Design, construction and performance of the most efficient biomass producing E.coli bacterium is reported by Trinh et al. (Met. Eng. 8 (2006) 628).

Summary ~~of the Invention~~

It has been found that by the deletion/inactivation of the ndh-gene, which codes for the enzyme NADH dehydrogenase II, a genetically modified prokaryotic organism can be obtained that has, when compared to the parent strain that is isogenic except for the ndh-gene, comparable oxygen uptake rates, comparable growth rates but has an increased productivity. Thus, it has been found that by the deletion/inactivation of the ndh-gene the specific productivity of a prokaryotic organism can be increased.

~~One aspect as~~As reported herein is a method for the recombinant production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene).

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the polypeptide is not an enzyme.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the polypeptide is not a respiratory chain pathway enzyme or a polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the polypeptide is not a NADH dehydrogenase, a SoxM type oxidase, a Sox type oxidase, a cytochrome bd type oxidase, a cytochrome bo type oxidase or any polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the prokaryotic cell has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd, wherein the acnA gene encoded polypeptide comprises a S68G mutation, the

aceA gene encoded polypeptide comprises a S522G mutation and the icd gene encoded polypeptide comprises a D398E and a D410E mutation.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the polypeptide is an immunoglobulin, an immunoglobulin fragment, an immunoglobulin-toxin conjugate, an immunoglobulin fragment-toxin conjugate, a toxin, a cytokine or a hormone.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the polypeptide is not an enzyme.

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- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

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wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the polypeptide is not a NADH dehydrogenase, a SoxM type oxidase, a Sox type oxidase, a cytochrome bd type oxidase, a cytochrome bo type oxidase or any polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

wherein the prokaryotic cell is deficient in the ndh-gene (i.e. the prokaryotic cell comprises/contains no functional copy of the ndh-gene),

and wherein the prokaryotic cell has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd, wherein the acnA gene encoded polypeptide comprises a S68G mutation, the aceA gene encoded polypeptide comprises a S522G mutation and the icd gene encoded polypeptide comprises a D398E and a D410E mutation.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in a prokaryotic cell comprising the following steps:

- cultivating a prokaryotic cell expressing the polypeptide (i.e. cultivating a cell that comprises a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

In one embodiment the incubating is at a temperature between 40°C and 60°C.

In one embodiment the incubating is at a temperature of 45°C or higher. In one embodiment the incubating is at a temperature of about 45°C.

In one embodiment the incubating is for 10 minutes to 180 minutes.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is not an enzyme.

One aspect as reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is not a respiratory chain pathway enzyme or a polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is not a NADH dehydrogenase, a SoxM type oxidase, a Sox type oxidase, a cytochrome bd type oxidase, a cytochrome bo type oxidase or any polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the NADH dehydrogenase II-deficient E.coli has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd, wherein the acnA gene encoded polypeptide comprises a S68G mutation, the aceA gene encoded polypeptide comprises a S522G mutation and the icd gene encoded polypeptide comprises a D398E and a D410E mutation.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is an immunoglobulin, an immunoglobulin fragment, an immunoglobulin-toxin conjugate, an immunoglobulin fragment-toxin conjugate, a toxin, a cytokine or a hormone.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is not an enzyme.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is not a respiratory chain pathway enzyme or a polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is not a NADH dehydrogenase, a SoxM type oxidase, a Sox type oxidase, a cytochrome bd type oxidase, a cytochrome bo type oxidase or any polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is a method for the production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the NADH dehydrogenase II-deficient E.coli has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd, wherein the acnA gene encoded polypeptide comprises a S68G mutation, the aceA gene encoded polypeptide comprises a S522G mutation and the icd gene encoded polypeptide comprises a D398E and a D410E mutation.

~~One aspect as~~ As reported herein is a method for the recombinant production of a polypeptide in E.coli comprising the following steps:

- cultivating an NADH dehydrogenase II-deficient E.coli expressing the polypeptide (i.e. cultivating an NADH dehydrogenase II-deficient E.coli comprising a nucleic acid encoding the polypeptide), and
- recovering the polypeptide from the cell or the cultivation medium,

and wherein the polypeptide is an immunoglobulin, an immunoglobulin fragment, an immunoglobulin-toxin conjugate, an immunoglobulin fragment-toxin conjugate, a toxin, a cytokine or a hormone.

In one embodiment the NADH dehydrogenase II-deficient E.coli has a comparable oxygen uptake rate as an E.coli with the same genotype except that it has a functional NADH dehydrogenase II.

In one embodiment the NADH dehydrogenase II-deficient E.coli has a comparable growth rate as an E.coli with the same genotype except that it has a functional NADH dehydrogenase II.

In one embodiment the method comprises after the cultivation step the following steps:

- incubating the cultivation medium including the cells at a temperature of 40°C or higher for at least 10 minutes, and
- recovering the insoluble polypeptide from the cells and/or the cultivation medium and thereby producing the polypeptide.

In one embodiment the incubating is at a temperature between 40°C and 60°C.

In one embodiment the incubating is at a temperature of 45°C or higher. In one embodiment the incubating is at a temperature of about 45°C.

In one embodiment the incubating is for 10 minutes to 180 minutes.

One aspect as reported herein is an E.coli K12 that has the genotype thi-1, ΔpyrF Δndh.

~~One aspect as~~ As reported herein is an E.coli K12 that has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd, wherein the acnA gene encoded polypeptide comprises a S68G mutation, the aceA gene encoded polypeptide comprises a S522G mutation and the icd gene encoded polypeptide comprises a D398E and a D410E mutation.

~~One aspect as~~ As reported herein is the use of an NADH dehydrogenase II-deficient E.coli in the production of a recombinant polypeptide.

One aspect as reported herein is the use of an NADH dehydrogenase II-deficient E.coli in the production of a recombinant polypeptide, wherein the polypeptide is not a respiratory chain pathway enzyme or a polypeptide encoded by an antibiotic resistance inducing gene.

~~One aspect as~~ As reported herein is the use of an NADH dehydrogenase II-deficient E.coli in the production of a polypeptide.

In one embodiment the NADH dehydrogenase II-deficient E.coli has the genotype Δndh, thi-1, ΔpyrF. In one embodiment the NADH dehydrogenase II-deficient E.coli has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd.

In one embodiment the NADH dehydrogenase II-deficient E.coli has the genotype thi-1, Δndh, ΔpyrF, acnA, aceA, icd, wherein the acnA gene encoded polypeptide comprises a S68G mutation, the aceA gene encoded polypeptide comprises a S522G mutation and the icd gene encoded polypeptide comprises a D398E and a D410E mutation.