BACTERIAL HOSTS FOR RECOMBINANT PROTEIN EXPRESSION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/209,239 filed June 10, 2021, the contents of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] Microbial host cell expression systems are used for production of recombinant proteins with varying degrees of success. Recombinant protein degradation and low yield remain challenges. Alterations of the host cell genome to optimize the production of high-quality protein often result in frustratingly low host cell growth and production yields. Recombinant host cells that achieve both high quality and high yield of recombinant proteins remain needed.

SUMMARY OF THE INVENTION

[0002a] In one aspect, provided herein is a recombinant gram-negative bacterial host cell for recombinant protein expression, wherein the host cell is: (a) deficient in a first protease activity, wherein the first protease activity is tail-specific protease activity, wherein the deficient first protease activity results from a mutation in at least one gene encoding a tail-specific protease; (b) deficient in a second protease activity, wherein the second protease activity is murein DD-endopeptidase activity, wherein the deficient second protease activity results from a mutation in at least one gene encoding a murein DD-endopeptidase.

[0002b] In one aspect, provided herein is a method for producing a recombinant protein of interest comprising: (a) recovering the recombinant protein of interest from a recombinant gram-negative bacterial host cell as described herein cultured under suitable fermentation conditions, wherein the recombinant gram-negative host cell is transformed with a plasmid comprising a nucleic acid encoding the recombinant protein of interest.

[0002c] In one aspect, provided herein is a recombinant polypeptide comprising: a secretion signal peptide operably linked to a heterologous protein or polypeptide of interest, wherein the secretion signal peptide has the amino acid sequence set forth in SEQ ID NO: 11.

[0002d] In one aspect, provided herein is a method of producing a protein or polypeptide of interest in a prokaryotic host cell, the method comprising: producing the protein or polypeptide of interest in the periplasm of a prokaryotic host cell cultured in a cell culture growth medium, wherein the prokaryotic host cell comprises an expression construct comprising a nucleic acid encoding a recombinant polypeptide comprising the protein or polypeptide of interest operably linked to a secretion signal peptide that directs expression of the protein or polypeptide of interest

to the periplasm of the prokaryotic host cell, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO: 11, and wherein the secretion signal peptide is not native to the protein or polypeptide of interest.

[0002e] In one aspect, provided herein is an expression vector comprising a nucleic acid sequence encoding a recombinant polypeptide as described herein.

[0002f] In one aspect, provided herein is a prokaryotic host cell comprising an expression vector as described herein.

[0003] The present invention provides recombinant bacterial host cells and methods for their use to produce high quality recombinant proteins at high yield. In particular, the compositions and methods provided by the invention are useful for producing recombinant proteins that are sensitive to proteolysis, including antibodies and antibody fragments. The invention includes recombinant gram-negative bacterial host cells comprising combinations of genetic modifications that result in reduced degradation of a recombinant protein while allowing growth to high cell density. In some embodiments, a recombinant gram-negative bacterial host cell of the present invention is deficient in first protease activity and a second protease activity, wherein the first protease activity is a tail-specific protease activity and the second protease activity is a murein DD-endopeptidase activity. In some embodiments, the invention relates to a recombinant gram-negative bacterial host cell that is deficient in tail-specific protease activity and a murein DD-endopeptidase activity, wherein the host cell produces high quality, undegraded, recombinant protein, and grows to a high cell density. In some embodiments, the murein DD-endopeptidase activity is a MepM activity. In some embodiments, the gram-negative bacterial host cell is not deficient in a MepS activity. In some embodiments, the recombinant gram-negative bacterial host cell deficient in a first protease activity and a second protease activity, wherein the first protease activity is a tail-specific protease activity, and the second protease activity is a MepM murein DD-endopeptidase activity, is further deficient in at least one additional protease activity, at least one autolytic factor activity, or both. In some embodiments, the at least one additional protease activity is a serralysin precursor activity. The deficiency in a protein activity, e.g., the first protease, the second protease, an additional protease, or an autolytic factor, can result from a