PRODUCTION AND SEPARATION OF 3-HYDROXYPROPIONIC ACID

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) of U.S. Provisional Application No. 62/577,361, filed on October 26, 2017 and Provisional Application No. 62/594,318, filed on December 4, 2017, the entire contents of each of which are incorporated herein by reference.

TECHNICAL FIELD

This disclosure relates to the production of 3-hydroxypropionic acid, including its production from glycerol by recombinant strains, as well as its removal from an aqueous solution (e.g., aqueous broth), and use thereof for producing various chemicals.

BACKGROUND

3-Hydroxypropionic acid (3-HP) and 3-hydroxypropanoate (a salt of 3-HP) are used in the industrial production of various chemicals, such as acrylic acid, which can be used as a cross-linking agent for polymer coatings, metal lubricants and antistatic agents for textiles.

SUMMARY

The disclosure provides methods and apparatus for producing 3-HP or a salt thereof, for removing 3-HP from aqueous solution (e.g., aqueous broth), and for using it to make various chemicals.

In one aspect of the present disclosure, there is provided an expression system, comprising a first promoter that is either constitutive or inducible by a small molecule, a second promoter that is either constitutive or ~~induced~~ inducible by a small molecule, a first gene encoding a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, a modified untranslated region (UTR), and a native sequence encoding up to 20 amino acids from the N-terminus of a native Pseudomonas denitrificans protein, wherein the native sequence is operably linked to the 3'-terminus of the modified UTR and operably linked to the 5'-terminus of the first gene, the first and second promoters are operably linked in tandem

upstream of the modified UTR, and the modified UTR comprises a sequence selected from the group consisting of SEQ ID NO: 22-28 (UTR0-6).

In another aspect of the present disclosure, there is provided a nucleic acid, comprising a first promoter, ~~and~~ a second promoter, and a modified untranslated region (UTR), wherein the first promoter is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, wherein the first promoter is operably linked to the 5'-terminus of the second promoter, the second promoter is operably linked to the 5'-terminus of the modified UTR, and the modified UTR is operably linked to the 5'terminus of the first gene, and wherein the ~~nucleic acid~~modified UTR comprises a sequence selected from the group consisting of SEQ ID NO:22-28 (UTR 0-6).

In ~~a further aspect of the present disclosure~~one example, there is provided a nucleic acid, comprising a first and a second promoter, wherein either the first is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, wherein at least the 10 codons at the 5'-terminus of the first gene are optimized for translation in Pseudomonas denitrificans, and wherein codon optimization comprises:

measuring a codon frequency of each amino acid in a gene encoding a native *Pseudomonas denitrificans* protein; and

using the codon frequency of the native protein to replace the 10 codons at the 5'-terminus of the first gene with an optimized 10 codons, wherein the codon for each amino acid of the optimized 10 codons is present at the same frequency as the codon frequency of the native protein,

wherein the gene encoding a native *Pseudomonas denitrificans* protein is mmsA.

CLAIMS

1. An expression system, comprising a first promoter that is either constitutive or inducible by a small molecule, a second promoter that is either constitutive or ~~induced~~ inducible by a small molecule, a first gene encoding a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, a modified untranslated region (UTR), and a native sequence encoding up to 20 amino acids from the N-terminus of a native Pseudomonas denitrificans protein, wherein the native sequence is operably linked to the 3'-terminus of the modified UTR and operably linked to the 5'-terminus of the first gene, the first and second promoters are operably linked in tandem upstream of the modified UTR, and the modified UTR comprises a sequence selected from the group consisting of SEQ ID NO: 22-28 (UTR0-6).

2. The expression system of claim 1, further comprising a third promoter; and a second gene encoding a transcriptional regulator configured to regulate expression of the first gene, wherein the third promoter is operably connected to the second gene, and the third promoter comprises a sequence that is 95% identical to the sequence selected from the group consisting of SEQ ID NO: 7-10 and 52-63.

3. The expression system of claim 1, wherein the first promoter is operably linked to the 5'-terminus of the second promoter, the second promoter is operably linked to the 5'-terminus of the modified UTR, and the modified UTR is operably linked to the 5'terminus of the first gene.

4. A nucleic acid, comprising a first promoter, ~~and~~ a second promoter, and a modified untranslated region (UTR), wherein the first promoter is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, wherein the first promoter is operably linked to the 5'-terminus of the second promoter, the second promoter is operably linked to the 5'-terminus of the modified

UTR, and the modified UTR is operably linked to the 5'terminus of the first gene, and wherein the ~~nucleic acid~~ modified UTR comprises a sequence selected from the group consisting of SEQ ID NO:22-28 (UTR 0-6).

5. The nucleic acid of claim 4, wherein the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP) and is selected from the group consisting of a glycerol dehydratase, a glycerol dehydratase reactivase, and an aldehyde dehydrogenase,

wherein:

the glycerol dehydratase is selected from the group consisting of dhaB1, dhaB2, dhaB3;

the glycerol dehydratase reactivase is selected from the group consisting of gdrA and gdrB; and

the aldehyde dehydrogenase is selected from the group consisting of kgsA.

6. The nucleic acid of claim 4, wherein the first gene comprises a dhaB1 gene, a dhaB2 gene, a dhaB3 gene, a gdrA gene, a gdrB gene, and a kgsA gene, and the sequence comprises a sequence that is at least 95% identical to a SEQ ID selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6.

7. The nucleic acid of claim 4, wherein the small molecule is an acid or alcohol, optionally wherein the acid or alcohol is selected from the group consisting of L-lactic acid (LAC), acetic acid (AcOH), propionic acid (PA), 3-hydroxypropionic acid (3-HP), 3-hydroxybutyrate (3-HB), 1,3-propanediol (1,3-PDO), 2,3-butanediol (2,3-BDO), L-valine (L-val), and 3-hydroxyisobutyrate (3-HIB).

8. The nucleic acid of claim 4, wherein the second promoter is derived from a PmmsA promoter, a PhbdH-1 promoter, a PhbdH-4 promoter, a PhpdH promoter, or a Pzwf promoter, and the promoter comprises a sequence that ~~it~~ is at least 95% identical to a SEQ ID selected from the group consisting of: SEQ ID NO: 7; SEQ ID

NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 52-63; and SEQ ID NO: 65-72.

9. The nucleic acid of claim 4, wherein the nucleic acid further comprises a gene encoding a transcriptional regulator that regulates expression of the first gene, wherein the transcriptional regulator binds to the first or second promoter,

optionally wherein the transcriptional regulator is a LysR-type transcriptional regulator (LTTR), a MmsR regulator or a HpdR regulator.

10. The nucleic acid of claim 4, wherein the first gene is fused at its 5'-terminus to a sequence encoding up to 20 (5, 10, 15, or 20) amino acids that are derived from the 5'terminus of a second gene encoding a native Pseudomonas denitrificans protein, thereby creating a fusion gene, and the second promoter is derived from the native promoter for the second gene, and the fusion gene comprises a sequence encoding at least 5, 10, 15, or 20 amino acids in the N-terminus of a native protein.

11. The nucleic acid of claim 10, wherein the second promoter comprises the PmmsA promoter and the fusion gene comprises a sequence encoding five or more amino acids from the N-terminus of a native MmsA gene, and the fusion gene is operably linked to the 3' end of the PmmsA promoter, and wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 32-35.

12. The nucleic acid of claim 10 or claim 11, wherein the mRNA of the fusion gene has a higher stability and increases the gene translation when compared to the first gene alone.

~~13. A nucleic acid, comprising a first and a second promoter, wherein either the first is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP,~~

~~or coenzyme B12, wherein at least the 10 codons at the 5'-terminus of the first gene are optimized for translation in Pseudomonas denitrificans, and wherein codon optimization comprises:~~

~~measuring a codon frequency of each amino acid in a gene encoding a native *Pseudomonas denitrificans* protein; and~~

~~using the codon frequency of the native protein to replace the 10 codons at the 5'-terminus of the first gene with an optimized 10 codons, wherein the codon for each amino acid of the optimized 10 codons is present at the same frequency as the codon frequency of the native protein,~~

~~wherein the gene encoding a native *Pseudomonas denitrificans* protein is mmsA.~~

~~14. The nucleic acid of claims 13, wherein wherein the nucleic acid comprises a sequence that is 95% identical to a sequence selected from the group consisting of SEQ ID Nos: 29, 30, and 31 (Opt1-3).~~