seen, e.g. in the increased productivity of such a strain compared to a corresponding prototrophic wild-type strain.

The present disclosure relates to an amino acid auxotrophy cured prokaryotic strain, characterized in that

- at least one deficiency in an essential amino acid metabolic pathway has been cured, and
- the strain can grow in chemically defined minimal growth medium that is free of the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic strain.

In one embodiment the auxotrophy cured prokaryotic strain is an auxotrophy cured E.coli strain.

The present disclosure relates to an amino acid auxotrophy cured prokaryotic cell, characterized in that

- at least one deficiency in an essential amino acid metabolic pathway has been cured, and
- the cell can grow in chemically defined minimal growth medium that is free of the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic cell.

In one embodiment the auxotrophy cured prokaryotic cell is an auxotrophy cured E.coli cell.

The auxotrophy cured prokaryotic strain/cell as reported herein can be used in a method for the recombinant production of a therapeutic polypeptide wherein compared to the non-auxotrophy cured strain/cell the supplementation of at least one amino acid residue is not required.

The present disclosure relates to is a method for producing a polypeptide in a (recombinant) prokaryotic cell, comprising the following step:

- cultivating a (recombinant) prokaryotic cell, which has been obtained by introducing into the genome of a parent prokaryotic cell a nucleic acid curing an amino acid auxotrophy of the parent prokaryotic cell, comprising one or more nucleic acids encoding the polypeptide in a

chemically defined minimal growth medium and recovering the polypeptide from the prokaryotic cell or the periplasm of the prokaryotic cell or from the medium.

One aspect as reported herein is a method for producing a polypeptide in a prokaryotic cell, comprising the following step:

- curing a prokaryotic cell that is auxotrophic for at least one amino acid from at least one amino acid auxotrophy by introducing into the genome of a parent prokaryotic cell a nucleic acid curing an amino acid auxotrophy of the parent prokaryotic cell,
- cultivating the the amino acid auxotrophy cured prokaryotic cell comprising one or more nucleic acids encoding the polypeptide in a chemically defined minimal growth medium and recovering the polypeptide from the prokaryotic cell or the periplasm of the prokaryotic cell or from the medium,

wherein the product titer when using the auxotrophy cured prokaryotic strain is higher than the product titer that can be obtained with a corresponding prototrophic wild-type strain.

The present disclosure relates to a method for producing a polypeptide in a (recombinant) prokaryotic cell, comprising the following steps:

- curing an prokaryotic cell that is auxotrophic for at least one amino acid from at least one amino acid auxotrophy,
- cultivating the amino acid auxotrophy cured (recombinant) prokaryotic cell comprising one or more nucleic acids encoding the polypeptide in a chemically defined minimal growth medium and recovering the polypeptide from the prokaryotic cell or the periplasm of the prokaryotic cell or from the medium.

The present disclosure relates to a method for producing a polypeptide in a (recombinant) prokaryotic cell, comprising the following step:

- cultivating a (recombinant) prokaryotic cell comprising one or more nucleic acids encoding the polypeptide in a chemically defined minimal growth medium and recovering the polypeptide from the prokaryotic cell or the periplasm of the prokaryotic cell or from the medium,

wherein the (recombinant) prokaryotic cell is an amino acid auxotrophy cured prokaryotic cell.

In one embodiment of all methods for producing a polypeptide the auxotrophy cured prokaryotic cell has at least one further (non-cured) amino acid auxotrophy. This auxotrophy can be used for the selection of recombinants/transformants after the introduction of one or more nucleic acids encoding a polypeptide of interest (the polypeptide).

In one embodiment of all methods for producing a polypeptide the growth of the amino acid auxotrophy cured prokaryotic cell compared to the non-cured prokaryotic cell (parent cell) under the same cultivation conditions and in the same growth medium requires the supplementation of fewer amino acids to the growth medium.

Patent Claims

1. A method for producing a polypeptide in a prokaryotic cell, comprising the following step:

   - curing a prokaryotic cell that is auxotrophic for at least one amino acid from at least one amino acid auxotrophy by introducing into the genome of a parent prokaryotic cell a nucleic acid curing an amino acid auxotrophy of the parent prokaryotic cell,

   - cultivating the the amino acid auxotrophy cured prokaryotic cell comprising one or more nucleic acids encoding the polypeptide in a chemically defined minimal growth medium and recovering the polypeptide from the prokaryotic cell or the periplasm of the prokaryotic cell or from the medium,

   wherein the product titer when using the auxotrophy cured prokaryotic strain is higher than the product titer that can be obtained with a corresponding prototrophic wild-type strain.

2. The method according to claim 1, characterized in that the cultivation of the auxotrophy cured prokaryotic cell requires supplementation of one or two or three or four amino acids less to the growth medium than required for the cultivation of the parent non-auxotrophy cured prokaryotic cell.

3. The method according to any one of claims 1 to 2, characterized in that the auxotrophy cured prokaryotic cell has at least one further amino acid auxotrophy.

4. The method according to any one of claims 1 to 3, characterized in that the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic cell is not added during the cultivation.

5. The method according to any one of claims 1 to 3, characterized in that the cultivation is in the absence of the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic cell.

6. The method according to any one of claims 1 to 5, characterized in that the prokaryotic cell is an E.coli cell.

7. The method according to any one of claims 1 to 6, characterized in that the polypeptide is a full length antibody chain, a single chain antibody, a single domain antibody, a scFv, a scFab, or a conjugate of one of the before with a non-antibody polypeptide.

8. The method according to claim 7, characterized in that the polypeptide is a conjugate of a scFv or scFab with a cytotoxic agent.