When, for example, E.coli is used as host cell line generally wild-type strains, such as MG1655, W3110 or BL21, are employed. These strains show good growth characteristics but inferior product titer.

Mutant prokaryotic strains, which have been obtained by non-directed mutagenesis and selection, show profound differences in their genomic DNA when compared to wild-type strains. The mutant strains have been selected based on the maximum product titer that can be obtained. As the mutant strains harbor a number of auxotrophies they cannot be used for cultivation in a chemically defined minimal growth medium. The mutant strains required the feeding of amino acids to complement their auxotrophies resulting in increased cultivation costs.

US 5,932,439 reports Escherichia coli K-12 strains for production of recombinant proteins.

Summary ~~of the Invention~~

By curing an auxotrophic prokaryotic strain of one or more of its auxotrophies the auxotrophy cured strain can grow on a chemically defined minimal growth medium *to which the substance corresponding to the auxotrophy that has been cured no* further needs to be added. In case of expensive substances a reduction of the costs of goods can be achieved and a recombinant polypeptide can be produced more economically.

In addition to the restoration of the ability to grow on a chemically defined minimal growth medium in the absence of the substance corresponding to the auxotrophy that has been cured it has been found that the obtainable product titer can be maintained or even increased compared to the non-cured strain. It has furthermore been found that the obtainable product titer using an auxotrophy cured prokaryotic strain is higher than the product titer that can be obtained with a corresponding prototrophic wild-type strain.

Without being bound by this theory it is assumed that an amino acid auxotrophy cured prokaryotic strain as reported herein has a different metabolism and metabolic fluxes when compared to a corresponding prototrophic wild-type strain in which such a deletion and cure has not been performed. Thus, the inactivation and re-activation of certain enzymes within a prokaryotic strain has a detectable/pronounced effect on the entire metabolism of the strain, which can be

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

The present disclosure relates to ~~One aspect as reported herein is~~ 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 ~~One aspect as reported herein is~~ 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.

One aspect as reported herein 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.

The present disclosure relates to ~~One aspect as reported herein is~~ 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~~One aspect as reported herein is~~ 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.

In one embodiment of all methods for producing a polypeptide the chemically defined minimal growth medium is free of the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic cell.

In one embodiment of all methods for producing a polypeptide 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 non-auxotrophy cured prokaryotic cell (parent/parent cell).

In one embodiment of all methods for producing a polypeptide the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic cell is not added during the cultivation.

In one embodiment of all methods for producing a polypeptide the cultivation is in the absence of the amino acid corresponding to the auxotrophy that has been cured in the auxotrophy cured prokaryotic cell.

In one embodiment of all methods for producing a polypeptide the prokaryotic cell is an E.coli cell.

In one embodiment of all methods for producing a polypeptide 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.

In one embodiment of all methods for producing a polypeptide the polypeptide is a conjugate of a scFv or scFab with a cytotoxic agent.

The present disclosure relates to ~~One aspect as reported herein is~~ the use of an amino acid auxotrophy cured prokaryotic strain for the (recombinant) production of a polypeptide.

In one embodiment

- in the amino acid auxotrophy cured prokaryotic strain at least one deficiency in an essential amino acid metabolic pathway has been cured, and
- the amino acid auxotrophy cured 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.