[0014] Thus, in one aspect, the present invention relates to a method for growing an attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette, comprising the step of culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH value at fermentation scale, wherein the amount of glucose in the medium during the fermentation is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase,

wherein no glucose is added to the medium during the fermentation,

wherein the starting glucose concentration is from about 0 g/l to about 3 g/l,

and wherein the volume of the medium is at least about 10 l.

~~a method for growing an attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising an expression cassette, comprising the step of culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH value at fermentation scale, wherein the amount of glucose in the medium during the fermentations is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase.~~

[0015] In a particular embodiment, no glucose is added to the medium during the fermentation and the starting amount of glucose is depleted before reaching the stationary phase.

[0016] In a particular embodiment, said attenuated mutant strain of Salmonella typhi is Salmonella typhi Ty21a.

[0017] In a particular embodiment, the expression cassette is a eukaryotic expression cassette. In a particular embodiment, the expression cassette encodes a VEGF receptor protein. In a particular embodiment, the VEGF receptor protein is selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith. In a preferred embodiment, human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

[0018] In a particular embodiment, the buffered medium comprises peptone of non-animal origin. In a preferred embodiment, said buffered medium is Tryptic Soy Broth (TSB) of non-animal origin.

[0026] In a particular embodiment, the cells are harvested before reaching an optical density of about 6, particularly at an optical density of about 5 to about 6.

[0027] In a particular embodiment, the attenuated mutant strain of Salmonella typhi is Salmonella typhi Ty21a and the recombinant DNA molecule comprises the kanamycin resistance gene, the pMB1 ori, and a eukaryotic expression cassette encoding human VEGFR-2, under the control of the CMV promoter. In a particular embodiment, human VEGFR-2 has the nucleic acid sequence as found in SEQ ID NO 2.

[0028] In another aspect, the present invention relates to an attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette,

obtainable by a method for growing the strain, comprising the step of culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH at fermentation scale,

wherein the amount of glucose in the medium during the fermentation is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase,

wherein no glucose is added to the medium during the fermentation,

wherein the starting glucose concentration is from about 0 g/l to about 3 g/l,

wherein the volume of the medium is at least about 10 l.~~an attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising an expression cassette, obtainable by a method for growing the strain, comprising the step of culturing the strain in a buffered medium comprising peptone at about neutral starting pH value at fermentation scale, wherein the amount of glucose in the medium during the fermentations is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase.~~

[0029] In a particular embodiment, no glucose is added to the medium during the fermentation and the starting amount of glucose is depleted before reaching the stationary phase.

[0030] In a particular embodiment, the expression cassette is a eukaryotic expression cassette encoding a VEGF receptor protein. In a particular embodiment, the eukaryotic

expression cassette encodes a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least

CLAIMS

1. A method for growing an attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette, comprising the step of culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH value at fermentation scale, wherein the amount of glucose in the medium during the fermentation is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase, wherein no glucose is added to the medium during the fermentation, and wherein the starting glucose concentration is from about 0 g/l to about 3 g/l.

2. The method of claim 1, wherein said attenuated mutant strain of Salmonella typhi is Salmonella typhi Ty21a.

3. The method of any one of claims 1 or 2, wherein the eukaryotic expression cassette encodes a VEGF receptor protein, preferably a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

4. The method of any one of claims 1 to 3, wherein the buffered medium comprises peptone of non-animal origin, preferably wherein said buffered medium is Tryptic Soy Broth (TSB) of non-animal origin.

5. The method of any one of claims 1 to 4, wherein the volume of the medium is at least about 10 l, preferably from about 10 l to about 10.000 l, more preferably from about 30 l to about 1.000 l, most preferably from about 100 l to about 500 l.

6. The method of any one of claims 1 to 5, wherein the starting glucose concentration corresponds to that of bacterial minimal medium or less.

7. The method of any one of claims 1 to 6, wherein the starting pH value is from about 5 to less than about 9, preferably from about 6 to about 8, more preferably from about 6.5 to about 7.5.

8. The method of any one of claims 1 to 7, wherein the pH value is adjusted during said culturing to a pH value of about 6 to about 8, preferably to a pH value of about 6.5 to about 7.5.

9. The method of any one of claims 1 to 8, wherein the progress of growth is determined by (i) measuring the optical density (OD), preferably by (ia) in-situ monitoring of the optical density of the culture or by (ib) taking samples and measuring the optical density of the samples, or by (ii) measuring the cell density, preferably (iia) microscopically or (iib) by measuring the electrical resistance, or (iic) by flow cytometry, or by (iii) measuring the colony forming units (CFU) value by taking samples and plating on agar plates.

10. The method of any one of claims 1 to 9, wherein the cells are harvested before reaching an optical density of about 6, preferably at an optical density of about 5 to about 6.

11. The method of any one of claims 1 to 10, wherein the attenuated mutant strain of Salmonella typhi is Salmonella typhi Ty21a and the recombinant DNA molecule comprises the kanamycin resistance gene, the pMB1 ori, and a eukaryotic expression cassette encoding human VEGFR-2, under the control of the CMV promoter, particularly wherein human VEGFR-2 has the nucleic acid sequence as found in SEQ ID NO 2.

12. An attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette,
obtainable by a method for growing the strain, comprising the step of
culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH at fermentation scale,
wherein the amount of glucose in the medium during the fermentation is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase,
wherein no glucose is added to the medium during the fermentation, and
wherein the starting glucose concentration is from about 0 g/l to about 3 g/l.

13. The attenuated mutant strain of claim 12, wherein the eukaryotic expression cassette encodes a VEGF receptor protein, preferably a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

14. The attenuated mutant strain of claim 13, wherein the attenuated mutant strain is Salmonella typhi Ty21a and the recombinant DNA molecule comprises the kanamycin resistance gene, the pMB1 ori, and an eukaryotic expression cassette encoding human

VEGFR-2 under the control of the CMV promoter, particularly wherein human VEGFR-2 has the nucleic acid sequence as found in SEQ ID NO 2.

15. An attenuated mutant strain of Salmonella typhi Ty21a comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette encoding a VEGF receptor protein, preferably a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1, for use as a vaccine.

CLAIMS

1. A method for growing an attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette, comprising the step of culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH value at fermentation scale, wherein the amount of glucose in the medium during the fermentation is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase,

   wherein no glucose is added to the medium during the fermentation,

   wherein the starting glucose concentration is from about 0 g/l to about 3 g/l,

   and wherein the volume of the medium is at least about 10 l.

2. The method of claim 1, wherein said attenuated mutant strain of Salmonella typhi is Salmonella typhi Ty21a.

3. The method of any one of claims 1 or 2, wherein the eukaryotic expression cassette encodes a VEGF receptor protein, preferably a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

4. The method of any one of claims 1 to 3, wherein the buffered medium comprises peptone of non-animal origin, preferably wherein said buffered medium is Tryptic Soy Broth (TSB) of non-animal origin.

5. The method of any one of claims 1 to 4, wherein the volume of the medium is from about 10 l to about 10.000 l, preferably from about 30 l to about 1.000 l, more preferably from about 100 l to about 500 l.

6. The method of any one of claims 1 to 5, wherein the starting glucose concentration corresponds to that of bacterial minimal medium or less.

7. The method of any one of claims 1 to 6, wherein the starting pH value is from about 5 to less than about 9, preferably from about 6 to about 8, more preferably from about 6.5 to about 7.5.

8. The method of any one of claims 1 to 7, wherein the pH value is adjusted during said culturing to a pH value of about 6 to about 8, preferably to a pH value of about 6.5 to about 7.5.

9. The method of any one of claims 1 to 8, wherein the progress of growth is determined by (i) measuring the optical density (OD), preferably by (ia) in-situ monitoring of the optical density of the culture or by (ib) taking samples and measuring the optical density of the samples, or by (ii) measuring the cell density, preferably (iia) microscopically or (iib) by measuring the electrical resistance, or (iic) by flow cytometry, or by (iii) measuring the colony forming units (CFU) value by taking samples and plating on agar plates.

10. The method of any one of claims 1 to 9, wherein the cells are harvested before reaching an optical density of about 6, preferably at an optical density of about 5 to about 6.

11. The method of any one of claims 1 to 10, wherein the attenuated mutant strain of Salmonella typhi is Salmonella typhi Ty21a and the recombinant DNA molecule comprises the kanamycin resistance gene, the pMB1 ori, and a eukaryotic expression cassette encoding human VEGFR-2, under the control of the CMV promoter, particularly wherein human VEGFR-2 has the nucleic acid sequence as found in SEQ ID NO 2.

12. An attenuated mutant strain of Salmonella typhi lacking galactose epimerase activity and comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette,

obtainable by a method for growing the strain, comprising the step of

culturing the strain in a buffered medium comprising peptone at approximately neutral starting pH at fermentation scale,

wherein the amount of glucose in the medium during the fermentation is adjusted such that the amount of glucose is reduced to zero before reaching the stationary phase,

wherein no glucose is added to the medium during the fermentation,

wherein the starting glucose concentration is from about 0 g/l to about 3 g/l,

wherein the volume of the medium is at least about 10 l.

13. The attenuated mutant strain of claim 12, wherein the eukaryotic expression cassette encodes a VEGF receptor protein, preferably a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

14. The attenuated mutant strain of claim 13, wherein the attenuated mutant strain is Salmonella typhi Ty21a and the recombinant DNA molecule comprises the kanamycin resistance gene, the pMB1 ori, and an eukaryotic expression cassette encoding human

VEGFR-2 under the control of the CMV promoter, particularly wherein human VEGFR-2 has the nucleic acid sequence as found in SEQ ID NO 2.

15. An attenuated mutant strain of Salmonella typhi Ty21a comprising at least one copy of a recombinant DNA molecule comprising a eukaryotic expression cassette encoding a VEGF receptor protein, preferably a VEGF receptor protein selected from the group consisting of human VEGFR-2 and a homolog thereof that shares at least about 80% homology therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1, for use as a vaccine.