CLAIMS

1. A method of producing a protein of interest comprising:

    a) culturing a recombinant Gram-negative bacterial cell in a medium comprising a magnesium salt, wherein a concentration of magnesium ions in the medium is from 6 mM to 20 mM, and wherein the recombinant Gram-negative bacterial cell comprises an exogenous gene encoding the protein of interest;

    b) inhibiting peptidoglycan biogenesis in the recombinant Gram-negative bacterial cell; and

    c) harvesting the protein of interest from the medium.

2. The method of claim 1, wherein b) and c) occur sequentially, or wherein c) is performed at least 1 hour after b), or wherein b) and c) occur simultaneously.

3. The method of claim 1 or 2, wherein the recombinant Gram-negative bacterial cell changes shape from bacillus to coccus after b).

4. The method of any one of claims 1 to 3, wherein the inhibiting of b) comprises adding an antibiotic to the medium, wherein the antibiotic inhibits peptidoglycan biogenesis in the recombinant Gram-negative bacterial cell.

5. The method of claim 4, wherein the antibiotic is selected from the group consisting of: a ß-lactam antibiotic, a phosphonic antibiotic, a polypeptide antibiotic, and a glycopeptide antibiotic.

6. The method of claim 5, wherein the ß-lactam antibiotic is selected from the group consisting of: a penicillin, a cephalosporin, a carbapenem, and a monobactam.

7. The method of claim 4, wherein the antibiotic is selected from the group consisting of: alafosfalin, amoxicillin, ampicillin, aztreonam, bacitracin, carbenicillin, cefamandole, cefotaxime, cefsulodin, cephalothin, fosmidomycin, methicillin, nafcillin, oxacillin, penicillin g, penicillin v, fosfomycin, primaxin, and vancomycin.

8. The method of any one of claims 1 to 7, wherein the inhibiting of b) comprises adding at least two structurally distinct antibiotics to the medium, and wherein the at least two structurally

distinct antibiotics inhibit different components of a peptidoglycan biogenesis pathway in the recombinant Gram-negative bacterial cell.

9. The method of any one of claims 1 to 8, wherein the recombinant Gram-negative bacterial cell is selected from the group consisting of: *gammaproteobacteria* and *alphaproteobacteria*.

10. The method of any one of claims 1 to 9, wherein the recombinant Gram-negative bacterial cell is selected from the group consisting of: *Escherichia coli, Vibrio natriegens, Pseudomonas fluorescens, Caulobacter crescentus, Agrobacterium tumefaciens,* and *Brevundimonas diminuta*.

11. The method of any one of claims 1 to 10, wherein the recombinant Gram-negative bacterial cell is *Escherichia coli*.

12. The method of any one of claims 1 to 11, wherein the concentration of magnesium ions in the medium is 10 mM.

13. The method of any one of claims 1 to 12, wherein the magnesium salt is selected from the group consisting of: magnesium sulfate and magnesium chloride.

14. The method of any one of claims 1 to 13, wherein the medium further comprises an osmotic stabilizer.

15. The method of claim 14, wherein the osmotic stabilizer is selected from the group consisting of: a sugar, a betaine, and a combination thereof.

16. The method of claim 15, wherein the sugar is selected from the group consisting of: arabinose, glucose, and sucrose.

17. The method of claim 15 or 16, wherein the betaine is trimethylglycine.

18. The method of any one of claims 14 to 17, wherein a concentration of the osmotic stabilizer in the medium is at least 5% (w/v).

19. The method of any one of claims 14 to 18, wherein a concentration of the osmotic stabilizer in the medium is from 5% (w/v) to 20% (w/v).

20. The method of any one of claims 14 to 19, wherein a concentration of the osmotic stabilizer in the medium is 10% (w/v).

21. The method of any one of claims 1 to 20, wherein the medium further comprises one or more ingredients selected from the group consisting of: ammonium chloride, ammonium sulfate, calcium chloride, casamino acids, iron(II) sulfate, magnesium sulfate, peptone, potassium phosphate, sodium chloride, sodium phosphate, and yeast extract.

22. The method of any one of claims 1 to 21, wherein the recombinant Gram-negative bacterial cell comprises an expression vector comprising an exogenous gene that encodes resistance to an antibiotic, wherein the antibiotic is not an inhibitor of bacterial cell peptidoglycan biogenesis.

23. The method of any one of claims 1 to 22, wherein the recombinant Gram-negative bacterial cell comprises an expression vector, wherein the expression vector is free of a marker encoding for resistance to an inhibitor of bacterial cell peptidoglycan biogenesis.

24. The method of any one of claims 1 to 23, wherein the exogenous gene is integrated into a chromosome of the recombinant Gram-negative bacterial cell.

25. The method of any one of claims 1 to 24, wherein the method further comprises inducing expression of the exogenous gene.

26. The method of any one of claims 1 to 25, wherein expression of the exogenous gene is inducible by an inducer selected from the group consisting of: isopropyl-ß-d-1-thiogalactopyranoside, lactose, copper, and zinc.

27. The method of any one of claims 1 to 26, wherein the recombinant Gram-negative bacterial cell further comprises a nucleic acid sequence encoding a signal peptide operably linked to the exogenous gene, wherein the signal peptide directs cotranslational export of the protein of interest from a cytoplasm to a periplasm of the recombinant Gram-negative bacterial cell.

28. The method of claim 27, wherein the signal peptide is a periplasmic disulfide bond oxidoreductase.

29. The method of claim 27, wherein the signal peptide is DsbA.

30. The method of any one of claims 1 to 29, wherein the protein of interest is a) collagen, optionally human collagen; or b) a therapeutic protein, optionally wherein the therapeutic protein is an antibody.