

CLAIMS

What is claimed is:

1. A recombinant microorganism having an improved ethylene producing ability, wherein the recombinant microorganism expresses at least one ethylene forming enzyme (EFE) protein having an amino acid sequence at least 95% identical to SEQ ID NO: 1 by expressing a non-native EFE expressing nucleotide sequence,
wherein an amount of EFE protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native EFE expressing nucleotide sequence.
- 10 2. The recombinant microorganism of claim 1, wherein the recombinant microorganism expresses at least one alpha-ketoglutarate permease (AKGP) protein having an amino acid sequence at least 95% identical to SEQ ID NO: 2 by expressing a non-native AKGP expressing nucleotide sequence,
15 wherein an amount of AKGP protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native AKGP expressing nucleotide sequence.
- 20 3. The recombinant microorganism of claim 1, wherein the amount of EFE protein produced by the recombinant microorganism is from about 5% to about 200% or more greater than that produced relative to the control microorganism lacking the non-native EFE expressing nucleotide sequence.
- 25 4. The recombinant microorganism of claim 1, wherein the recombinant microorganism includes a microorganism selected from the group consisting of a *Cyanobacteria*, a *Synechococcus*, *Synechococcus elongatus*, *Synechococcus leopoliensis*, *Synechocystis*, *Anabaena*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*, *Chlamydomonas*, *Chlamydomonas reinhardtii*, *Escherichia*, *Escherichia coli*, *Geobacteria*, algae, microalgae, electrosynthesis bacteria, a photosynthetic microorganism, yeast,
30 filamentous fungi, and a plant cell.
5. The recombinant microorganism of claim 1, wherein the non-native EFE expressing nucleotide sequence is inserted into a bacterial vector plasmid, a high copy number bacterial vector plasmid, a bacterial vector plasmid having an inducible promoter, a nucleotide guide

of a homologous recombination system, a CRISPR CAS system, a phage display system, or a combination thereof.

6. The recombinant microorganism of claim 2, wherein the non-native EFE expressing nucleotide sequence and the non-native AKGP expressing nucleotide sequence are inserted into a bacterial vector plasmid, a high copy number bacterial vector plasmid, a bacterial vector plasmid having an inducible promoter, a nucleotide guide of a homologous recombination system, a CRISPR CAS system, a phage display system, or a combination thereof.

7. The recombinant microorganism of claim 1, wherein the non-native EFE expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 3, and the non-native EFE expressing nucleotide sequence is inserted into a vector plasmid of a *Chlamydomonas* sp. bacterium.

8. The recombinant microorganism of claim 2, wherein the non-native EFE expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 4, and the non-native EFE expressing nucleotide sequence and the AKGP expressing nucleotide sequence are inserted into a vector plasmid of an *Escherichia* sp. bacterium.

9. The recombinant microorganism of claim 1, further comprising a non-native AKGP expressing nucleotide sequence, wherein the non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence express a combined amino acid sequence at least 95% identical to SEQ ID NO. 5, and the non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence are inserted into a bacterial plasmid of a *Synechococcus* sp. bacterium.

10. The recombinant microorganism of claim 1, further comprising a non-native AKGP expressing nucleotide sequence, wherein the non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence express a combined amino acid sequence at least 95% identical to SEQ ID NO. 6, and the non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence are inserted into a bacterial plasmid of a *Synechococcus* sp. bacterium.

11. The recombinant microorganism of claim 1, wherein the recombinant microorganism expresses at least one phosphoenolpyruvate synthase (PEP) protein having an amino acid sequence at least 95% identical to SEQ ID NO. 15 by expressing a non-native PEP expressing nucleotide sequence,

- 5 wherein an amount of PEP protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native PEP expressing nucleotide sequence, and
wherein an amount of AKG produced by the recombinant microorganism is greater than that produced relative to the control microorganism.

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12. The recombinant microorganism of claim 11, wherein the recombinant microorganism expresses at least one citrate synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 17 by expressing a non-native citrate synthase expressing nucleotide sequence, wherein an amount of citrate synthase protein produced by
15 the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native citrate synthase expressing nucleotide sequence.

13. The recombinant microorganism of claim 1, wherein the recombinant microorganism expresses at least one isocitrate dehydrogenase (IDH) protein having an amino acid sequence
20 at least 95% identical to SEQ ID NO. 20 by expressing a non-native IDH expressing nucleotide sequence,
wherein an amount of IDH protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native IDH expressing nucleotide sequence, and
25 wherein an amount of AKG produced by the recombinant microorganism is greater than that produced relative to the control microorganism.

14. The recombinant microorganism of claim 13, wherein the recombinant microorganism contains a deletion in a glucose-1-phosphate adenylyltransferase expressing
30 nucleotide sequence, wherein an amount of glucose-1-phosphate adenylyltransferase protein produced by the recombinant microorganism is less than that produced relative to a control microorganism lacking the deletion.

15. The recombinant microorganism of claim 11, wherein the recombinant microorganism includes a microorganism selected from the group consisting of a *Cyanobacteria*, a *Synechococcus*, *Synechococcus elongatus*, *Synechococcus leopoliensis*, *Synechocystis*, *Anabaena*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*,
5 *Chlamydomonas*, *Chlamydomonas reinhardtii*, *Escherichia*, *Escherichia coli*, *Geobacteria*, algae, microalgae, electrosynthesis bacteria, a photosynthetic microorganism, yeast, filamentous fungi, and a plant cell.

16. The recombinant microorganism of claim 1, wherein the recombinant microorganism
10 expresses at least one sucrose permease protein having an amino acid sequence at least 95% identical to SEQ ID NO. 24 by expressing a non-native sucrose permease expressing nucleotide sequence,
wherein an amount of sucrose permease protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native
15 sucrose permease expressing nucleotide sequence.

17. The recombinant microorganism of claim 1, wherein the recombinant microorganism expresses at least one protein selected from the group consisting of a sucrose phosphate synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 26, a
20 sucrose-6-phosphatase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 28, a glycogen phosphorylase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 30, and a UTP-glucose-1-phosphate uridylyltransferase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 32, by expressing a non-native nucleotide sequence encoding the at least one protein,
25 wherein an amount of the at least one protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native nucleotide sequence encoding the at least one protein,
wherein an amount of sucrose produced by the recombinant microorganism is greater than that produced relative to the control microorganism.

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18. The recombinant microorganism of claim 17, wherein the recombinant microorganism contains at least one deletion in at least one nucleotide sequence, wherein the at least one nucleotide sequence encodes at least one protein selected from an invertase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 34, a

glucosylglycerol-phosphate synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 36, and a glycogen synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 38, wherein an amount of the at least one protein produced by the recombinant microorganism is less than that produced relative to a control microorganism lacking the at least one deletion.

19. A method of producing a recombinant microorganism having an improved ethylene producing ability comprising:

producing the recombinant microorganism by inserting a non-native EFE expressing nucleotide sequence or a combined non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence into a bacterial plasmid of a microorganism,

wherein the non-native EFE expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 3 or SEQ ID NO. 4, or

wherein the combined non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence expresses an amino acid sequence at least 95% identical to SEQ ID NO. 5 or SEQ ID NO. 6; or

wherein the combined non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 7.

20. The method of claim 19, wherein the microorganism is selected from the group consisting of a *Cyanobacteria*, a *Synechococcus*, *Synechococcus elongatus*, *Synechococcus leopoliensis*, *Synechocystis*, *Anabaena*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*, *Chlamydomonas*, *Chlamydomonas reinhardtii*, *Escherichia*, *Escherichia coli*, *Geobacteria*, algae, microalgae, electrosynthesis bacteria, a photosynthetic microorganism, yeast, filamentous fungi, and a plant cell.

21. The method of claim 19, wherein the non-native EFE expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 3 and the microorganism is a *Chlamydomonas* sp. bacterium; or

wherein the non-native EFE expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 4 and the microorganism is an *Escherichia* sp. bacterium; or

the combined non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence expresses an amino acid sequence at least 95% identical to SEQ ID NO. 5 or SEQ ID NO. 6 and the microorganism is a *Synechococcus* sp. bacterium; or

the combined non-native EFE expressing nucleotide sequence and non-native AKGP
5 expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 7 and the microorganism is *Synechococcus* sp. bacterium.

22. A method of producing ethylene comprising:

providing a recombinant microorganism having an improved ethylene producing
10 ability, wherein the recombinant microorganism expresses at least one ethylene forming enzyme (EFE) protein having an amino acid sequence at least 95% identical to SEQ ID NO: 1 by expressing a non-native EFE expressing nucleotide sequence,

wherein an amount of EFE protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native EFE
15 expressing nucleotide sequence;

culturing the recombinant microorganism in a bioreactor culture vessel under conditions sufficient to produce ethylene in the bioreactor culture vessel; and

harvesting ethylene from the bioreactor culture vessel.

23. The method of claim 22, wherein the recombinant microorganism contains a non-native EFE expressing nucleotide sequence or a combined non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence inserted into a bacterial plasmid of the microorganism,

wherein the non-native EFE expressing nucleotide sequence has a nucleotide
25 sequence at least 95% identical to SEQ ID NO. 3 or SEQ ID NO. 4, or

wherein the combined non-native EFE expressing nucleotide sequence and non-native AKGP expressing nucleotide sequence has a nucleotide sequence at least 95% identical to SEQ ID NO. 7; or

wherein the combined non-native EFE expressing nucleotide sequence and non-native
30 AKGP expressing nucleotide sequence expresses an amino acid sequence at least 95% identical to SEQ ID NO. 5 or SEQ ID NO. 6.

24. The method of claim 22, wherein the microorganism is selected from the group consisting of a *Cyanobacteria*, a *Synechococcus*, *Synechococcus elongatus*, *Synechococcus*

leopoliensis, *Synechocystis*, *Anabaena*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*, *Chlamydomonas*, *Chlamydomonas reinhardtii*, *Escherichia*, *Escherichia coli*, *Geobacteria*, algae, microalgae, electrosynthesis bacteria, a photosynthetic microorganism, yeast, filamentous fungi, and a plant cell.

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25. The method of claim 22, further comprising increasing an amount of ethylene production by adding at least one activator to a culture containing the recombinant microorganism located within the bioreactor culture vessel; or adding CO₂ to a culture atmosphere contained within the bioreactor culture vessel at rate of between about 100 ml/minute and about 500 ml/minute.

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26. The method of claim 22, further comprising decreasing an amount of ethylene production by removing at least one molecular switch from the cell culture containing the recombinant microorganism located within the bioreactor culture vessel.

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27. The method of claim 22, further comprising controlling the amount of ethylene produced from the microbial culture by increasing or decreasing the concentration of at least one nutrient or the amount of at least one stimulus when culturing the recombinant microorganism.

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28. The method of claim 22, wherein the concentration of at least one nutrient and the amount of at least one stimulus are at a ratio of from about 0.5-1.5 gr./liter to about 0.1 mM in the microbial culture.

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29. The method of claim 22, further comprising removing the amount of ethylene produced from the microbial culture by condensing the ethylene from a gaseous to a liquid state, or wherein the amount of ethylene recovered is from about 0.5 ml to about 10 ml/liter/h.

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30. A recombinant microorganism having an improved alpha-ketoglutarate (AKG) producing ability, wherein the recombinant microorganism expresses at least one phosphoenolpyruvate synthase (PEP) protein having an amino acid sequence at least 95% identical to SEQ ID NO. 15 by expressing a non-native PEP expressing nucleotide sequence, and

wherein an amount of PEP protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native PEP expressing nucleotide sequence; or

wherein the recombinant microorganism expresses at least one isocitrate dehydrogenase

- 5 (IDH) protein having an amino acid sequence at least 95% identical to SEQ ID NO. 20 by expressing a non-native IDH expressing nucleotide sequence, and

wherein an amount of IDH protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native IDH expressing nucleotide sequence;

- 10 wherein an amount of AKG produced by the recombinant microorganism is greater than that produced relative to the control microorganism.

31. The recombinant microorganism of claim 30, wherein the recombinant microorganism expresses at least one citrate synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO. 17 by expressing a non-native citrate synthase
15 expressing nucleotide sequence, wherein an amount of citrate synthase protein produced by the recombinant microorganism is greater than that produced relative to a control microorganism lacking the non-native citrate synthase expressing nucleotide sequence.

- 20 32. The recombinant microorganism of claim 30, wherein the recombinant microorganism contains a deletion in a glucose-1-phosphate adenylyltransferase expressing nucleotide sequence, wherein an amount of glucose-1-phosphate adenylyltransferase protein produced by the recombinant microorganism is less than that produced relative to a control microorganism lacking the deletion.

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33. The recombinant microorganism of claim 30, wherein the recombinant microorganism includes a microorganism selected from the group consisting of a *Cyanobacteria*, a *Synechococcus*, *Synechococcus elongatus*, *Synechococcus leopoliensis*, *Synechocystis*, *Anabaena*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*,
30 *Chlamydomonas*, *Chlamydomonas reinhardtii*, *Escherichia*, *Escherichia coli*, *Geobacteria*, algae, microalgae, electrosynthesis bacteria, a photosynthetic microorganism, yeast, filamentous fungi, and a plant cell.

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