Claims

1. A method to produce microorganisms which resist the phenomenon of lactose killing when grown in an environment in which lactose is combined with another carbon source, wherein said method comprises:

a. altering the expression of a lactose transporter within microorganisms by mutation comprising one or more of: introducing a heterologous promoter in front of an endogenous or exogenous lactose transporter gene; mutating the untranslated region in front of the coding sequence that contains the ribosome binding or Kozak sequences; and modifying the codon usage of the endogenous lactose transporter gene,
b. growing said mutated microorganisms on a medium comprising a carbon-source which is not lactose,
c. adding lactose to said medium during growth of said mutated microorganisms, and
d. selecting the microorganisms which resist the phenomenon of lactose killing growing on said medium comprising lactose and which retain at least 50% of the lactose influx obtained with the wild type expression cassette of said lactose transporter.

2. A method according to claim 1 wherein said introduction of a heterologous promoter in front of an endogenous or exogenous lactose transporter gene is undertaken by: a) deleting the endogenous lactose transporters from the genome and reintroducing them at another location within the genome of said microorganism, or, b) by introducing a heterologous promoter in front of the endogenous lactose transporters, or, c) by knocking out the endogenous lactose promoter and introducing a heterologous promoter at the same location in the genome of said microorganism.

3. A method according to claims 1-2 wherein said expressed lactose transporter is detected via translational coupling with a reporter gene and/or via aptamer coupling.

4. A method according to claims 1-3wherein said expressed lactose transporter is detected via genetic constructs as given in by SEQ ID no 2, 3, 4 and /or 5.

5. A method according to claims 1-4 wherein said lactose transporter is a lactose permease.

6. A method according to claims 1-5 wherein said microorganism is a bacterium, a yeast or a fungus cell.

7. A lactose transporter expression cassette that leads to the expression of a lactose transporter and that does not result into lactose killing phenotype,, wherein said expression cassette comprises a sequence as given by SEQ ID no 1, 7, 8, 12, 13, 14, 15, 16, 17, 18, 19,20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 57.

8. A microorganism wherein the expression of a lactose transporter has been altered within said microorganism, which microorganism resists the phenomenon of lactose killing when grown in an environment in which lactose is combined with (an)other carbon source(s) and which retains at least 50% of the lactose influx obtained with the wild type expression cassette of said lactose transporter, and, is obtainable by a method according to claims 1-6, wherein said microorganism comprises a lactose transporter expression cassette comprising a sequence as given by SEQ ID no 1, 7, 8, 12, 13, 14, 15, 16, 17, 18, 19,20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 57.

9. A microorganism according to claim 8 in which the genes coding for the enzymes from the lactose and/or galactose degradation pathways are rendered less functional or non-functional.

10. Use of a microorganism and/or a lactose transporter expression cassette according to claims 8-9 and/or claim 7, respectively for the production of lactose- and/or galactose-based specialty products.

11. Use of a microorganism and/or a lactose transporter expression cassette according to claim 10 wherein said lactose-based specialty products are specialty carbohydrates, glycolipids and galactosylated compounds.

12. Use of a microorganism according to claim 11 wherein said specialty carbohydrates are 2-fucosyllactose or 2'-fucosyllactose or 3-fucosyllactose or 2',3-difucosyllactose or lacto*N*triose or lacto-N-tetraose, or lacto-nN-tetraose or 3'sialyllactose or 6'sialyllactose.

13. A method for producing a lactose transporter expression cassette that does not result in lactose killing, comprising:

- creating mutants of a lactose transporter expression cassette;

- screening the mutant expression cassettes for expression cassettes that ensure lactose transporter expression in a microorganism transformed with the expression cassette;

- screening the mutant expression cassettes that ensure lactose transporter expression for expression cassettes that do not lead to lactose killing in a microorganism transformed with the expression cassette by growing microorganisms that have been transformed with the expression cassettes in a medium to which lactose is added during the mid exponential phase; and

isolating the expression cassette from the transformed microorganisms that resist the phenomenon of lactose killing.

14. The method according to claim 13, wherein lactose transporter expression from the expression cassette is detected via translational coupling with a reporter gene and/or via aptamer coupling.

15. Use of a lactose transporter expression cassette produced by the method according to claim 13 or 14, for the production of lactose- and/or galactose-based specialty products.

16. Use according to claim 15, wherein said lactose-based specialty products are specialty carbohydrates, glycolipids and galactosylated compounds.

17. Use according to claim 16, wherein said specialty carbohydrates are 2-fucosyllactose or 2'-fucosyllactose or 3-fucosyllactose or 2',3-difucosyllactose or lacto*N*triose or lacto-N-tetraose, or lacto-nN-tetraose or 3'sialyllactose or 6'sialyllactose.