum* with SEQ ID NO 170, Sweet-like protein from *Flavobacterium bacterium* MS024-2A with SEQ ID NO 172, rnd-like from *Sinorhizobium medicae* WSM419 with SEQ ID NO 182, arabinose efflux from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 184 or functional homolog or functional fragment of any of the above
- 5 porter membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said MdfA, IceT, YnfM, Yhhs, EmrD, YdhC, YbdA, YdeE, MhpT, YebQ, YjhB, Bcr, FucP, Wzx, EmrE, Wzx, Blon_2331, Blon_2232, Blon_0247, Blon_0245, Blon_0345, NAPO, NAm, Nap, mdtD, YegB, Tcr_1_D38215, cmr, MFS, CDT2, rnd, Sweet-like or arabinose efflux membrane proteins with SEQ ID NOs 02, 06, 04, 08, 10, 12, 14, 16,
- 10 18, 20, 22, 24, 26, 32, 38, 40, 42, 46, 48, 50, 52, 54, 58, 60, 62, 64, 66, 86, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 182 or 184 respectively.
54. Host cell according to any one of the specific embodiments 50 or 51, wherein said P-P-bond-
- 15 hydrolysis-driven transporter is selected from LmrA from *Lactococcus lactis* strain SRCM103457 with SEQ ID NO 28, OppF from *Escherichia coli* strain K12 MG1655 with SEQ ID NO 30, Wzk from *Helicobacter pylori* (strain ATCC 700392 / 26695) with SEQ ID NO 36, Blon_2475 from *Bifidobacterium longum* subsp. *Infantis* (strain ATCC 15697) with SEQ ID NO 44, LpsE from *Flavobacterium spartansii* with SEQ ID NOs 68 or 72, LpsE from
- 20 *Sporomusa sphaeroides* DSM 2875 with SEQ ID NOs 70 or 74, TolC from *Candidatus Planktophila sulfonica* with SEQ ID NO 76, TolC from *Butyrivibrio hungatei* XBD2006 with SEQ ID NO 78, MsbA from *Roseburia intestinalis* CAG:13 with SEQ ID NO 80, MsbA from *Pedobacter ginsengisoli* with SEQ ID NO 82, MsbA from *Verrucomicrobia bacterium* CG1_02_43_26 with SEQ ID NO 84, Wzm from *Rhizobium* sp. Root149 with SEQ ID NO 174,
- 25 Wzm from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 176, Wzm from *Escherichia coli* 113303 with SEQ ID NO 196, Wzt from *Rhizobium* sp. Root149 with SEQ ID NO 178, Wzt from *Azospirillum brasiliense* LMG 04375 with SEQ ID NO 180, Wzt from *Escherichia coli* 113303 with SEQ ID NO 194, Nodj from *Bradyrhizobium japonicum* USDA 110 with SEQ ID NOs 188 or 190, malE from *Escherichia coli* K-12 MG1655 with SEQ ID NO 206, malK
- 30 from *Escherichia coli* K-12 MG1655 with SEQ ID NO 208, araF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 214, xylF from *Escherichia coli* K-12 MG1655 with SEQ ID NO 216, or ytfQ from *Escherichia coli* K-12 MG1655 with SEQ ID NO 218 or functional homolog or functional fragment of any one of the above P-P-bond-hydrolysis-driven transporter
- 35 membrane protein or a protein having an amino acid sequence having at least 80% sequence identity to any of said LmrA, OppF, Wzk, Blon_2475, LpsE, TolC, MsbA, Wzm, Wzt, Nodj, malE, malK, araF, xylF or ytfQ membrane proteins with SEQ ID NOs 28, 30, 36, 44, 68, 72, 70, 74, 76, 78, 80, 82, 84, 174, 176, 196, 178, 180, 194, 188, 190, 206, 208, 214, 216, or 218 respectively.

55. Host cell according to any one of the specific embodiments 50 or 51, wherein said putative transport protein is selected from Cytochrome C biogenesis protein from *Helicobacter pylori* with SEQ ID NO 56, CutC from *Clostridium* sp. CAG: 1013 with SEQ ID NO 90, CutC from *Odoribacter splanchnicus* DSM 20712 with SEQ ID NO 92, CutC from *Mitsuaria* sp. PDC51 with SEQ ID NO 94, CutC from *Prevotella intermedia* ATCC 25611 (DSM 20706) with SEQ ID NO 96, ybjM from *Escherichia coli* K12 MG1655 with SEQ ID NO 190, ybjM from *Enterobacteriaceae bacterium* ENNIH1 with SEQ ID NO 192 or functional homolog or functional fragment of any one of the above putative transport protein or protein having an amino acid sequence having at least 80% sequence identity to any one of said CytC, CutC or ybjM membrane protein with SEQ ID NOs 56, 90, 92, 94, 96, 190 or 192, respectively.
56. Host cell according to any one of the specific embodiments 50 or 51, wherein said β -barrel porin is selected from Wza from *Escherichia coli* K12 MG1655 with SEQ ID NO 34 or lamB from *Escherichia coli* K12 MG1655 with SEQ ID NO 204 or functional homolog or functional fragment to any one of said Wza or lamB protein or a sequence having at least 80% sequence identity to any one of said Wza or lamB membrane protein with SEQ ID NO 34 or 204, respectively.
57. Host cell according to any one of the specific embodiments 50 or 51, wherein said auxiliary transport protein is selected from Wzc from *Thermotoga maritima* (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099) with SEQ ID NO 88, or functional homolog or functional fragment thereof or a sequence having at least 80% sequence identity to said Wzc membrane protein with SEQ ID NO 88.
58. Host cell according to any one of the specific embodiments 50 or 51, wherein said phosphotransfer-driven group translocators is selected from nagE from *Escherichia coli* K12 MG1655 with SEQ ID NO 210 or srlB from *Escherichia coli* K12 MG1655 with SEQ ID NO 212 or functional homolog or functional fragment of any of said nagE or srlB membrane protein or a sequence having at least 80% sequence identity to any one of said nagE or srlB membrane protein with SEQ ID NOs 210 or 212, respectively.
59. Cell according to any one of the previous specific embodiments 49 to 58, wherein said membrane protein is a transporter protein involved in transport of compounds across the outer membrane of the cell wall.
60. Cell according to any one of the specific embodiments 49 to 59, wherein the cell is stably cultured in a medium.
61. Cell according to any one of the specific embodiments 49 to 60, wherein said cell is selected from the group consisting of microorganism, plant, or animal cells, preferably said microorganism is a bacterium, fungus or a yeast, preferably said plant is a rice, cotton, rapeseed, soy, maize or corn plant, preferably said animal is an insect, fish, bird or non-human mammal.
62. Host cell according to specific embodiment 61, wherein the cell is an *Escherichia coli* cell.

63. Cell according to any one of the specific embodiments 49 to 62 wherein the cell comprises a catabolic pathway for selected mono-, di- or oligosaccharides which is at least partially inactivated, the mono-, di-, or oligosaccharides being involved in and/or required for the synthesis of fucosyllactose.
- 5 64. Cell according to any one of the specific embodiments 49 to 63 wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose or difucosyllactose.
65. Method for the production of fucosyllactose, comprising the steps of:
- a) providing a cell according to any one of the specific embodiments 49 to 64,
 - b) culturing the cell in a medium under conditions permissive for the production of said
 - 10 fucosyllactose,
 - c) separating said fucosyllactose from the cultivation.
66. Use of a membrane protein selected from the group of membrane proteins as defined in any one of the specific embodiments 1 to 38, for fucosyllactose transport, in the fermentative production of fucosyllactose.
- 15 67. Use of a cell according to any one of the specific embodiments 49 to 64, for the production of fucosyllactose.
68. Use of a cell according to specific embodiment 67 wherein said fucosyllactose is 2'-fucosyllactose, 3-fucosyllactose or difucosyllactose.
- 20 The following drawings and examples will serve as further illustration and clarification of the present invention and are not intended to be limiting.

Description of the figures

Figure 1: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 58 till SEQ ID NO 96 (excl SEQ ID NO 90) in TU2, with SEQ ID NO 90 in TU3 or with SEQ ID NO 02 till SEQ ID NO 44 in TU10 and expressing an α 1,3-fucosyltransferase. Strains with membrane proteins SEQ ID NO 04 till SEQ ID NO 34 produce 3-FL from FT1, whereas strains with membrane proteins with SEQ ID NO 02 and with SEQ ID NO 40 till SEQ ID NO 96 produce 3-FL from FT2. The CPI data refer to 3-FL measurements in whole

25 broth samples. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

30

Figure 2: 3-FL export ratio in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 58 till SEQ ID NO 104 (excluding SEQ ID NO 90) in TU2, SEQ ID NO 90 in TU3 or SEQ ID NO 02 till SEQ ID NO 34 in TU10 and expressing an α 1,3-fucosyltransferase. Strains with membrane proteins with SEQ ID NO 08 till SEQ ID NO 30 produce 3-FL from FT1, whereas strains with membrane proteins with SEQ ID NO 58 till SEQ ID NO 104 produce 3-FL from FT2. The strain with the membrane protein with SEQ ID NO 02

35

was tested in combination with either FT1 or FT2. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 3: Growth speed in relative percentages (%) obtained in a growth experiment with strains expressing the membrane proteins with SEQ ID NO 08, 14, 18 or 22 in TU10 and expressing the α 1,3-fucosyl transferase FT1. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 4: 3-FL export ratio in relative percentages (%) obtained in a growth experiment with a strain expressing the membrane protein with SEQ ID NO 28 in TU10 and expressing the α 1,3-fucosyl transferase FT1. The growth experiment was performed in minimal medium supplemented with 45 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 5: CPI in relative percentages (%) obtained in a growth experiment with the strain expressing the membrane protein with SEQ ID NO 28 in TU10 and expressing the α 1,3-fucosyl transferase FT1. The CPI data refer to 3-FL measurements in whole broth samples. The growth experiment was performed in minimal medium supplemented with 90 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 6: 3FL export ratio in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 10 or 16 in TU10 and expressing the α 1,3-fucosyl transferase FT1. The growth experiment was performed in minimal medium supplemented with 90 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 7: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 10, 16 or 28 in TU10 and expressing the α 1,3-fucosyl transferase FT1. The growth experiment was performed in minimal medium supplemented with 100 g/L sucrose and 90 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 8: Growth speed in relative percentages (%) obtained in a growth experiment with a strain expressing the membrane protein with SEQ ID NO 28 in TU10 and the α 1,3-fucosyl transferase FT1. The growth experiment was performed in minimal medium supplemented with 100 g/L sucrose and 90 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 9: CPI in relative percentages (%) obtained in a growth experiment with the strain expressing the membrane protein with SEQ ID NO 22 in TU10 and the α 1,3-fucosyl transferase FT1. The growth experiment was performed in minimal medium supplemented with 5 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 10: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 02 or 28 in TU10 from the host's genome and expressing an α 1,3-fucosyl transferase being either FT1 or FT2 from plasmid. Hereby, the gene with SEQ ID NO 01 was integrated in the EcLdhA locus, the gene with SEQ ID NO 27 was integrated in the EcSetA locus. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 11: 3-FL export ratio in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 02 or 28 in TU10 from the host's genome and expressing the α 1,3-fucosyl transferase FT2 from plasmid. Hereby, the gene with SEQ ID NO 01 was integrated in the EcLdhA locus, the gene with SEQ ID NO 27 was integrated in the EcSetA locus. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 12: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 02, 06, 10, 16, 22, 28, 32, 34, 36, 38, 40, 42, 44 or 50 in different transcriptional units (TU) from plasmid and expressing the α 1,3-fucosyl transferase FT2 from plasmid. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 13: 3-FL export ratio in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 02, 06, 10, 16, 22, 28, 32, 34, 36, 38, 40, 42, 44 or 50 in different transcriptional units (TU) from plasmid, and expressing the α 1,3-fucosyl transferase FT2 from plasmid. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 14: CPI (left panel) and 3-FL export ratio (right panel) in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 40, 42, 46 or 48, either cloned as single genes in TU10 or cloned in their native transcriptional operon structure containing 2 membrane protein genes and presented on plasmid, and expressing the α 1,3-fucosyl transferase FT2 from plasmid. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 15: CPI for the hosts with 2'FL (panel A) or DiFL (panel B) production and DiFL export ratio (panel C) in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 02, 06 or 28 in TU10, being integrated in the host's genome in the EcSetA locus (for membrane protein with SEQ ID NO 28) or in the EcLdhA locus (for membrane protein with SEQ ID NO 02 and 06) and expressing the α 1,2-fucosyl transferase FT3

from plasmid. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 2'-FL and DiFL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 16: Productivity parameters enhanced in the batch and fed-batch phase measured in 8 independent fermentation runs that were performed with an 3-FL E. coli production host over-expressing the membrane protein with SEQ ID NO 02 from genome and the α 1,3-fucosyl transferase FT2 from plasmid. The dashed horizontal line indicates the setpoint to which all adaptations were normalized. A reference fermentation was performed with an identical strain lacking the over-expression cassette of the membrane protein gene. Fermentations were performed as described in Example 3. CPI, cell performance index (g 3-FL / g biomass); br, whole broth; sn, supernatant; Qp, specific productivity (g 3-FL / g biomass / h); Qs, specific productivity (g sucrose/ g biomass / h); Ys, yield on sucrose (g 3-FL / g sucrose); Yx, biomass yield (g biomass / g sucrose); rate, production rate (g 3-FL / L / h); lac_rate, lactose conversion rate (g lactose consumed / h).

Figure 17: Productivity parameters enhanced in the batch and fed-batch phase measured in a fermentation run that was performed with an 3-FL E. coli production host over-expressing the membrane protein with SEQ ID NO 06 from a first plasmid and the α 1,3-fucosyl transferase FT2 from a second plasmid. The dashed horizontal line indicates the setpoint to which all adaptations were normalized. A specific reference fermentation was performed with an identical strain lacking the membrane protein gene. Fermentations were performed as described in Example 3. CPI, cell performance index (g 3-FL / g biomass); br, whole broth; sn, supernatant; Ys, yield on sucrose (g 3-FL / g sucrose); Yx, biomass yield (g biomass / g sucrose).

Figure 18: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 02, 06, 120, 126, 128, 140, 146 or 150 and expressing α 1,3-fucosyl transferase FT1 (for membrane proteins with SEQ ID NO 02 and SEQ ID NO 06) or FT2 (for the other membrane proteins) from plasmid. Membrane proteins with SEQ ID NO 02 and SEQ ID NO 06 were cloned in TU10. The membrane protein with SEQ ID NO 126 was cloned in TU2. Membrane proteins with SEQ ID NO 120, 140 and 150 were cloned in TU3. The membrane proteins with SEQ ID NO 128 and SEQ ID NO 146 were cloned either in TU2 (version v1) or TU3 (version v2). The CPI data refer to 3-FL measurements in whole broth samples. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 19: 3-FL export ratio in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 126 and SEQ ID NO 128 (version v1) cloned in TU2, SEQ ID NO 128 (version 2) cloned in TU3 and SEQ ID NO 02 in TU10 and expressing α 1,3-fucosyl transferase FT1 (for strain with membrane protein with SEQ ID NO 02) or FT2 (for strains with membrane proteins with SEQ ID NO 126 and 128) from plasmid. The

growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 20: Growth speed in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 120 and 140 in TU3 and expressing the α 1,3-fucosyl transferase FT2 from plasmid. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 21: MATGAT table of example 20 relating to EcMdfA.

Figure 22: MATGAT table of example 20 relating to EclceT.

Figure 23: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 54 cloned in TU1, with SEQ ID NO 62, 66, 70, 76, 84, 92, 96, 104 cloned in TU2, with SEQ ID NO 58, 64, 72, 74, 94 cloned in TU3, with SEQ ID NO 184, 204, 208 cloned in TU11, with SEQ ID NO 52, 56, 60, 80, 82, 88, 90, 98 cloned in TU12 and expressing the α 1,3-fucosyltransferase FT2. The CPI data refer to 3-FL measurements in whole broth samples. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 3-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 24: CPI in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 204 or 214 cloned in TU11 and expressing the α 1,2-fucosyltransferase FT4. The CPI data refer to 2'-FL measurements in whole broth samples. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 2'-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Figure 25: 2'-FL export ratio in relative percentages (%) obtained in a growth experiment with strains expressing membrane proteins with SEQ ID NO 206, 208, 214, 216, 218 cloned in TU11 and expressing the α 1,2-fucosyltransferase FT4. The growth experiment was performed in minimal medium supplemented with 20 g/L lactose as precursor for 2'-FL. The dashed horizontal line indicates the setpoint to which all adaptations were normalized.

Examples

Example 1: Identification of membrane protein families

An HMM is a probabilistic model called profile hidden Markov models. It characterizes a set of aligned proteins into a position-specific scoring system. Amino acids are given a score at each position in the sequence alignment according to the frequency by which they occur (Eddy, S.R.1998. Profile hidden Markov models. Bioinformatics. 14: 755-63). HMMs have wide utility, as is clear from the numerous databases that use this method for protein classification, including Pfam, InterPro, SMART, TIGRFAM, PIRSF, PANTHER, SFLD, Superfamily and Gene3D.

HMMsearch from the HMMER package 3.2.1 (<http://hmmer.org/>) as released on 13th June 2019 can use this HMM to search sequence databases for sequence homologs. Sequence databases that can be used are for example, but not limited to: the NCBI nr Protein Database (NR; <https://www.ncbi.nlm.nih.gov/protein>), UniProt Knowledgebase (UniProtKB, <https://www.uniprot.org/help/uniprotkb>) and the SWISS-PROT database (https://web.expasy.org/docs/swiss-prot_guideline.html).

Membrane protein families were classified based on the eggNOG database 1.0.2 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6324079/>; <http://eggnoget.org/>) as released on 3rd Nov 2017, the TCDB database (<http://www.tcdb.org/public/tcdb>) as released on 17th June 2019, InterPro 75.0 (<https://www.ebi.ac.uk/interpro/>) as released on 4th July 2019 and PFAM domains using Pfam 32.0 (<https://pfam.xfam.org/>) as released on Sept 2018. The eggNOG database is a public database of orthology relationships, gene evolutionary histories and functional annotations. The Transporter Classification DataBase (TCDB) is analogous to the Enzyme Commission (EC) system for classifying enzymes and incorporates both functional and phylogenetic information. The Pfam and InterPro databases are a large collection of protein families. Other protein domains like SMART (<http://smart.embl-heidelberg.de/>), TIGRFAM (<https://www.jcvi.org/tigrfams>), PIRSF (<https://proteininformationresource.org/pirwww/dbinfo/pirsf.shtml>), PANTHER (<http://pantherdb.org/>), SFLD (<http://sfld.rbvi.ucsf.edu/archive/django/index.html>), Superfamily (<http://supfam.org/>) and Gene3D (<http://gene3d.biochem.ucl.ac.uk/Gene3D/>), NCBI Conserved Domains (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) can also be used.

Identification of eggNOG families was done by using a standalone version of eggNOG-mapper (<https://github.com/eggnoget/eggnoget-mapper>) based on eggnoget 1.0.2 as released on 3rd Nov 2017. For each of the eggNOG families an HMM can be downloaded on the eggNOG website and can be used for HMMsearch to the protein databases.

Identification of the TCDB family was done by blasting (blastp) to the TCDB database as released on 17th June 2019. New members of the obtained family can be retrieved on the website (<http://www.tcdb.org/download.php>). Fasta files can be used as input in blastp to the protein databases.

Identification of the PFAM domains was done by an online search on <https://pfam.xfam.org/search#tabview=tab1> as released on Sept 2018. The HMM for the obtained family was downloaded in 'Curation & model'. HMMsearches with this model to the protein databases will identify new family members. Sequences comprising the InterPro hit can also be downloaded from the PFAM website.

Identification of the InterPro (super)families, domains and sites was done by using the online tools on <https://www.ebi.ac.uk/interpro/> or a standalone version of InterProScan (<https://www.ebi.ac.uk/interpro/download.html>), both based on InterPro 75.0 as released on 4th July 2019. InterPro is a composite database combining the information of many databases of

protein motifs and domains. The HMM of the InterPro domain and/or (super)families can be obtained from InterProScan and can be used to identify new family members in the protein databases. Sequences comprising the InterPro hit can also be downloaded from the InterPro website ('Protein Matched') or can be queried on the UniProt website (<https://www.uniprot.org>).

5

Example 2: Identification of membrane proteins or protein sequences useful in the methods of the invention

A first set of membrane proteins or protein sequences were found by identifying the PFAM domains of the membrane proteins found in the neighbourhood of fucosyltransferases and selecting membrane proteins having any one of the PFAM domains identified, as exemplified in Example 1. Protein identifiers belonging to fucosyltransferase families IPR001053 (GT10) and IPR002516 (GT11) were extracted from UniProtKB/trembl, as defined by InterPro 75.0 as released on 4th July 2019. These identifiers were used as input in the genome neighborhood tool <https://efi.igb.illinois.edu/efi-gnt/> as released on 19th June 2019. EFI-GNT (EFI Genome Neighborhood Tool) allows exploration of the genome neighborhoods and is focused on placing protein families and superfamilies into a genomic context. A sequence similarity network (SSN) is used as an input. Each sequence within an SSN is used as a query for interrogation of its genome neighborhood. EFI-GNT enables exploration of the genome neighborhoods for sequences in SSN clusters in order to facilitate their assignment of function.

A neighborhood window size of 14 was selected. Neighboring genes were classified based on their PFAM domain. Membrane proteins with the following PFAM domains are present near GT10 (IPR001503) and GT11 (IPR002516) fucosyltransferases: PF00005, PF00006, PF00023, PF00083, PF00092, PF00115, PF00116, PF00122, PF00209, PF00213, PF00230, PF00231, PF00254, PF00359, PF00375, PF00381, PF00391, PF00401, PF00403, PF00474, PF00484, PF00520, PF00528, PF00529, PF00543, PF00571, PF00593, PF00625, PF00654, PF00664, PF00689, PF00690, PF00702, PF00860, PF00873, PF00892, PF00893, PF00902, PF00909, PF00916, PF00939, PF00999, PF01032, PF01061, PF01103, PF01203, PF01235, PF01384, PF01496, PF01544, PF01547, PF01554, PF01566, PF01578, PF01614, PF01618, PF01656, PF01699, PF01740, PF01741, PF01758, PF01810, PF01813, PF01891, PF01895, PF01899, PF01943, PF02026, PF02080, PF02133, PF02136, PF02225, PF02254, PF02277, PF02302, PF02321, PF02355, PF02378, PF02386, PF02417, PF02447, PF02501, PF02563, PF02632, PF02652, PF02653, PF02690, PF02706, PF02874, PF02896, PF03030, PF03083, PF03186, PF03222, PF03412, PF03459, PF03471, PF03544, PF03547, PF03548, PF03567, PF03605, PF03606, PF03610, PF03616, PF03814, PF03840, PF03865, PF03932, PF04193, PF04277, PF04389, PF04966, PF05134, PF05140, PF05524, PF05552, PF05977, PF06251, PF06826, PF06835, PF07264, PF07549, PF07660, PF07670, PF07685, PF07690, PF07715, PF07885, PF07969, PF08239, PF08279, PF08334, PF08352, PF08402, PF08479, PF10531, PF11356, PF11612, PF12156, PF12399, PF12796, PF12822, PF12848, PF12974, PF13306, PF13347,

PF13409, PF13410, PF13416, PF13417, PF13440, PF13442, PF13462, PF13466, PF13473, PF13499, PF13505, PF13520, PF13531, PF13599, PF13609, PF13637, PF13807, PF13855, PF14524, PF14667, PF16327, PF17912 and PF18412.

5 Example 3: Materials and methods *Escherichia coli*

Media

The Luria Broth (LB) medium consisted of 1% tryptone peptone (Difco, Erembodegem, Belgium), 0.5% yeast extract (Difco) and 0.5% sodium chloride (VWR, Leuven, Belgium). The minimal medium used in the cultivation experiments in 96-well plates or in shake flasks contained 2.00
10 g/L NH₄Cl, 5.00 g/L (NH₄)₂SO₄, 2.993 g/L KH₂PO₄, 7.315 g/L K₂HPO₄, 8.372 g/L MOPS, 0.5 g/L NaCl, 0.5 g/L MgSO₄·7H₂O, 30 g/L sucrose or another carbon source when specified in the examples, 1 ml/L vitamin solution, 100 µL/L molybdate solution, and 1 mL/L selenium solution. As specified in the respective examples, 20 or 45 g/L lactose was additionally added to the medium as precursor. The medium was set to a pH of 7 with 1M KOH. Vitamin solution consisted
15 of 3.6 g/L FeCl₂·4H₂O, 5 g/L CaCl₂·2H₂O, 1.3 g/L MnCl₂·2H₂O, 0.38 g/L CuCl₂·2H₂O, 0.5 g/L CoCl₂·6H₂O, 0.94 g/L ZnCl₂, 0.0311 g/L H₃BO₄, 0.4 g/L Na₂EDTA·2H₂O and 1.01 g/L thiamine·HCl. The molybdate solution contained 0.967 g/L NaMoO₄·2H₂O. The selenium solution contained 42 g/L SeO₂.

The minimal medium for fermentations contained 6.75 g/L NH₄Cl, 1.25 g/L (NH₄)₂SO₄, 2.93 g/L
20 KH₂PO₄ and 7.31 g/L K₂HPO₄, 0.5 g/L NaCl, 0.5 g/L MgSO₄·7H₂O, 30 g/L sucrose, 1 mL/L vitamin solution, 100 µL/L molybdate solution, and 1 mL/L selenium solution with the same composition as described above. As specified in the respective examples, 20 g/L lactose was additionally added to the medium as precursor.

Complex medium was sterilized by autoclaving (121°C, 21') and minimal medium by filtration
25 (0.22 µm Sartorius). When necessary, the medium was made selective by adding an antibiotic (e.g. chloramphenicol (20 mg/L), carbenicillin (100 mg/L), spectinomycin (40 mg/L) and/or kanamycin (50 mg/L)).

Plasmids

30 pKD46 (Red helper plasmid, Ampicillin resistance), pKD3 (contains an FRT-flanked chloramphenicol resistance (cat) gene), pKD4 (contains an FRT-flanked kanamycin resistance (kan) gene), and pCP20 (expresses FLP recombinase activity) plasmids were obtained from Prof. R. Cunin (Vrije Universiteit Brussel, Belgium in 2007).

Plasmids for membrane protein and for fucosyltransferase expression were constructed in a
35 pSC101 ori (Rep10-v3) and pMB1 ori containing backbone vector, respectively, using Golden Gate assembly. All membrane protein and fucosyltransferase encoding genes were synthetically synthesized at Twist Biosciences (San Francisco, USA). Polynucleotide sequences of the membrane proteins and the corresponding membrane protein polypeptides are shown in SEQ ID

NOs 1 to 196 and SEQ ID NOs 204 to 218 and enlisted in Table 1. The fucosyltransferases used in the enclosed examples are 3-fucosyltransferases FT1 with nucleic acid and protein sequence SEQ ID NOs 197 and 198 respectively and FT2 with SEQ ID NOs 199 and 200. The 2-fucosyltransferases used are HpFutC with SEQ ID NOs 201 and 202, referred to here as FT3, and FT4 with nucleic acid and protein sequence SEQ ID NOs 219 and 220, respectively. Both membrane protein and fucosyltransferase genes were expressed in different transcriptional units (TUs) using specific promoter, UTR and terminator combinations as enlisted in Table 2. The genes were expressed using promoters MutalikP5 (PROM0005_MutalikP5), MutalikP12 (PROM0012_MutalikP12), apFAB146 ("PROM0032") and MutalikP10 ("PROM0010_MutalikP10") (as described by Mutalik et al. (Nat. Methods 2013, No. 10, 354-360)) and promoters p22 (PROM0015_p22) and p14 (PROM0016_p14) both as described by De Mey *et al.* (BMC Biotechnology 2007, 7:34)). UTR's used comprise Gene10-LeuAB-BCD2 ("UTR0002_Gene10-LeuAB-BCD2"), BCD1 ("UTR003_BCD1"), Gene10_LeuL ("UTR0011_Gene10_LeuL"), ThrA_BCD2 ("UTR0013_ThrA_BCD2"), GalE_LeuAB ("UTR0014_GalE_LeuAB"), GalE_IptFG ("UTR0038_GalE_IptFG") and uspF_IptFG ("UTR0055_uspF_IptFG") (as described by Mutalik et al. (Nat. Methods 2013, No. 10, 354-360)). Terminator used in the examples is TER0010_T7 Early as described by Dunn et al. (Nucleic Acids Res. 1980, 8(10), 2119-32). Table 3 shows the overview of the transcriptional units used in the examples by combination of the above promoter UTRs and terminator. Expression could be further facilitated by optimizing the codon usage to the codon usage of the expression host. Genes were optimized using the tools of the supplier.

Table 1

SEQ ID NO (protein)	Name / TCDB group	Organism	Origin	Country of origin of digital sequence information
2	EcMdfA	E. coli K12 MG1655	Synthetic	USA
4	EcYnfM	E. coli K12 MG1655	Synthetic	USA
6	EcIceT	E. coli K12 MG1655	Synthetic	USA
8	EcYhhs	E. coli K12 MG1655	Synthetic	USA
10	EcEmrD	E. coli K12 MG1655	Synthetic	USA
12	EcYdhC	E. coli K12 MG1655	Synthetic	USA
14	EcYbdA	E. coli K12 MG1655	Synthetic	USA
16	EcYdeE	E. coli K12 MG1655	Synthetic	USA
18	EcMhpT	E. coli K12 MG1655	Synthetic	USA
20	EcYebQ	E. coli K12 MG1655	Synthetic	USA
22	EcYjhB	E. coli K12 MG1655	Synthetic	USA
24	EcBcr	E. coli K12 MG1655	Synthetic	USA
26	EcFucP	E. coli K12 MG1655	Synthetic	USA

28	LlImrA	Lactococcus lactis strain SRCM103457	Synthetic	South Korea
30	EcOppF	E. coli strain K12 (MG1655)	Synthetic	USA
32	EcWzxE	E. coli strain K12 (MG1655)	Synthetic	USA
34	EcWza	E. coli strain K12 (MG1655)	Synthetic	USA
36	HpWzk	Helicobacter pylori strain ATCC 700392 / 26695	Synthetic	United Kingdom
38	EcEmrE	E. coli strain K12	Synthetic	USA
40	Blon_2331	B. longum subsp. Infantis (strain ATCC 15697)	Synthetic	Germany
42	Blon_2332	B. longum subsp. Infantis (strain ATCC 15697)	Synthetic	Germany
44	Blon_2475	B. longum subsp. Infantis (strain ATCC 15697)	Synthetic	Germany
46	Blon_0247	B. longum subsp. Infantis (strain ATCC 15697)	Synthetic	Germany
48	Blon_0245	B. longum subsp. Infantis (strain ATCC 15697)	Synthetic	Germany
50	Blon_0345	B. longum subsp. Infantis (strain ATCC 15697)	Synthetic	Germany
52	NcCDT2	Neurospora crassa OR74A	Synthetic	USA
54	AoCDT2	Aspergillus oryzae RIB40	Synthetic	Japan
56	HpCytC	Helicobacter pylori (Campylobacter pylori)	Synthetic	United Kingdom
58	ChWzx	Chitinophaga sp. CF118	Synthetic	USA
60	EuWzx	Eubacterium sp. CAG:581	Synthetic	Denmark
62	DsWzx	Dyadobacter soli DSM 25329 contig Ga0069981_102	Synthetic	South Korea
64	LrWzx	Lactococcus raffinolactis (ATCC 43920)	Synthetic	Unknown
66	PrWzx	Prevotella ruminicola (AR32)	Synthetic	USA
68	FsLpsE	Flavobacterium spartansii	Synthetic	USA
70	SsLpsE	Sporomusa sphaeroides DSM 2875	Synthetic	Germany
72	FsLpsE	Flavobacterium spartansii	Synthetic	USA
74	SsLpsE	Sporomusa sphaeroides DSM 2875	Synthetic	Germany
76	PsTolC	Candidatus Planktophilia sulfonica	Synthetic	Switzerland

78	BhTolC	Butyrivibrio hungatei XBD2006	Synthetic	USA
80	RiMsbA	Roseburia intestinalis CAG:13	Synthetic	Denmark
82	PgMsbA	Pedobacter ginsengisoli	Synthetic	South Korea
84	VbMsbA	Verrucomicrobia bacterium CG1_02_43_26	Synthetic	USA
86	BhNAPO	Brachyspira hampsonii P280/1	Synthetic	UK
88	TmWzc	Thermotoga maritima (strain ATCC 43589 / MSB8 / DSM 3109 / JCM 10099)	Synthetic	Italy
90	CiCutC	Clostridium sp. CAG:1013	Synthetic	Denmark
92	OsCutC	Odoribacter splanchnicus DSM 20712	Synthetic	Unknown
94	MiCutC	Mitsuaria sp. PDC51	Synthetic	USA
96	PiCutC	Prevotella intermedia ATCC 25611 (DSM 20706)	Synthetic	USA
98	AsNA _m	Actinobaculum suis DSM 20639	Synthetic	Unknown
100	RgNA _m	Ruminococcus gnavus	Synthetic	USA
102	CuNA _m	Curtobacterium sp. 314Chir4.1	Synthetic	USA
104	NdNap	Niabella drilacis DSM25811	Synthetic	Germany
106	SfNap	Saccharicrinis fermentans DSM 9555 = JCM 21142	Synthetic	unknown
108	mdtD	Citrobacter freundii MGH152	Synthetic	USA
110	mdtD	Citrobacter werkmanii NBRC 105721	Synthetic	Belgium, not further specified
112	mdtD	Citrobacter amalonaticus	Synthetic	USA
114	mdtD	Klebsiella oxytoca	Synthetic	United Kingdom
116	mdtD	Escherichia albertii B156	Synthetic	USA
118	yegB	Salmonella enterica subsp. Salamae	Synthetic	United Kingdom
120	mdtD	Klebsiella pneumoniae 30684/NJST258_2	Synthetic	USA
122	Tcr_1_D38215	Klebsiella pneumoniae	Synthetic	USA
124	mdtD	Pseudocitrobacter faecalis	Synthetic	USA
126	Cmr	Yokenella regensburgei ATCC43003	Synthetic	United Kingdom
128	MdfA	Cronobacter muytjensii	Synthetic	USA

130	MdfA	Klebsiella oxytoca	Synthetic	Zweden
132	MFS	Citrobacter koseri (Citrobacter diversus)	Synthetic	USA
134	MdfA	Escherichia marmotae	Synthetic	USA
136	Cmr	Shigella flexneri	Synthetic	United Kingdom
138	MdfA	Salmonella enterica subsp. Salamae	Synthetic	Denmark
140	Cmr/MdfA	Citrobacter youngae ATCC 29220	Synthetic	USA
142	MdfA	Citrobacter freundii	Synthetic	USA
144	MdfA/DHA1/MFS	Enterobacter kobei	Synthetic	USA
146	MdfA	Enterobacter sp.	Synthetic	Australia
148	MdfA	Lelliottia sp. WB101	Synthetic	USA
150	MdfA	Enterobacter ludwigii EcWSU1	Synthetic	USA
152	Sweet-like	Actinoplanes utahensis	Synthetic	USA
154	Sweet-like	Chitinophagaceae bacterium PMG_246	Synthetic	United Kingdom
156	Sweet-like	Rhizobium sp. PDC82	Synthetic	USA
158	Sweet-like	Kineococcus rhizosphaerae DSM 19711	Synthetic	South-Korea
160	Sweet-like	Morganella morganii IS15	Synthetic	Austria
162	Sweet-like	Geodermatophilus obscurus (strain ATCC 25078 / DSM 43160 / JCM 3152 / G-20)	Synthetic	USA
164	Sweet-like	Bradyrhizobium sp. BTAi1	Synthetic	USA
166	Sweet-like	Bradyrhizobium japonicum USDA 110	Synthetic	USA
168	Sweet-like	Xanthomonas campestris pv. vesicatoria str. 85-10	Synthetic	South Korea
170	Sweet-like	Herbaspirillum aquaticum	Synthetic	USA
172	Sweet-like	Flavobacteria bacterium MS024-2A	Synthetic	USA
174	wzm	Rhizobium sp. Root149	Synthetic	Germany
176	wzm	Azospirillum brasiliense LMG 04375	Synthetic	Belgium, Flanders
178	wzt	Rhizobium sp. Root149	Synthetic	Germany
180	wzt	Azospirillum brasiliense LMG 4375	Synthetic	Belgium, Flanders
182	rnd-like	Sinorhizobium medicae WSM419	Synthetic	USA
184	arabinose efflux	Azospirillum brasiliense LMG 4375	Synthetic	Belgium, Flanders
186	nodj	Bradyrhizobium japonicum USDA 110	Synthetic	USA

188	nodi	Bradyrhizobium japonicum USDA 110	Synthetic	USA
190	ybjM	E. coli K12 MG1655	Synthetic	USA
192	ybjM-like	Enterobacteriaceae bacterium ENNIH1	Synthetic	USA
194	wzt	Escherichia coli 113303	Synthetic	USA
196	wzm	Escherichia coli 113303	Synthetic	USA
198	FT1	Helicobacter pylori	Synthetic	Australia
200	FT2	Basilea psittaculmonis JF4266 (DSM 24701)	Synthetic	Switzerland
202	HpFutC (FT3)	Helicobacter pylori	Synthetic	Australia
204	lamB	E. coli K12 MG1655	Synthetic	USA
206	malE	E. coli K12 MG1655	Synthetic	USA
208	malK	E. coli K12 MG1655	Synthetic	USA
210	nagE	E. coli K12 MG1655	Synthetic	USA
212	srlB	E. coli K12 MG1655	Synthetic	USA
214	araF	E. coli K12 MG1655	Synthetic	USA
216	xylF	E. coli K12 MG1655	Synthetic	USA
218	ytfQ	E. coli K12 MG1655	Synthetic	USA
220	FT4	Helicobacter sp. MIT 01-6242	Synthetic	USA

Table 2

SEQ ID NO (protein)	Family	EggNOG	PFAM	Interpro	TCDB
2	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
4	porters	05D94	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.36
6	porters	05C0R	PF07690	IPR004638 IPR011701 IPR020846 IPR036259 IPR023721 IPR001411	2.A.1.3
8	porters	05F9N	PF07690	IPR011701 IPR020846 IPR036259 IPR023008	2.A.1.46
10	porters	05E5M	PF07690	IPR011701 IPR020846 IPR036259 IPR004734	2.A.1.2
12	porters	05CZQ	PF07690	IPR004812 IPR011701 IPR020846 IPR036259	2.A.1.2
14	porters	05E8G	PF05977	IPR023722 IPR020846 IPR036259 IPR010290	2.A.1.38
16	porters	05E5W	PF07690	IPR011701 IPR020846 IPR036259	2.A.1.2
18	porters	08SC4	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.15
20	porters	08H15	PF07690	IPR011701 IPR020846 IPR036259 IPR001411	2.A.1.3
22	porters	05JHE	PF07690	IPR011701 IPR020846 IPR036259	2.A.1.12

24	porters	05C2C	PF07690	IPR004812 IPR020846 IPR004734	IPR011701 IPR036259	2.A.1.2
26	porters	05EDR	PF07690	IPR005275 IPR020846	IPR011701 IPR036259	2.A.1.7
28	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00664 PF00005	IPR003593 IPR036640 IPR017871 IPR039421	IPR011527 IPR003439 IPR027417	3.A.1.11
30	P-P-bond-hydrolysis-driven transporters	08JQ7	PF00005 PF08352	IPR003593 IPR017871 IPR027417	IPR003439 IPR013563	3.A.1.5
32	porters	05EGZ	PF01943	IPR002797	IPR032896	2.A.66
34	β -Barrel Porins	05DAY	PF02563 PF10531 PF18412	IPR003715 IPR040716	IPR019554	1.B.18
36	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00005	IPR003593 IPR036640 IPR017871 IPR039421	IPR011527 IPR003439 IPR027417	3.A.1.10
38	porters	0814C	PF00893	IPR000390		2.A.7.1
40	porters	07RBJ	PF13347	IPR039672 IPR001927	IPR036259	2.A.2
42	porters	07RBJ	PF13347	IPR039672 IPR001927	IPR036259	2.A.2
44	P-P-bond-hydrolysis-driven transporters	08IJ9	PF00005 PF17912	IPR003593 IPR017871 IPR040582	IPR003439 IPR008995 IPR027417	3.A.1.1
46	porters	08N8A	PF07690	IPR011701 IPR036259	IPR020846	2.A.1.68
48	porters	05DXI	PF07690	IPR011701 IPR036259	IPR020846	2.A.1.81
50	porters	08N8A	PF07690	IPR011701 IPR036259	IPR020846	2.A.1.68
52	porters	07QNK	PF00083	IPR020846 IPR036259 IPR005829	IPR005828 IPR003663	2.A.1.1
54	porters	07QNK	PF00083	IPR020846 IPR036259	IPR005828	2.A.1.1
56	Putative transport proteins	05CRE	PF01578 PF05140	IPR002541	IPR007816	9.B.14
58	porters	07T5E	PF14667 PF13440	IPR029303		2.A.66
60	porters	07VQ3	PF01943 PF14667	IPR029303	IPR002797	2.A.66
62	porters	08Z4Q	PF13440			2.A.66
64	porters	07VQ3	PF01943	IPR002797		2.A.66
66	porters	05PSV	PF13440			2.A.66
68	P-P-bond-hydrolysis-driven transporters	05EY8	PF00005 PF14524	IPR003593 IPR027417	IPR003439 IPR029439	3.A.1.10
70	P-P-bond-hydrolysis-driven transporters	05EY8	PF00005	IPR003593 IPR027417	IPR003439	3.A.1.10
72	P-P-bond-hydrolysis-driven transporters	07V1T	PF01061	IPR013525	IPR000412	3.A.1.10
74	P-P-bond-hydrolysis-driven transporters	07V1T	PF01061	IPR013525	IPR000412	3.A.1.10
76	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00005	IPR003593 IPR036640 IPR017871 IPR039421	IPR011527 IPR003439 IPR027417	3.A.1.11

78	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00664 PF00005	IPR003593 IPR036640 IPR017871 IPR039421	IPR011527 IPR003439 IPR027417	3.A.1.10
80	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00664 PF00005	IPR003593 IPR036640 IPR017871 IPR039421	IPR011527 IPR003439 IPR027417	3.A.1.10
82	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00664 PF00005	IPR003593 IPR036640 IPR017871 IPR027417	IPR011527 IPR003439	3.A.1.10
84	P-P-bond-hydrolysis-driven transporters	05BZ1	PF00664 PF00005	IPR003593 IPR036640 IPR017871 IPR027417	IPR011527 IPR003439	3.A.1.10
86	porters	05EAM	PF02690 PF01895	IPR003841 IPR038078 IPR026022	IPR004633	2.A.58
88	Auxiliary transport proteins	07SYR	PF13807 PF02706	IPR032807 IPR027417	IPR003856	8.A.3
90	Putative transport proteins	06N3A	PF03932	IPR005627 IPR036822	IPR023648	9.B.158
92	Putative transport proteins	06N3A	PF03932	IPR005627 IPR036822	IPR023648	9.B.158
94	Putative transport proteins	06N3A	PF03932	IPR005627 IPR036822	IPR023648	9.B.158
96	Putative transport proteins	06N3A	PF03932	IPR005627 IPR036822	IPR023648	9.B.158
98	porters	05CT4	PF13347	IPR039672 IPR036259	IPR020846	2.A.2
100	porters	088QT	PF13347	IPR039672 IPR036259 IPR001927	IPR020846	2.A.2
102	porters	05C0R	PF07690	IPR004638 IPR020846 IPR036259 IPR001411	IPR011701 IPR023721	2.A.1.3
104	porters	07CWC	PF00474	IPR038377	IPR001734	2.A.21
106	porters	07RJ1	PF00474	IPR038377	IPR001734	2.A.21
108	porters	05C0R	PF07690	IPR011701 IPR036259 IPR001411	IPR020846 IPR023721	2.A.1.3
110	porters	05C0R	PF07690	IPR004638 IPR020846 IPR023721 IPR001411	IPR011701 IPR036259	2.A.1.3
112	porters	05C0R	PF07690	IPR011701 IPR036259 IPR001411	IPR020846 IPR023721	2.A.1.3
114	porters	05C0R	PF07690	IPR004638 IPR020846 IPR023721 IPR001411	IPR011701 IPR036259	2.A.1.3
116	porters	05C0R	PF07690	IPR004638 IPR020846 IPR023721 IPR001411	IPR011701 IPR036259	2.A.1.3
118	porters	05C0R	PF07690	IPR011701 IPR036259 IPR001411	IPR020846 IPR023721	2.A.1.3
120	porters	05C0R	PF07690	IPR011701 IPR036259 IPR001411	IPR020846 IPR023721	2.A.1.3
122	porters	05C0R	PF07690	IPR004638 IPR020846 IPR023721 IPR001411	IPR011701 IPR036259	2.A.1.3

124	porters	05C0R	PF07690	IPR004638 IPR011701 IPR020846 IPR036259 IPR023721 IPR001411	2.A.1.3
126	porters	07QF7	PF07690 PF07690	IPR011701 IPR011701 IPR020846 IPR020846 IPR036259 IPR036259 IPR005829 IPR005829	2.A.1.2
128	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
130	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
132	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
134	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
136	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
138	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
140	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
142	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
144	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
146	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
148	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
150	porters	07QF7	PF07690	IPR011701 IPR020846 IPR036259 IPR005829	2.A.1.2
152	porters	070Q9	PF04193	IPR006603	2.A.123
154	porters	05W3H	PF04193	IPR006603	2.A.123
156	porters	05W3H	PF03083	IPR004316	2.A.123
158	porters	05W3H	PF03083 PF04193	IPR006603 IPR004316	2.A.123
160	porters	05W2Y	PF03083	IPR004316	2.A.123
162	porters	070Q9	PF04193	IPR006603	2.A.123
164	porters	05W3H	PF04193	IPR006603	2.A.123
166	porters	05W3H	PF04193	IPR006603	2.A.123
168	porters	05XJ5	PF04193	IPR006603	2.A.123
170	porters	05XJ5	PF03083	IPR004316	2.A.123
172	porters	05W3H	PF04193	IPR006603	2.A.123
174	P-P-bond-hydrolysis-driven transporters	05MFV	PF01061	IPR013525 IPR000412	3.A.1.10
176	P-P-bond-hydrolysis-driven transporters	05MFV	PF01061	IPR013525 IPR000412	3.A.1.10
178	P-P-bond-hydrolysis-driven transporters	05EY8	PF00005 PF14524	IPR003593 IPR003439 IPR027417 IPR029439	3.A.1.10
180	P-P-bond-hydrolysis-driven transporters	05EY8	PF14524	IPR003593 IPR003439 IPR017871 IPR027417 IPR029439	3.A.1.10
182	porters	05BZS	PF00873	IPR027463 IPR001036	2.A.6.3
184	porters	05CXP	PF07690	IPR011701 IPR020846 IPR036259	2.A.1.2

186	P-P-bond-hydrolysis-driven transporters	05HAC	PF01061	IPR013525 IPR005981	IPR000412	3.A.1.10
188	P-P-bond-hydrolysis-driven transporters	05CJ1	PF00005	IPR003593 IPR017871 IPR005978	IPR003439 IPR015851 IPR027417	3.A.1.10
190	Putative transport proteins	05GWF	PF11045	IPR020368		N/A
192	Putative transport proteins	05GWF	PF11045	IPR020368		N/A
194	P-P-bond-hydrolysis-driven transporters	05EY8	PF00005 PF14524	IPR003593 IPR017871 IPR029439	IPR003439 IPR027417	3.A.1.10
196	P-P-bond-hydrolysis-driven transporters	05MFV	PF01061	IPR013525	IPR000412	3.A.1.10
204	β -Barrel Porins	08KDD	PF02264	IPR003192 IPR036998	IPR023738	1.B.3.1
206	P-P-bond-hydrolysis-driven transporters	07FKK	PF13416	IPR006059 IPR006061	IPR006060	3.A.1.1
208	P-P-bond-hydrolysis-driven transporters	172T7	PF00005 PF17912	IPR003439 IPR008995 IPR017871 IPR040582	IPR003593 IPR015855 IPR027417	3.A.1.1
210	Phosphotransfer-driven Group Translocators	05CI1	PF00367 PF00358 PF02378	IPR001127 IPR003352 IPR011055 IPR018113	IPR001996 IPR010974 IPR013013 IPR036878	4.A.1.1
212	Phosphotransfer-driven Group Translocators	05VI0	PF03829	IPR004716 IPR036665	IPR018454	4.A.4.1
214	P-P-bond-hydrolysis-driven transporters	05DMK	PF00532	IPR001761 IPR028082	IPR026266	3.A.1.2
216	P-P-bond-hydrolysis-driven transporters	05DFW	PF13407	IPR013456 IPR028082	IPR025997	3.A.1.2
218	P-P-bond-hydrolysis-driven transporters	07R5U	PF13407	IPR025997 IPR033893	IPR028082	3.A.1.2

Table 3

Transcriptional units for expression of the membrane protein genes			
TU number	Promoter part	UTR part	Terminator part
TU1	PROM0005_MutalikP5	UTR0014_GalE_LeuAB	TER0010_T7early
TU2	PROM0005_MutalikP5	UTR0038_GalE_IptFG	TER0010_T7early
TU3	PROM0005_MutalikP5	UTR0055_uspF_ipFG	TER0010_T7early
TU4	PROM0012_MutalikP12	UTR0014_GalE_LeuAB	TER0010_T7early
TU5	PROM0012_MutalikP12	UTR0038_GalE_IptFG	TER0010_T7early
TU6	PROM0012_MutalikP12	UTR0055_uspF_ipFG	TER0010_T7early
TU7	PROM0032_apFAB146	UTR0014_GalE_LeuAB	TER0010_T7early

TU8	PROM0032_apFAB146	UTR0038_GalE_lptFG	TER0010_T7early
TU9	PROM0032_apFAB146	UTR0055_uspF_lptFG	TER0010_T7early
TU10	PROM0015_p22	UTR0003_BCD1	TER0010_T7early
TU11	PROM0010_MutalikP10	UTR0013_ThrA_BCD2	TER0010_T7early
TU12	PROM0005_MutalikP5	UTR0011_Gene10_LeuL	TER0010_T7early

Transcriptional units for expression of fucosyltransferase genes			
TU number	Promoter part	UTR part	Terminator part
TU13	PROM0016_p14	UTR0002_Gene10-LeuAB-BCD2	TER0010_T7early

Plasmids were maintained in the host *E. coli* DH5alpha (F⁻, phi80dlacZdeltaM15, delta(lacZYAargF) U169, *deoR*, *recA1*, *endA1*, *hsdR17*(rk⁻, mk⁺), *phoA*, *supE44*, lambda⁻, *thi-1*, *gyrA96*, *relA1*) bought from Invitrogen.

5

Strains and mutations

Escherichia coli K12 MG1655 [lambda⁻, F⁻, rph-1] was obtained from the Coli Genetic Stock Center (US), CGSC Strain#: 7740, in March 2007. Gene disruptions as well as gene introductions were performed using the technique published by Datsenko and Wanner (PNAS 97 (2000), 6640-6645). This technique is based on antibiotic selection after homologous recombination performed by lambda Red recombinase. Subsequent catalysis of a flippase recombinase ensures removal of the antibiotic selection cassette in the final production strain.

Transformants carrying a Red helper plasmid pKD46 were grown in 10 ml LB media with ampicillin, (100 mg/L) and L-arabinose (10 mM) at 30 °C to an OD_{600nm} of 0.6. The cells were made electrocompetent by washing them with 50 ml of ice-cold water, a first time, and with 1ml ice cold water, a second time. Then, the cells were resuspended in 50 µl of ice-cold water. Electroporation was done with 50 µl of cells and 10-100 ng of linear double-stranded-DNA product by using a Gene Pulser™ (BioRad) (600 Ω, 25 µFD, and 250 volts).

After electroporation, cells were added to 1 ml LB media incubated 1 h at 37 °C, and finally spread onto LB-agar containing 25 mg/L of chloramphenicol or 50 mg/L of kanamycin to select antibiotic resistant transformants. The selected mutants were verified by PCR with primers upstream and downstream of the modified region and were grown in LB-agar at 42 °C for the loss of the helper plasmid. The mutants were tested for ampicillin sensitivity.

The linear ds-DNA amplicons were obtained by PCR using pKD3, pKD4 and their derivatives as template. The primers used had a part of the sequence complementary to the template and another part complementary to the side on the chromosomal DNA where the recombination must take place. For the genomic knock-out, the region of homology was designed 50-nt upstream and 50-nt downstream of the start and stop codon of the gene of interest. For the genomic knock-in,

25

the transcriptional starting point (+1) had to be respected. PCR products were PCR-purified, digested with DpnI, repurified from an agarose gel, and suspended in elution buffer (5 mM Tris, pH 8.0).

The selected mutants (chloramphenicol or kanamycin resistant) were transformed with pCP20
5 plasmid, which is an ampicillin and chloramphenicol resistant plasmid that shows temperature-sensitive replication and thermal induction of FLP synthesis. The ampicillin-resistant transformants were selected at 30 °C, after which a few were colony purified in LB at 42 °C and then tested for loss of all antibiotic resistance and of the FLP helper plasmid. The gene knock outs and knock ins are checked with control primers (Fw/Rv-gene-out).

10 A mutant strain derived from *E. coli* K12 MG1655 was created by knocking out the genes *lacZ*, *lacY* *lacA*, *glgC*, *agp*, *pfkA*, *pfkB*, *pgi*, *arcA*, *iclR*, *wcaJ*, *lon* and *thyA*. Additionally, the *E. coli* *lacY* gene, a fructose kinase gene (*frk*) originating from *Zymomonas mobilis*, an *E. coli* W sucrose transporter (*cscB*) and a sucrose phosphorylase (*SP*) originating from *Bifidobacterium adolescentis* were knocked in into the genome and expressed constitutively. The constitutive
15 promoters originate from the promoter library described by De Mey et al. (BMC Biotechnology, 2007). These genetic modifications are also described in WO2016075243 and WO2012007481. The α 1,3- or α 1,2-fucosyltransferase genes were presented to the mutant strain from a plasmid as described in the same. All membrane protein genes were evaluated in this mutant strain derived from *E. coli* K12 MG1655. Membrane protein genes were evaluated either present on
20 plasmid or integrated in the host's genome (in the *setA* or *ldhA* locus). All strains are stored in cryovials at -80°C (overnight LB culture mixed in a 1:1 ratio with 70% glycerol).

An alternative mutant strain can be derived from *E. coli* K12 JM109 wherein the genes *lacZ*, *rcaA* and *wcaJ* are knocked out. α 1,3- or α 1,2-fucosyltransferase genes are presented to said mutant strain from a plasmid as described above resulting in the production of 2'fucosyllactose, 3-
25 fucosyllactose or 2',3-difucosyllactose. Membrane protein genes are evaluated in the same way as described above. Said strain is enabled to internalize lactose by means of allo-lactose or IPTG, inducing the lactose permease gene *lacY*.

Another alternative mutant strain can be derived from *E. coli* BL21. The genes *lacZ*, *fucI*, *fucK* and *wzxC-wcaJ* are knocked out in said strain. In order to improve the synthesis of GDP-fucose in said mutant strain the genes encoding for phosphomannomutase (*manB*), mannose-1-phosphate
30 guanosyltransferase (*manC*), GDP-mannose-4,6-dehydratase (*gmd*), and GDP-L-fucose synthase (*wcaG*) from *E. coli* K12 were overexpressed in a similar way as described above. Intracellular lactose synthesis is accomplished by overexpression of the gene encoding for beta-1,4-galactosyltransferase encoded by the gene *lgtB*. To enhance the synthesis of UDP-galactose
35 the operon encoding for *galETKM* is knocked out and the gene encoding for UDP-glucose epimerase is overexpressed. α 1,3- or α 1,2-fucosyltransferase genes are presented to said mutant strain from a plasmid as described above resulting in the production of 2'fucosyllactose, 3-

fucosyllactose or 2',3-difucosyllactose. Membrane protein genes are evaluated in the same way as described above.

Another alternative mutant strain can be derived from *E. coli* K12. The genes *lacZ*, *fucl*, *fucK* and *wxC-wcaJ* are knocked out in said strain. In order to improve the synthesis of GDP-fucose in said mutant strain the genes encoding for phosphomannomutase (*manB*), mannose-1-phosphate guanosyltransferase (*manC*), GDP-mannose-4,6-dehydratase (*gmd*), and GDP-L-fucose synthase (*wcaG*) from *E. coli* K12 were overexpressed in a similar way as described above. In addition, said strain is modified with genomic knock-ins of the fucose permease (*fucP*) gene from *E. coli* and the bifunctional fucose kinase/fucose-1-phosphate guanylyltransferase (*fkp*) gene from *Bacteroides fragilis*. α 1,3- or α 1,2-fucosyltransferase genes are presented to said mutant strain from a plasmid as described above resulting in the production of 2'fucosyllactose, 3-fucosyllactose or 2',3-difucosyllactose. Membrane protein genes are evaluated in the same way as described above. Said strain is enabled to internalize lactose by means of allo-lactose or IPTG, inducing the lactose permease gene *lacY*.

Another alternative mutant strain can be derived from *E. coli* K12. The genes *lacZ*, and *wxC-wcaJ* are knocked out in said strain. In order to improve the synthesis of GDP-fucose in said mutant strain the genes encoding for phosphomannomutase (*manB*), mannose-1-phosphate guanosyltransferase (*manC*), GDP-mannose-4,6-dehydratase (*gmd*), and GDP-L-fucose synthase (*wcaG*) from *E. coli* K12 were overexpressed in a similar way as described above. To improve the formation of fructose-6-phosphate from gluconeogenic substrates such as glycerol, acetate, lactate, ethanol, succinate, pyruvate, the genes encoding for phosphofructokinase (*pfkA* and *pfkB*) are knocked out and the genes encoding for fructose-1,6-bisphosphate aldolase (*fbaB*) and a heterologous fructose-1,6-bisphosphate phosphatase (*fbpase*) from *Pisum sativum* were overexpressed. α 1,3- or α 1,2-fucosyltransferase genes are presented to said mutant strain from a plasmid as described above resulting in the production of 2'fucosyllactose, 3-fucosyllactose or 2',3-difucosyllactose. Membrane protein genes are evaluated in the same way as described above.

Cultivation conditions

A preculture of 96well microtiter plate experiments was started from a cryovial, in 150 μ L LB and was incubated overnight at 37 °C on an orbital shaker at 800 rpm. This culture was used as inoculum for a 96well square microtiter plate, with 400 μ L minimal medium by diluting 400x. Each strain was grown in multiple wells of the 96-well plate as biological replicates. These final 96-well culture plates were then incubated at 37°C on an orbital shaker at 800 rpm for 72h, or shorter, or longer. At the end of the cultivation experiment samples were taken from each well to measure sugar concentrations in the broth supernatant (extracellular sugar concentrations, after 5 spinning down the cells), or by boiling the culture broth for 15 min at 90°C before spinning down the cells (= whole broth measurements, average of intra- and extracellular sugar concentrations).

Also a dilution of the cultures was made to measure the optical density at 600 nm. The cell performance index or CPI was determined by dividing the fucosyllactose concentrations measured in the whole broth by the biomass, in relative percentages compared to the reference strain. The biomass is empirically determined to be approximately $1/3^{\text{rd}}$ of the optical density measured at 600 nm. The fucosyllactose export ratio was determined by dividing the fucosyllactose concentrations measured in the supernatant by the fucosyllactose concentrations measured in the whole broth, in relative percentages compared to the reference strain.

A preculture for the bioreactor was started from an entire 1 mL cryovial of a certain strain, inoculated in 250 mL or 500 mL of minimal medium in a 1 L or 2.5 L shake flask and incubated for 24 h at 37°C on an orbital shaker at 200 rpm. A 5 L bioreactor was then inoculated (250 mL inoculum in 2 L batch medium); the process was controlled by MFCS control software (Sartorius Stedim Biotech, Melsungen, Germany). Culturing condition were set to 37 °C, and maximal stirring; pressure gas flow rates were dependent on the strain and bioreactor. The pH was controlled at 6.8 using 0.5 M H₂SO₄ and 20% NH₄OH. The exhaust gas was cooled. 10% solution of silicone antifoaming agent was added when foaming raised during the fermentation.

Optical density

Cell density of the cultures was frequently monitored by measuring optical density at 600 nm (Implen Nanophotometer NP80, Westburg, Belgium or with a Spark 10M microplate reader, Tecan, Switzerland).

Productivity

The specific productivity Q_p is the specific production rate of the fucosyllactose product, typically expressed in mass units of product per mass unit of biomass per time unit (= g fucosyllactose / g biomass / h). The Q_p value has been determined for each phase of the fermentation runs, i.e. Batch and Fed-Batch phase, by measuring both the amount of product and biomass formed at the end of each phase and the time frame each phase lasted.

The specific productivity Q_s is the specific consumption rate of the substrate, e.g. sucrose, typically expressed in mass units of substrate per mass unit of biomass per time unit (= g sucrose / g biomass / h). The Q_s value has been determined for each phase of the fermentation runs, i.e. Batch and Fed-Batch phase, by measuring both the total amount of sucrose consumed and biomass formed at the end of each phase and the time frame each phase lasted.

The yield on sucrose Y_s is the fraction of product that is made from substrate and is typically expressed in mass unit of product per mass unit of substrate (= g fucosyllactose / g sucrose). The Y_s has been determined for each phase of the fermentation runs, i.e. Batch and Fed-Batch phase, by measuring both the total amount of fucosyllactose produced and total amount of sucrose consumed at the end of each phase.

The yield on biomass Y_x is the fraction of biomass that is made from substrate and is typically expressed in mass unit of biomass per mass unit of substrate (= g biomass / g sucrose). The Y_p has been determined for each phase of the fermentation runs, i.e. Batch and Fed-Batch phase, by measuring both the total amount of biomass produced and total amount of sucrose consumed at the end of each phase.

The rate is the speed by which the product is made in a fermentation run, typically expressed in concentration of product made per time unit (= g fucosyllactose / L / h). The rate is determined by measuring the concentration of fucosyllactose that has been made at the end of the Fed-Batch phase and dividing this concentration by the total fermentation time.

The lactose conversion rate is the speed by which lactose is consumed in a fermentation run, typically expressed in mass units of lactose per time unit (= g lactose consumed / h). The lactose conversion rate is determined by measurement of the total lactose that is consumed during a fermentation run, divided by the total fermentation time.

Growth rate/speed measurement

The maximal growth rate (μ_{Max}) was calculated based on the observed optical densities at 600nm using the R package grofit.

Liquid chromatography

Standards for 2'-fucosyllactose, 3-fucosyllactose and 2', 3-difucosyllactose were synthesized in house. Other standards such as but not limited to lactose, sucrose, glucose, glycerol, fructose were purchased from Sigma. Carbohydrates were analyzed via a HPLC-RI (Waters, USA) method, whereby RI (Refractive Index) detects the change in the refraction index of a mobile phase when containing a sample. The sugars were separated in an isocratic flow using an X-Bridge column (Waters X-bridge HPLC column, USA) and a mobile phase containing 75 ml acetonitrile and 25 ml Ultrapure water and 0.15 ml triethylamine. The column size was 4.6 x 150mm with 3.5 μ m particle size. The temperature of the column was set at 35°C and the pump flow rate was 1 mL/min.

Normalization of the data

For all types of cultivation conditions, data obtained from the mutant strains was normalized against data obtained in identical cultivation conditions with reference strains having an identical genetic background as the mutant strains but lacking the membrane protein expression cassettes. The dashed horizontal line on each plot that is shown in the examples, indicates the setpoint to which all adaptations were normalized. All data is given in relative percentages to that setpoint.

Example 4: Membrane proteins identified that enhance 3-fucosyllactose (3-FL) production in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate membrane proteins for their ability to enhance fucosyllactose production of a host cell growing in minimal media supplemented with 20 g/L lactose. The membrane proteins with SEQ ID NO 02, 04, 06, 18, 20, 22, 26, 28, 30, 32, 34, 40, 42, 44, 58, 62, 64, 66, 70, 72, 74, 82, 84, 90, 92, 94 and 96 showed that they are able to enhance 3-FL production that is being produced in a 3-FL production host expressing the α 1,3-fucosyl transferases FT1 or FT2. Candidate genes were combined in transcriptional unit TU2, TU3 or TU10 and presented to the 3-FL production hosts on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 1 presents the CPI of the strains, in relative percentages compared to the respective reference strain.

Example 5: Membrane proteins identified that enhance 3-FL secretion in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate membrane proteins for their ability to enhance fucosyllactose secretion of a host cell growing in minimal media supplemented with 20 g/L lactose. The membrane proteins with SEQ ID NO 02, 08, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30, 34, 58, 62, 64, 66, 70, 72, 74, 76, 82, 84, 90, 92, 94, 96 and 104 showed that they are able to enhance secretion of 3-FL that is being produced intracellularly in a 3-FL bacterial production host expressing the α 1,3-fucosyl transferases FT1 or FT2. Candidate genes were combined in transcriptional unit TU2, TU3 or TU10 and presented to the 3-FL production hosts on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 2 demonstrates the export ratio of 3-FL in the strains, in relative percentages compared to the respective reference strain.

Example 6: Membrane proteins identified that enhance growth speed in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate membrane proteins for their ability to influence growth speed of a host cell growing in minimal media supplemented with 20 g/L lactose. Membrane proteins with SEQ ID NO 08, 14, 18 and 22 showed to be able to enhance the growth speed of a 3-FL production host expressing the α 1,3-fucosyl transferase FT1 or FT2. Candidate genes were combined in transcriptional unit TU2, TU3 or TU10 and presented to the 3-FL production hosts on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 3 demonstrates the growth speed of the strains, in relative percentages compared to the respective reference strain.

Example 7: Membrane protein identified that enhances 3-FL secretion in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 45 g/L lactose

An experiment was set up to evaluate the effectiveness of a range of identified membrane proteins to enhance fucosyllactose secretion by a host cell growing in minimal media supplemented with 45 g/L lactose. Membrane protein with SEQ ID NO 28 showed to be able to enhance 3-FL secretion in a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT1. The gene with SEQ ID NO 27 was combined in transcriptional unit TU10 and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3 using minimal medium supplemented with 45 g/L lactose. Figure 4 shows the CPI of the strain, in relative percentages compared to the respective reference strain.

Example 8: Membrane protein identified that enhances 3-FL production in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 90 g/L lactose

An experiment was set up to evaluate the ability of a range of membrane proteins to enhance fucosyllactose production in minimal media supplemented with 90 g/L lactose. The membrane protein with SEQ ID NO 28 showed to be able to enhance 3-FL production in a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT1. The gene with SEQ ID NO 27 was combined in transcriptional unit TU10 and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3 using minimal medium supplemented with 90 g/L lactose. Figure 5 shows the CPI, in relative percentages compared to the respective reference strain.

Example 9: Membrane proteins identified that increase 3-FL secretion in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 90 g/L lactose

An experiment was set up to evaluate the ability of a range of membrane proteins to increase fucosyllactose secretion by a host cell growing in minimal media supplemented with 90 g/L lactose. The membrane proteins with SEQ ID NO 10 and 16 showed to be able to enhance 3-FL secretion in a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT1. The candidate genes were combined in transcriptional unit TU10 and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3 using minimal medium supplemented with 90 g/L lactose. Figure 6 demonstrates the export ratio of 3-FL, in relative percentages compared to the respective reference strain.

Example 10: Membrane proteins identified that increase 3-FL production in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 100 g/L sucrose and 90 g/L lactose

5 An experiment was set up to evaluate the ability of a range of membrane proteins for their ability to increase fucosyllactose production in a host cell growing in minimal media supplemented with 100 g/L sucrose and 90/L of lactose. The membrane proteins with SEQ ID NO 10, 16 and 28 showed to be able to enhance 3-FL production in a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT1. All candidate genes were combined in transcriptional unit TU10 and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was
10 performed according to the cultivation conditions provided in Example 3 using minimal medium supplemented with 100 g/L sucrose and 90 g/L lactose. Figure 7 demonstrates the CPI, in relative percentages compared to the respective reference strain.

Example 11: Membrane protein identified that increases the growth speed of E. coli hosts when cultivated 72 h in a growth experiment in minimal media supplemented with 100 g/L sucrose and 90 g/L lactose

15 An experiment was set up to evaluate membrane proteins for their ability to influence growth speed of a host cell growing in minimal media supplemented with 100 g/L sucrose and 90/L of lactose. The membrane protein with SEQ ID NO 28 showed to be able to enhance the growth speed of a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT1. The gene with SEQ ID NO 27 was combined in transcriptional unit TU10 and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3 using minimal medium supplemented with 100 g/L sucrose and 90 g/L lactose. Figure 8 demonstrates the growth speed, in relative percentages
20 compared to the respective reference strain.

Example 12: Membrane protein identified that increases 3-FL production in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 5 g/L lactose

30 An experiment was set up to evaluate the ability of membrane proteins for their ability to influence fucosyllactose production by a host cell growing in minimal media supplemented with 5 g/L lactose. The membrane protein with SEQ ID NO 22 showed that it is able to enhance 3-FL production in a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT1. The gene with SEQ ID NO 21 was combined in transcriptional unit TU10 and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3 using minimal medium supplemented with 5 g/L
35 lactose. Figure 9 demonstrates the CPI, in relative percentages compared to the respective reference strain.

Example 13: Membrane proteins identified that, when integrated in the host's genome, increase 3-FL production and/or secretion in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

Another series of experiments was set up to evaluate the ability of membrane proteins integrated in the genome to increase fucosyllactose production and/or secretion in/by a host cell cultivated for 72h in minimal media supplemented with 20g/L lactose. The membrane proteins with SEQ ID NO 02 and 28 showed that they are able to enhance 3-FL production and/or secretion of 3-FL that is being produced intracellularly in a 3-FL production host either expressing the α 1,3-fucosyl transferase enzymes FT1 or FT2. The genes with SEQ ID NO 01 and 27 were combined in the transcriptional unit TU10 and presented to the genome of the 3-FL production hosts as genomic KI in the *EcLdhA* or the *EcSetA* locus, respectively. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 10 demonstrates the CPI whereas Figure 11 shows the 3-FL export, both times in relative percentages compared to the respective reference strain.

Example 14: Membrane proteins that independently from the transcriptional unit they are cloned in, enhance 3-FL production and/or 3-FL secretion in an E. coli host

Another series of experiments was set up to evaluate the ability of membrane proteins to influence fucosyllactose production and/or secretion, of a host cell. In this example also several transcriptional units were used for cloning. The membrane proteins with SEQ ID NO 02, 06, 10, 16, 22, 28, 32, 34, 36, 38, 40, 42, 44 and 50 showed that they are able to enhance 3-FL production and/or secretion of 3-FL that is being produced intracellularly in a 3-FL production host by the α 1,3-fucosyl transferase enzyme FT2. The exporter genes were cloned in different transcriptional units and presented as clonal vector (pSC101 ori) to the 3-FL production host. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 12 demonstrates the CPI whereas Figure 13 shows the 3-FL export, both times in relative percentages compared to the respective reference strain.

Example 15: Membrane proteins identified that, when cloned in their native transcriptional operon structure, enhance 3-FL production and/or 3-FL secretion in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate the ability of membrane proteins to enhance fucosyllactose production and/or secretion of a host cell cultivated for 72h in minimal media supplemented with 20 g/L lactose. This time the membrane proteins were cloned in their native transcriptional operon structure. The membrane proteins with SEQ ID NO 40, 42, 46 and 48 showed to be able to enhance 3-FL production and/or 3-FL secretion in a 3-FL production host expressing the α 1,3-fucosyl transferase enzyme FT2. All candidate exporters genes were cloned either in TU10 as single genes or in their native transcriptional operon structure containing 2 membrane protein

genes and presented to the 3-FL production host on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 14 shows the CPI (left panel) and the 3-FL export ratio (right panel), in relative percentages compared to the respective reference strain.

5

Example 16: Membrane proteins identified that enhance 2'-FL and/or DiFL production, and/or DiFL secretion in a 2'-FL *E. coli* host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate the ability of a range of membrane proteins to enhance fucosyllactose production and/or secretion of a host cell cultivated for 72h in minimal media supplemented with 20 g/L lactose. This time the membrane proteins were tested for 2'-FL and/or diFL production. The membrane proteins with SEQ ID NO 02, 06 and 28 showed to be able to enhance 2'-FL and/or DiFL production and/or DiFL secretion in a 2'-FL production host expressing the α 1,2-fucosyltransferase FT3. Candidate genes were cloned in different TUs and presented on the genome of the 2'-FL production host, using either the *SetA* locus (for the gene with SEQ ID NO 27) or the *IdhA* locus (for the gene with SEQ ID NO 01 and 05). A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 15 shows the CPI for the hosts with 2'-FL (panel A) or DiFL (panel B) production and the DiFL export ratio (panel C), every time in relative percentages compared to the respective reference strain.

20

Example 17: The membrane protein MdfA enhances the productivity of a 3-FL production *E. coli* host in (30L) fermentation runs.

A 3-FL producing *E. coli* host having the membrane protein gene with SEQ ID NO NO 01 expressed in TU1 and presented in the host's genome in the *EcdhA* locus, and expressing the α 1,3-fucosyl transferase FT2 from plasmid was evaluated for its productivity in bioreactor settings. Eight fermentation runs were performed according to the conditions provided in Example 3. Also, a reference strain identical to the 3-FL production host but lacking the membrane protein gene was analyzed in identical fermentation settings. Figure 16 demonstrates the enhanced productivity of the strain over-expressing the membrane protein EcMdfA with SEQ ID NO 02 in the eight different fermentation runs, relatively compared to this reference strain.

30

Example 18: The membrane protein IceT enhances the productivity of a 3-FL production *E. coli* host in (30L) fermentation runs.

Another 3-FL production *E. coli* host expressing a membrane protein was evaluated for its productivity in 30L bioreactors. The 3-FL strain had the membrane protein gene EcIceT with SEQ ID NO 05 expressed in TU3 from a first plasmid and the α 1,3-fucosyl transferase FT2 expressed from a second plasmid. A specific reference strain identical to the 3-FL production hosts but lacking the membrane protein gene construct was used to analyze the 3-FL productivity in

35

identical fermentation settings. Figure 17 demonstrates the enhanced productivity of the strain over-expressing the membrane protein relatively compared to the specific reference strain.

Example 19: Membrane proteins homologous to EcMdfA or EclceT enhance 3-FL production, and/or 3-FL secretion, and/or growth speed in a 3-FL *E. coli* host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate the membrane protein with SEQ ID NO 120 (homologous to EclceT with SEQ ID NO 06), and membrane proteins with SEQ ID NO 126, 128, 140, 146 and 150 (homologous to membrane protein EcMdfA with SEQ ID NO 02) that are able to enhance 3-FL production and/or secretion of 3-FL that is being produced intracellularly in a 3-FL production host by the α 1,3-fucosyl transferase enzymes FT1 or FT2 and/or that improve growth speed of the 3-FL *E. coli* host. The exporter genes were cloned in different transcriptional units and presented as clonal vector (pSC101 ori) to the 3-FL production host. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 18 demonstrates the CPI whereas Figure 19 shows the 3-FL export and Figure 20 presents the strains with elevated growth speed, each time in relative percentages compared to the respective reference strains.

Example 20: Calculation of global percentage identity between polypeptide sequences.

Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch ((1970) J Mol Biol 48: 443-453) to find the global (i.e. spanning the complete sequences) alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. The BLAST algorithm (Altschul et al. (1990) J Mol Biol 215: 403-10) calculates percentage sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (NCBI). Homologues may readily be identified using, for example, the ClustalW multiple sequence alignment algorithm (version 1.83), with the default pairwise alignment parameters, and a scoring method in percentage. Global percentages of similarity and identity may also be determined using one of the methods available in the MatGAT software package (Campanella et al., BMC Bioinformatics. 2003 Jul 10;4:29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences.). Minor manual editing may be performed to optimize alignment between conserved motifs, as would be apparent to a person skilled in the art. Furthermore, instead of using full-length sequences for the identification of homologues, specific domains may also be used. The sequence identity values may be determined over the entire nucleic acid or amino acid sequence or over selected domains or conserved motif(s), using the programs mentioned above using the

default parameters. For local alignments, the Smith-Waterman algorithm is particularly useful (Smith TF, Waterman MS (1981) J. Mol. Biol 147(1);195-7).

Global percentages of similarity and identity between full length polypeptide sequences useful in performing the methods of the invention were determined using MatGAT (Matrix Global Alignment

5 Tool) software (BMC Bioinformatics. 2003 4:29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences. Campanella JJ, Bitincka L, S malley J; software hosted by Ledion Bitincka). MatGAT generates similarity/identity matrices for DNA or protein sequences without needing pre-alignment of the data. The program performs a series of pair-wise alignments using the Myers and Miller global alignment algorithm, calculates similarity and identity, and then places the results in a distance matrix. 1. CYP704-like polypeptides

10 Results of an exemplary analysis are shown in Figures 21 and 22 for the global identity over the full length of the polypeptide sequences relating to EcMdfA (SEQ ID NO 2) and EclceT (SEQ ID NO 6). Sequence identity is shown in the top half of the diagonal dividing line. Parameters used in the comparison were: Scoring matrix: Blosum62, First Gap: 12, Extending Gap: 2. The sequence identity (in percent) between the EcMdfA membrane protein and its homologs useful in performing the methods of the invention is generally higher than 80%. The sequence identity in percent between the EclceT membrane protein and its homologs useful in performing the methods of the invention is generally higher than 80%.

20 Example 21: Membrane proteins identified that enhance 3-fucosyllactose (3-FL) production in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate membrane proteins for their ability to enhance fucosyllactose production of a host cell growing in minimal media supplemented with 20 g/L
25 lactose. The membrane proteins with SEQ ID NO 52, 54, 56, 58, 60, 62, 64, 66, 70, 72, 74, 76, 80, 82, 84, 88, 90, 92, 94, 96, 98, 104, 184, 204 and 208 showed that they are able to enhance 3-FL production that is being produced in a 3-FL production host expressing the α 1,3-fucosyltransferase FT2. Candidate genes were combined in transcriptional unit TU1, TU2, TU3, TU11 or TU12 and presented to the 3-FL production hosts on a pSC101 plasmid. A growth
30 experiment was performed according to the cultivation conditions provided in Example 3. Figure 23 presents the CPI of the strains, in relative percentages compared to the respective reference strain.

35 Example 22: Membrane proteins identified that enhance 2'-fucosyllactose (2'-FL) production in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate membrane proteins for their ability to enhance fucosyllactose production of a host cell growing in minimal media supplemented with 20 g/L

lactose. The membrane proteins with SEQ ID NO 204 and 214 showed that they are able to enhance 2'-FL production that is being produced in a 2'-FL production host expressing the α 1,2-fucosyltransferase FT4. Candidate genes were combined in transcriptional unit TU11 and presented to the 2'-FL production hosts on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 24 presents the CPI of the strains, in relative percentages compared to the respective reference strain.

Example 23: Membrane proteins identified that enhance 2'-FL secretion in an E. coli host cultivated 72 h in a growth experiment in minimal media supplemented with 20 g/L lactose

An experiment was set up to evaluate membrane proteins for their ability to enhance fucosyllactose secretion of a host cell growing in minimal media supplemented with 20 g/L lactose. The membrane proteins with SEQ ID NO 206, 208, 214, 216 and 218 showed that they are able to enhance secretion of 2'-FL that is being produced intracellularly in a 2'-FL bacterial production host expressing the α 1,2-fucosyltransferase FT4. Candidate genes were combined in transcriptional unit TU11 and presented to the 2'-FL production hosts on a pSC101 plasmid. A growth experiment was performed according to the cultivation conditions provided in Example 3. Figure 25 demonstrates the export ratio of 2'-FL in the strains, in relative percentages compared to the respective reference strain.