identical to SEQ ID NO: 1, and wherein the recombinant type II asparaginase is produced in the periplasm at a yield of about 20% to about 40% TCP in soluble form.

[0004] Provided herein are methods for producing a recombinant type II asparaginase. In some examples, the method comprises: culturing a *Pseudomonadales* host cell in a culture medium and expressing the recombinant asparaginase in the cytoplasm of the *Pseudomonadales* host cell from an expression construct comprising a nucleic acid encoding the recombinant asparaginase, wherein the recombinant asparaginase is produced in the cytoplasm at a yield of about 20% TCP to about 40% TCP soluble asparaginase. In some examples, the recombinant asparaginase is produced in the cytoplasm at a yield of about 10 g/L to about 25 g/L. In some examples, the method further comprises measuring the activity of an amount of the soluble recombinant type II asparaginase produced, using an activity assay. In some examples, the nucleic acid encoding the recombinant asparaginase is optimized for expression in the host cell. In some examples, the recombinant asparaginase is an *Erwinia chrysanthemi* L-asparaginase type II (crisantaspase). In some examples, the nucleic acid encoding the recombinant asparaginase comprises a sequence at least 85% homologous to SEQ ID NO: 1. In some examples, the recombinant asparaginase has an amino acid sequence at least 85% homologous to SEQ ID NO: 2. In some examples, expression of the recombinant asparaginase is induced by addition of IPTG to the culture medium. In some examples, the IPTG is at a concentration in the culture medium of about 0.05 mM to about 2.5 mM. In some examples, expression of the recombinant asparaginase is induced when the *Pseudomonad* host cell has grown to a wet cell weight of about 0.1 g/g to about 0.5 g/g. In some examples, the *Pseudomonadales* host cell is cultured at a pH of about 5.0 to about 8.0. In some examples, the *Pseudomonadales* host cell is cultured at a temperature of about 22 °C to about 33 °C. In any example herein, the *Pseudomonadales* host cell is a *Pseudomonas fluorescens* cell. In some examples, the *Pseudomonadales* host cell is deficient in the expression of one or more asparaginases. In some examples, the *Pseudomonadales* host cell is deficient in the expression of one or more native asparaginases. In some examples, the deficiently expressed native asparaginase is a type I asparaginase. In some examples, the deficiently expressed native asparaginase is a type II asparaginase. In some examples, the *Pseudomonadales* host cell is deficient in the expression of one or more proteases. In some examples, the *Pseudomonadales* host cell overexpresses one or more folding modulators. In some examples, the *Pseudomonadales* host cell is deficient in the expression of one or more native asparaginases, is deficient in the expression of one or more proteases and/or overexpresses one or more folding modulators.

[0005] In examples, the method comprises: culturing a *Pseudomonadales* host cell in a culture medium and expressing the recombinant asparaginase in the periplasm of the *Pseudomonadales* host cell from an expression construct comprising a nucleic acid encoding the recombinant asparaginase; wherein the recombinant asparaginase is produced in the periplasm at a yield of about 20% to about 40% TCP soluble asparaginase, e.g., monomeric asparaginase. In some examples, the recombinant asparaginase is produced in the periplasm at a yield of about 5 g/L to about 20 g/L. In some examples, the method further comprises measuring the activity of an amount of the recombinant type II asparaginase produced,

using an activity assay. In some examples, the nucleic acid encoding the recombinant asparaginase is optimized for expression in the host cell. In some examples, the recombinant asparaginase is an *Erwinia chrysanthemi* L-asparaginase type II (crisantaspase). In some examples, the nucleic acid encoding the recombinant asparaginase comprises a sequence at least 85% homologous to SEQ ID NO: 1. In some examples, the recombinant asparaginase has an amino acid sequence at least 85% homologous to SEQ ID NO: 2. In some examples, expression of the recombinant asparaginase is induced by addition of IPTG to the culture medium. In some examples, he IPTG is at a concentration in the culture medium of about 0.05 mM to about 2.5 mM. In some examples, expression of the recombinant asparaginase is induced when the *Pseudomonadales* host cell has grown to a wet weight of about 0.05 g/g to about 0.5 g/g. In some examples, the *Pseudomonadales* host cell is cultured at a pH of about 5.0 to about 8.0. In some examples, the *Pseudomonadales* host cell is cultured at a temperature of about 22 °C to about 33 °C. In some examples, the *Pseudomonadales* host cell is a *Pseudomonas fluorescens* cell. In some examples, the *Pseudomonadales* host cell is deficient in the expression of one or more native asparaginases. In

some examples, the deficiently expressed native asparaginase is a type I asparaginase. In some examples, the deficiently expressed native asparaginase is a type II asparaginase. In some examples, the *Pseudomonadales* host cell is deficient in the expression of one or more proteases. In some examples, the *Pseudomonadales* host cell overexpresses one or more folding modulators. In some examples, the expression construct comprises a secretion leader. In some examples, the secretion leader is selected from the group comprising the *Pseudmonadales* secretion leaders FlgI, Ibps31A, PbpA20V, DsbC, 8484, and 5193. In some examples, the secretion leader directs transfer of the recombinant asparaginase produced to the periplasm of the *Pseudomonad* host cell. In some examples, the method further comprises comparing the measured activity of the recombinant type II asparaginase produced, with an activity measured in the same amount of a control type II asparaginase using the same activity assay. In some examples, the control type II asparaginase comprises an *Erwinia* type II asparaginase that has been commercially approved for use in patients in at least one country. In some examples, the recombinant type II asparaginase produced is selected for use in patients when it has about 80% to about 120% of the activity of the type II asparaginase control sample. In some examples, the recombinant type II asparaginase is modified to increase half-life in patients. In examples, the host cell is selected from at least one of: a host cell that is deficient in HslUV protease, a host cell that is deficient in PrtB protease, a host cell that is deficient in Prc protease, a host cell that is deficient in DegP protease, a host cell that is deficient in AprA protease, a host cell that is deficient in Lon protease, a host cell that is deficient in La protease, a host cell that is deficient in Deg P1, a host cell that is deficient in Deg P2, and a host cell that overexpresses DegP2 S219A.

INCORPORATION BY REFERENCE

[0006] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0008] FIG. 1. SDS-CGE Gel-like Images - Tier 1 Expression Plasmid Screen. Crisantaspase small scale growth whole broth sonicate soluble samples from DC454 (upper panel) and DC441 (lower panel) were analyzed by reduced SDS-CGE. The lane at the far left shows molecular weight marker ladder 1 (upper panel MW ladder 48 KD, 29 KD; lower panel MW ladder 48 KD, 29 KD, 21 KD), and the lane at the far right shows the same ladders. From left to right (lanes 1 to 46), beginning immediately to the right of ladder 1 are lanes showing the expression patterns observed when the following secretion leader peptides were fused to the N-terminus of crisantaspase protein (high RBS except as otherwise indicated): no leader; DsbD; Leader A; DsbA; DsbA-Medium RBS; Azu; Azu-Medium RBS; Lao; Ibp-S31A; TolB;

CLAIMS

WHAT IS CLAIMED IS:

1. A method for producing a recombinant type II asparaginase, the method comprising culturing a *Pseudomonadales* host cell in a culture medium and expressing the recombinant type II asparaginase in the cytoplasm of the *Pseudomonadales* host cell from an expression construct comprising a nucleic acid encoding the recombinant type II asparaginase, wherein the recombinant type II asparaginase encoded by the nucleic acid comprises an amino acid sequence at least about 85% identical to SEQ ID NO: 1, and wherein the recombinant type II asparaginase is produced in the cytoplasm at a yield of about 20% to about 50% total cell protein (TCP) in soluble form.

2. The method of claim 1, wherein the recombinant type II asparaginase is produced in the cytoplasm at a yield of about 10 g/L to about 25 g/L.

3. A method for producing a recombinant type II asparaginase, the method comprising: culturing a *Pseudomonadales* host cell in a culture medium and expressing the recombinant type II asparaginase in the periplasm of the host cell from an expression construct comprising a nucleic acid encoding the recombinant type II asparaginase, wherein the recombinant type II asparaginase encoded by the nucleic acid comprises an amino acid sequence at least about 85% identical to SEQ ID NO: 1, and wherein the recombinant type II asparaginase is produced in the periplasm at a yield of about 20% to about 40% TCP in soluble form.

4. The method of claim 3, wherein the recombinant type II asparaginase is produced in the periplasm at a yield of about 5 g/L to about 30 g/L.

5. The method of claim 1 or 3, wherein the method further comprises measuring the activity of an amount of the recombinant type II asparaginase produced, using an activity assay.

6. The method of claim 1 or 3, wherein the recombinant type II asparaginase is an *Erwinia chrysanthemi* L-asparaginase type II (crisantaspase).

7. The method of claim 1 or 3, wherein the nucleic acid encoding the recombinant type II asparaginase comprises a sequence having at least 85% sequence identity to SEQ ID NO: 2, or wherein the recombinant type II asparaginase comprises an amino acid sequence as set forth in SEQ ID NO: 1.

8. The method of claim 1 or 3, wherein the *Pseudomonadales* host cell is a *Pseudomonas fluorescens* cell.

9. The method of claim 1 or 3, wherein the host cell is deficient in the expression of one or more asparaginases, preferably wherein the deficiently expressed asparaginase is selected from: a type I asparaginase, a type II asparaginase, or both.

10. The method of claim 1 or 3, wherein the expression construct comprises a secretion leader that directs transfer of the recombinant type II asparaginase produced to the periplasm of the host cell, preferably wherein the secretion leader is selected from the group consisting of FlgI, Ibps31A, PbpA20V, DsbC, 8484, and 5193.

11. The method of claim 5, further comprising comparing the measured activity of the recombinant type II asparaginase produced with a measured activity of the same amount of a control type II asparaginase using the same activity assay, wherein the measured activity of the recombinant type II asparaginase produced is comparable to the activity of the control type II asparaginase.

12. The method of claim 1 or 3, wherein the recombinant type II asparaginase is modified to increase half-life in patients.

13. The method of claim 1 or 3, wherein the host cell: is deficient in the expression of one or more proteases; overexpresses one or more folding modulators; or both, and optionally is deficient in HslUV protease, is deficient in PrtB protease; is deficient in Prc protease; is deficient in DegP protease; is deficient in AprA protease; is deficient in Lon protease; is deficient in La protease; is deficient in DegP1; is deficient in DegP2; overexpresses DegP S219A; or a combination thereof.

14. The method of claim 11, wherein the control type II asparaginase is an *E. chrysanthemi* L-asparaginase type II or an *E. coli* L-asparaginase type II.

15. The method of claim 12, wherein the modification is pegylation.