Claims

1. Method for producing a heterologous polypeptide in a recombinant bacterial host cell,
wherein a recombinant bacterial host cell is used, whose catabolism of carbon sources is under the control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source, and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter, which is inducible by the secondary carbon source and is regulated by the transcriptional regulator protein,
said method comprising the steps of growing the bacterial host cell in a cell culture medium, that does not comprise the inducing secondary carbon source but a different carbon source, inducing the expression of said polypeptide by the different carbon source at a time, when the concentration of the different carbon source decreases under a level necessary for carbon catabolite repression,
and recovering the polypeptide from the cells or from the cell culture,

2. Method according to claims 1 or 2,
wherein a bacterial host cell is used, in which by genetical alteration deactivation of the transcriptional regulator protein by phosphorylation by the enzyme EII, specific for said transcriptional regulator protein, is prevented.

3. Method according to claim 3,
wherein i) in the genome of the bacterial host cell the gene is deleted which encodes for the phosphoryl group transferring enzyme EII specific for the transcriptional regulator protein, or
wherein ii) in the genome of the bacterial host cell the gene encoding for the transcriptional regulator protein is genetically altered so that the transcriptional regulator protein expressed by said gene is incapable of binding a phosphoryl group transferred from enzyme EII.

4. Method according to claim 4,
wherein in case i) into the vector is integrated the gene encoding for the transcriptional regulator protein controlling the promoter of the vector; or
wherein in case ii) into the vector is integrated the genetically manipulated gene of the bacterial host cell encoding for the transcriptional regulator protein incapable of binding a phosphoryl group transferred from the corresponding enzyme EII.

5. Method according to any of the claims 1 to 5,
wherein the bacterial host cell is selected from Bacilli, Chlostridia and Escherichia.

6. Method according to any of the claims 1 and 5,
wherein the culture medium comprises casamino acids.

7. Method according to any of the claims 1 to 7,
wherein expression of the polypeptide is started without induction by the inducer.

8. Method according to any of the claims 1 to 8,
wherein said at least carbon source is added to the culture in an amount sufficient to maintain a pre-determined growth rate of the bacterial host cell without induction of carbon catabolite repression.

9. Method according to any of the claims 1 to 8,
wherein the carbon source inducible promoter is a promoter of the mannose operon and wherein the inducing carbon source is mannose.

10. Method according to claim 10,
wherein the promoter is the P_{manP} or the P_{manR} promoter of the mannose operon.

11. Method according to claim 11,
wherein i) in the genome of the bacterial host cell the gene encoding for manP is deleted, or
wherein ii) in the genome of the bacterial host cell the gene encoding for the transcriptional regulater protein ManR is genetically altered so that ManR expressed by said gene is incapable of binding a phosporyl group transferred from the specific enzyme EII.

12. Method for the production of a heterologous polypeptide by culturing a recombinant bacterial host cell, wherein a recombinant bacterial host cell is used whose catabolism of carbon sources is under control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system, and which is genetically altered such, that it is incapable of metabolizing the inducing carbon source of a carbon source inducible promoter, wherein the inducing carbon source is a secondary carbon source for the bacterial host cell, and which comprises a vector with the promoter inducible by said secondary carbon source and a heterologous nucleic acid sequence encoding a polypeptide operably linked to the promoter,
wherein the process is a fed-batch process, wherein after the batch phase induction is started by adding a first portion of the inducing secondary carbon source and, simultaneously, feeding of a second portion is started.

13. Method according to claim 13,
wherein in the genome of the bacterial host cell the gene encoding for the inducer specific 6-phosphate isomerase is deleted.

14. Method according to claim 14,
wherein this gene is the gene *manA* of the mannose operon.

15. Method of the claims 1 to 15,
wherein the bacterial host cell is selected from *Bacillus*.

16. Recombinant bacterial host cell whose catabolism of carbon sources is under control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system, and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source by deleting in the genome of the bacterial host cell the gene which encodes for a phosporyl group transferring enzyme EII specific for the transcriptional regulator protein, and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter, which is regulated by the transcriptional regulator protein for which the bacterial host cell is genetically altered to be incapable of deactivation, and wherein into the vector is integrated the gene encoding for the specific transcriptional regulator protein.

17. Recombinant bacterial host cell, whose catabolism of carbon sources is under control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system,
and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source by genetically altering in the genome of the bacterial host cell the gene encoding for the transcriptional regulator protein so that the transcriptional regulator protein expressed by said gene is incapable of binding a phosphoryl group transferred by enzyme EII, specific for said transcriptional regulator protein,
and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter regulated by the transcriptional regulator protein for which the bacterial host cell is genetically altered to be incapable of deactivation, and
wherein into the vector is additionally integrated the manipulated gene encoding for the transcriptional regulator protein incapable of binding a phosphoryl group transferred by the corresponding enzyme EII.

9200-9329 EP Ah/sä
01.06.2012 – Claims

Claims

1. Method for producing a heterologous polypeptide in a recombinant bacterial host cell,
wherein a recombinant bacterial host cell is used, whose catabolism of carbon sources is under the control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source, and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter, which is inducible by the secondary carbon source and is regulated by the transcriptional regulator protein,
said method comprising the steps of growing the bacterial host cell in a cell culture medium, that does not comprise the inducing secondary carbon source but a different carbon source, inducing the expression of said polypeptide by the different carbon source at a time, when the concentration of the different carbon source decreases under a level necessary for carbon catabolite repression,
and recovering the polypeptide from the cells or from the cell culture,

2. Method according to claim 1,
wherein a bacterial host cell is used, in which by genetical alteration deactivation of the transcriptional regulator protein by phosphorylation by the enzyme EII, specific for said transcriptional regulator protein, is prevented.

3. Method according to claim 2,
wherein i) in the genome of the bacterial host cell the gene is deleted which encodes for the phosphoryl group transferring enzyme EII specific for the transcriptional regulator protein, or
wherein ii) in the genome of the bacterial host cell the gene encoding for the transcriptional regulator protein is genetically altered so that the transcriptional regulator protein expressed by said gene is incapable of binding a phosphoryl group transferred from enzyme EII.

4. Method according to claim 3,
wherein in case i) into the vector is integrated the gene encoding for the transcriptional regulator protein controlling the promoter of the vector; or
wherein in case ii) into the vector is integrated the genetically manipulated gene of the bacterial host cell encoding for the transcriptional regulator protein incapable of binding a phosphoryl group transferred from the corresponding enzyme EII.

5. Method according to any of the claims 1 to 4,
wherein the bacterial host cell is selected from Bacilli, Chlostridia and Escherichia.

6. Method according to any of the claims 1 and 5,
wherein the culture medium comprises casamino acids.

7. Method according to any of the claims 1 to 6,
wherein expression of the polypeptide is started without induction by the inducer.

8. Method according to any of the claims 1 to 7,
wherein said at least one carbon source is added to the culture in an amount sufficient to maintain a pre-determined growth rate of the bacterial host cell without induction of carbon catabolite repression.

9. Method according to any of the claims 1 to 8,
wherein the carbon source inducible promoter is a promoter of the mannose operon and wherein the inducing carbon source is mannose.

10. Method according to claim 9,
wherein the promoter is the P_{manP} or the P_{manR} promoter of the mannose operon.

11. Method according to claim 10,
wherein i) in the genome of the bacterial host cell the gene encoding for manP is deleted, or
wherein ii) in the genome of the bacterial host cell the gene encoding for the transcriptional regulater protein ManR is genetically altered so that ManR expressed by said gene is incapable of binding a phosporyl group transferred from the specific enzyme EII.

12. Method for the production of a heterologous polypeptide by culturing a recombinant bacterial host cell, wherein a recombinant bacterial host cell is used whose catabolism of carbon sources is under control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system, and which is genetically altered such, that it is incapable of metabolizing the inducing carbon source of a carbon source inducible promoter, wherein the inducing carbon source is a secondary carbon source for the bacterial host cell, and which comprises a vector with the promoter inducible by said secondary carbon source and a heterologous nucleic acid sequence encoding a polypeptide operably linked to the promoter,
wherein the process is a fed-batch process, wherein after the batch phase induction is started by adding a first portion of the inducing secondary carbon source and feeding of a second portion is started during exponential growth simultaneously with the feeding solution.

13. Method according to claim 12,
wherein in the genome of the bacterial host cell the gene encoding for the inducer specific 6-phosphate isomerase is deleted.

14. Method according to claim 13,
wherein this gene is the gene *manA* of the mannose operon.

15. Method of the claims 1 to 14,
wherein the bacterial host cell is selected from *Bacillus*.

Claims

1. Method for producing a heterologous polypeptide in a recombinant bacterial host cell,
   wherein a recombinant bacterial host cell is used, whose catabolism of carbon sources is under the control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source, and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter, which is inducible by the secondary carbon source and is regulated by the transcriptional regulator protein,
   said method comprising the steps of growing the bacterial host cell in a cell culture medium, that does not comprise the inducing secondary carbon source but a different carbon source, inducing the expression of said polypeptide by the different carbon source at a time, when the concentration of the different carbon source decreases under a level necessary for carbon catabolite repression,
   and recovering the polypeptide from the cells or from the cell culture,

2. Method according to claim 1,
   wherein a bacterial host cell is used, in which by genetical alteration deactivation of the transcriptional regulator protein by phosphorylation by the enzyme EII, specific for said transcriptional regulator protein, is prevented.

3. Method according to claim 2,
   wherein i) in the genome of the bacterial host cell the gene is deleted which encodes for the phosphoryl group transferring enzyme EII specific for the transcriptional regulator protein, or
   wherein ii) in the genome of the bacterial host cell the gene encoding for the transcriptional regulator protein is genetically altered so that the transcriptional regulator protein expressed by said gene is incapable of binding a phosphoryl group transferred from enzyme EII.

4. Method according to claim 3,
wherein in case i) into the vector is integrated the gene encoding for the transcriptional regulator protein controlling the promoter of the vector; or
wherein in case ii) into the vector is integrated the genetically manipulated gene of the bacterial host cell encoding for the transcriptional regulator protein incapable of binding a phosphoryl group transferred from the corresponding enzyme EII.

5. Method according to any of the claims 1 to 4,
wherein the bacterial host cell is selected from Bacilli, Chlostridia and Escherichia.

6. Method according to any of the claims 1 and 5,
wherein the culture medium comprises casamino acids.

7. Method according to any of the claims 1 to 6,
wherein expression of the polypeptide is started without induction by the inducer.

8. Method according to any of the claims 1 to 7,
wherein said at least one carbon source is added to the culture in an amount sufficient to maintain a pre-determined growth rate of the bacterial host cell without induction of carbon catabolite repression.

9. Method according to any of the claims 1 to 8,
wherein the carbon source inducible promoter is a promoter of the mannose operon and wherein the inducing carbon source is mannose.

10. Method according to claim 9,
wherein the promoter is the P_{manP} or the P_{manR} promoter of the mannose operon.

11. Method according to claim 10,
wherein i) in the genome of the bacterial host cell the gene encoding for manP is deleted, or
wherein ii) in the genome of the bacterial host cell the gene encoding for the transcriptional regulator protein ManR is genetically altered so that ManR expressed by said gene is incapable of binding a phosporyl group transferred from the specific enzyme EII.

12. Recombinant bacterial host cell whose catabolism of carbon sources is under control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system, and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source by deleting in the genome of the bacterial host cell the gene which encodes for a phosporyl group transferring enzyme EII specific for the transcriptional regulator protein, and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter, which is regulated by the transcriptional regulator protein for which the bacterial host cell is genetically altered to be incapable of deactivation, and wherein into the vector is integrated the gene encoding for the specific transcriptional regulator protein.

13. Recombinant bacterial host cell, whose catabolism of carbon sources is under control of carbon catabolite repression and phosphoenolpyruvate: carbohydrate phosphotransferase system,
and which is genetically altered such, that it is incapable to deactivate the transcriptional regulator protein specific for a promoter inducible by a secondary carbon source in the absence of the inducing secondary carbon source by genetically altering in the genome of the bacterial host cell the gene encoding for the transcriptional regulator protein so that the transcriptional regulator protein expressed by said gene is incapable of

binding a phosphoryl group transferred by enzyme EII, specific for said transcriptional regulator protein,

and which comprises a vector with a heterologous nucleic acid sequence encoding a polypeptide operably linked to a promoter regulated by the transcriptional regulator protein for which the bacterial host cell is genetically altered to be incapable of deactivation, and

wherein into the vector is additionally integrated the manipulated gene encoding for the transcriptional regulator protein incapable of binding a phosphoryl group transferred by the corresponding enzyme EII.