E COLI STRAINS HAVING AN OXIDATIVE CYTOPLASM

RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 62/757,498, filed November 8, 2018. The entire content of said provisional application is hereby incorporated by reference for all purposes.

BACKGROUND OF THE INVENTION

[0002] Commercially valuable proteins, such as therapeutic proteins, often possess disulfide bonds. These disulfide bonds are important for protein stability and function. Conventional bacterial hosts for protein production have a reductive cytosol and thus are unable to form disulfide bonds in proteins expressed in the cytosol. As a result, currently many proteins cannot readily be expressed in bacterial cytosol and are instead expressed in eukaryotic or periplasmic expression systems. Although attempts to promote disulfide bond formation in bacterial cytosol have been made by introducing mutations into the bacterial host genome to disrupt reductive pathways, these efforts have yielded limited success. Thus, cytosolic production of disulfide-bonded proteins in bacteria remains a challenge.

SUMMARY OF INVENTION

[0003] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, sequence accession numbers, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

[0003a] In one aspect, provided herein is an *E. coli* strain, wherein : i) the strain lacks the activity of a thioredoxin reductase due to genetic modification of *trxB*; ii) the strain lacks the activity of a thioredoxin 1 due to genetic modification of *trxA*; iii) the strain lacks the activity of a glutathione reductase due to genetic modification of *gor*; iv) the strain expresses a mutated AhpC protein, wherein the mutated AhpC protein has glutathione reductase activity but lacks peroxyreductase activity due to the mutation; and v) the strain expresses a cytosolic prokaryotic disulfide isomerase.

[0003b] In one aspect, provided herein is a method for expressing soluble, recombinant proteins of interest in *E. coli* bacterial strains comprising the steps of: a. culturing an *E. coli* bacterial strain comprising an oxidizing cytosol and an expression cassette for expressing a protein of interest under conditions that permit expression of the protein of interest as a soluble protein, wherein the strain: i) lacks thioredoxin reductase activity due to genetic modification of *trxB*; ii) lacks thioredoxin 1 activity due to genetic modification of *trxA*; iii) lacks the activity of a glutathione reductase due to genetic modification of *gor*; iv) expresses an *ahpC* gene that has been mutated such that an enzyme expressed from the *ahpC* gene it has glutathione reductase activity but lacks peroxyreductase activity due to the mutation; and, v) expresses a gene encoding a cytosolic prokaryotic disulfide isomerase.

[0004] In some embodiments, the disclosure provides an E. coli strain, which lacks thioredoxin reductase activity encoded by trxB and thioredoxin 1 activity encoded by trxA, and glutathione reductase activity encoded by gor. Said E. coli strain expresses a mutated AhpC protein having glutathione reductase activity and a cytosolic prokaryotic disulfide isomerase. In some embodiments, the mutated AhpC protein is AhpC*. In some embodiments, the E. coli strain

WHAT IS CLAIMED IS:

1. An *E. coli* strain, wherein :

i) the strain lacks the activity of a thioredoxin reductase due to genetic modification of *trxB*;

ii) the strain lacks the activity of a thioredoxin 1 due to genetic modification of *trxA*;

iii) the strain lacks the activity of a glutathione reductase due to genetic modification of *gor*;

iv) the strain expresses a mutated AhpC protein, wherein the mutated AhpC protein has glutathione reductase activity but lacks peroxyreductase activity due to the mutation; and

v) the strain expresses a cytosolic prokaryotic disulfide isomerase.

2. The *E. coli* strain of claim 1 wherein the strain further comprises a gene encoding a protein of interest.

3. The *E. coli* strain of claim 2 wherein the protein of interest is selected from the group consisting of: an antibody, a fragment thereof or an antibody light chain from an IgG.

4. The *E. coli* strain of claim 1 wherein the cytosolic disulfide isomerase is DsbC.

5. The *E. coli* strain of claim 1 wherein the *E. coli* further expresses a recombinant prolyl isomerase and/or a deaggregase.

6. The *E. coli* strain of claim 5, wherein the prolyl isomerase is selected from the group consisting of cyclophilin, FKBPs, parvulin, SlyD, Tig, yCpr6;
and wherein the deaggregase is selected from the group consisting of Skp, GroEL, GroES, DnaK, DnaJ, and GrpE.

7. The *E. coli* strain of claim 2 wherein the gene encoding the protein of interest is operably linked to an inducible promoter.

8. The *E. coli* strain of claim 7 wherein the inducible promoter is a T7 promoter.

9. The E. coli strain of claim 1, wherein the expression of the mutated *ahpC* gene is controlled by a Pc0 promoter.

10. The E. coli strain of claim 1, wherein the expression of the cytosolic prokaryotic disulfide isomerase is controlled by a MTL promoter.

11. The E. coli strain of claim 1, wherein the E. coli strain is a K-12 strain.

12. A method for expressing soluble, recombinant proteins of interest in *E. coli* bacterial strains comprising the steps of:

a. culturing an *E. coli* bacterial strain comprising an oxidizing cytosol and an expression cassette for expressing a protein of interest under conditions that permit expression of the protein of interest as a soluble protein, wherein the strain:

i) lacks thioredoxin reductase activity due to genetic modification of *trxB*;

ii) lacks thioredoxin 1 activity due to genetic modification of *trxA*;

iii) lacks the activity of a glutathione reductase due to genetic modification of *gor*;

iv) expresses an *ahpC* gene that has been mutated such that an enzyme expressed from the *ahpC* gene it has glutathione reductase activity but lacks peroxyreductase activity due to the mutation; and,

v) expresses a gene encoding a cytosolic prokaryotic disulfide isomerase.

13. The method of claim 12, wherein the *E. coli* strain contains a null mutation in *trxC*.

14. The method of claim 12 wherein the *E. coli* strain contains a null mutation in *trx B*.

15. The method of claim 12, wherein the E. coli strain contains a null mutation in *trxA*.

16. The method of claim 12 wherein the protein of interest is selected from the group consisting of: an IgG, a light chain from an IgG or a heavy chain from an IgG.

17. The method of claim 12 wherein cytosolic disulfide isomerase is DsbC or yeast protein disulfide isomerase (yPDI).

18. The method of claim 12, wherein the E. coli strain further expresses an recombinant prolyl isomerase and/or a recombinant deaggregase.

19. The method of claim 18 wherein the recombinant prolyl isomerase is selected from the group consisting of cyclophilin, FKBPs, parvulin, SlyD, Tig and yCpr6; and the deaggregase is selected from the group consisting of Skp, GroEL, GroES, DnaK, DnaJ, and GrpE.

20. The method of claim 12 wherein the gene encoding the protein of interest is operably linked to a inducible promoter.

21. The method of claim 20 wherein the inducible promoter is a T7 promoter.

22. The method of claim 16, wherein the light chain from an IgG is a light chain of an anti-HER2 antibody.

23. The method of claim 12, wherein the E. coli strain expresses a GshA protein encoded by the *gshA* gene.

24. The method of claim 23, wherein the *gshA* gene is inserted into the locus of *TrxB*.

25. The method of claim 12, wherein the E. coli strain further express a T7 polymerase.

26. The method of claim 25, wherein the T7 polymerase is under the control of an inducible promoter.

27. The method of claim 26, wherein the inducible promoter is a P_{araBAD}, lac, lacUV5 phoA, tetA, xylAB, tac, or rhamnose promoter.

28. A kit comprising the *E. coli* of any of claims 1-11, wherein the kit further comprises a growth medium.

29. The kit of claim 27, wherein the kit further comprises a plasmid encoding a protein of interest.