conventional belief has been that this plant produces high levels of steviol glycoside, so the Sr.KO enzyme should have evolved to have a higher conversion rate, or handle a higher flux, than most other KO enzymes.

[0006] In a yeast strain with high carbon flux to RebM, the Sr.KO was found to have a low conversion efficiency rate to kaurenoic acid (25.6%), and very high levels of the upstream intermediate metabolites (*ent*-kaurene, kaurenol and kaurenal) were formed (**FIG. 1C**).

[0007] To produce RebM efficiently and at high purity, improved enzymes capable of producing kaurenoic acid at high efficiency are needed. The compositions and methods provided herein address this need and provide related advantages as well.

4. SUMMARY

[0008] Provided herein are compositions and methods for the improved conversion of kaurene to kaurenoic acid. These compositions and methods are based in part on the surprising discovery of certain kaurene oxidases (KOs) are capable of converting kaurene to kaurenoic acid with remarkably high efficiency. Even a modest improvement in strain performance (*e.g.*, ten percent) with new KOs can potentially save over ten million dollars in production cost in the future, assuming that the market demand for RebM is 5000 million tons per year.

[0009] Certain KOs described herein are also capable of producing kaurenoic acid with little or no residual kaurenol or kaurenal. As such, in certain examples, the compositions and methods described herein can reduce the costs of downstream processing to obtain a composition with high yield steviol glycosides such as RebM.

[0009a] In one aspect, the present disclosure refers to a genetically modified *Saccharomyces cerevisiae* host cell capable of producing one or more steviol glycosides comprising a heterologous nucleic acid encoding a kaurene oxidase comprising an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO: 1.

[0009b] In another aspect, the present disclosure refers to a method for producing kaurenoic acid: (a) culturing a population of the genetically modified Saccharomyces cerevisiae host cells of any the preceding claims in a medium with a carbon source under conditions suitable for making RebD; and (b) recovering said kaurenoic acid compound from the medium.

[0009c] In another aspect, the present disclosure refers to a method for producing RebD: (a) culturing a population of the genetically modified host cells of any the preceding claims in a medium with a carbon source under conditions suitable for making RebD; and (b) recovering said RebD compound from the medium.

[0009d] In another aspect, the present disclosure refers to a method for producing RebM: (a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and (b) recovering said RebM compound from the medium.

[0009e] In another aspect, the present disclosure refers to an *in vitro* method for producing kaurenoic acid: (a) contacting kaurene with a non-naturally occurring kaurene oxidase of any of the preceding claims, capable of converting kaurene to kaurenoic acid, under conditions suitable for forming kaurenoic acid; and (b) recovering said kaurenoic acid compound from the medium.

[0009f] In another aspect, the present disclosure refers to a fermentation composition comprising: (a) a genetically modified *Saccharomyces cerevisiae* host cell comprising: i. a heterologous nucleic acid encoding a non-natural kaurene oxidase of any of the preceding claims, capable of converting kaurene to kaurenoic acid; and (b) steviol glycosides produced from the genetically modified host cell.

[0009g] In another aspect, the present disclosure refers to a non-naturally occurring kaurene oxidase having at least 80%, 85%, 90%, 95% sequence identity to SEQ ID NO:1.

[0009h] In another aspect, the present disclosure refers to a non-naturally occurring nucleic acid encoding the non-naturally occurring kaurene oxidase as disclosed herein.

[0009i] In another aspect, the present disclosure refers to a method for producing a steviol glycoside: (a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making a steviol glycoside.

[0010] In one example, provided herein are genetically modified host cells and methods of their use for the production of industrially useful compounds. In one example, provided herein is a genetically modified host cell comprising: a heterologous nucleic acid encoding a *Pisum sativum* kaurene oxidase. In some examples, the genetically modified host cell further comprises one or more enzymatic pathways capable of producing steviol and/or steviol glycosides.

[0011] In certain examples, provided herein are genetically modified host cells comprising a heterologous nucleic acid encoding a kaurene oxidase comprising an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to the sequence of *Pisum sativum* kaurene oxidase (*e.g.*, SEQ ID NO:1). In certain examples, the genetically

modified host cell is capable of converting kaurene to kaurenoic acid at an efficiency greater than 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, or 98%. In certain examples the genetically modified host cells are yeast cells. In certain examples, the genetically modified host cells are *Saccharomyces cerevisiae* cells.

[0012] In another example, provided herein are methods for producing a heterologous steviol glycoside, the method comprising: culturing a population of genetically modified host cells provided herein, capable of producing the steviol glycoside as described herein, in a medium with a carbon source under conditions suitable for making said steviol glycoside compound; and recovering said steviol glycoside from the medium. In some examples, heterologous steviol glycoside is selected from the group consisting of RebD and RebM.

[0013] In another example, provided herein are methods for producing RebD, the method comprising: culturing a population of genetically modified host cells provided herein, capable of producing RebD as described herein, in a medium with a carbon source under conditions suitable for making said RebD; and recovering said RebD from the medium.

[0014] In another example, provided herein are methods for producing RebM, the method comprising: culturing a population of genetically modified host cells provided herein, capable of producing RebM as described herein, in a medium with a carbon source under conditions suitable for making said RebM; and recovering said RebM from the medium.

[0015] In another example, provided herein are methods for producing kaurenoic acid, the method comprising: contacting kaurene with a kaurene oxidase described herein, capable of converting kaurene to kaurenoic acid, under conditions suitable for forming kaurenoic acid.

[0016] In some examples, the host cell is a yeast cell. In some examples, the yeast is *Saccharomyces cerevisiae*. In some examples, the host cell produces RebD or RebM at high efficiency. In some examples, the host cell produces an increased amount of RebD or RebM compared to a yeast cell not comprising the *Pisum sativum* kaurene oxidase enzyme.

5. BRIEF DESCRIPTION OF THE FIGURES

[0017] **FIG. 1A** provides a schematic representation of the conversion of farnesyl pyrophosphate to steviol.

[0018] **FIG. 1B** provides a schematic representation of the conversion of geranyl geranyl pyrophosphate (GGPP) to RebM.

WHAT IS CLAIMED:

1. A genetically modified *Saccharomyces cerevisiae* host cell capable of producing one or more steviol glycosides comprising a heterologous nucleic acid encoding a kaurene oxidase comprising an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO: 1.

2. The genetically modified *Saccharomyces cerevisiae* host cell of claim 1 that is capable of converting kaurene to kaurenoic acid at an efficiency of greater than 30%, 35%, 40%, 45%, 50%, or 55%.

3. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the kaurene oxidase comprises an amino acid sequence having the sequence of SEQ ID NO:1.

4. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the kaurene oxidase is capable of oxidation of the C19 position of kaurene, kaurenol, and/or kaurenal.

5. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the kaurene oxidase is encoded by a heterologous nucleic acid, wherein the heterologous nucleic acid comprises a nucleotide sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO:15.

6. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the kaurene oxidase is encoded by a heterologous nucleic acid having the sequence of SEQ ID NO:15.

7. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, that is capable of converting kaurene to kaurenoic acid at an efficiency of greater than 30%, 35%, 40%, 45%, 50%, or 55%, and wherein the kaurene oxidase comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:1.

8. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the kaurene oxidase is a polypeptide having a functional domain with at least 80%, 85%, 90%, or 95% sequence identity to a functional domain of SEQ ID NO:1.

9. The genetically modified *Saccharomyces cerevisiae* host cell of claim 7 that is capable of converting kaurene to kaurenoic acid at an efficiency of greater than 55%.

10. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims that is capable of producing kaurenoic acid.

11. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims that is capable of producing steviol.

12. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims that is capable of producing RebD.

13. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims that is capable of producing RebM.

14. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims that is capable of producing at RebM and RebM2 at a ratio of at least 10:1, 100:1, or 1000:1.

15. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the genetically modified host cell produces an undetectable level of RebM2.

16. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making steviol.

17. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more

heterologous nucleic acids encoding one or more enzymes of a pathway for making a steviol glycoside.

18. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making a RebA.

19. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making RebM.

20. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making RebE.

21. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a geranylgeranyl diphosphate synthase.

22. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a copalyl diphosphate synthase.

23. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise an *ent*-kaurene synthase.

24. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a kaurenoic acid hydroxylase.

25. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a cytochrome P450 reductase.

26. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise one or more uridine 5'-diphosphate-dependent glycosyltransferases.

27. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims wherein the one or more enzymes of the pathway comprise Os_UGT_91C1, Sl_UGT_101249881, UGT40087, sr.UGT_9252778, Bd_UGT10840, Hv_UGT_V1, Bd_UGT10850, or Ob_UGT91B1_like.

28. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims wherein the one or more enzymes of the pathway comprise UGT74G1, UGT76G1, UGT85C2, UGT91D, or UGT40087.

29. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a geranylgeranyl diphosphate synthase, a copalyl diphosphate synthase, a ent-kaurene synthase, a kaurene oxidase, a kaurenoic acid hydroxylase, a cytochrome P450 reductase, a UGT_{AD}, UGT74G1, UGT76G1, UGT85C2, and UGT91D.

30. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a geranylgeranyl diphosphate synthase, a copalyl diphosphate synthase, a *ent*-kaurene synthase, a kaurene oxidase, a kaurenoic acid hydroxylase, a cytochrome P450 reductase, UGT40087, UGT74G1, UGT76G1, UGT85C2, and UGT91D.

31. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the UGT40087 is according to SEQ ID NO:17, 18, or 33.

32. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprises a bifunctional copalyl diphosphate synthase and kaurene synthase.

33. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the pathway are under control of a single transcriptional regulator.

34. The genetically modified *Saccharomyces cerevisiae* host cell of any of the preceding claims, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the pathway are under control of multiple heterologous transcriptional regulators.

35. A method for producing kaurenoic acid:

(a) culturing a population of the genetically modified *Saccharomyces cerevisiae* host cells of any the preceding claims in a medium with a carbon source under conditions suitable for making RebD; and

(b) recovering said kaurenoic acid compound from the medium.

36. A method for producing RebD:

(a) culturing a population of the genetically modified host cells of any the preceding claims in a medium with a carbon source under conditions suitable for making RebD; and

(b) recovering said RebD compound from the medium.

37. A method for producing RebM:

(a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and

(b) recovering said RebM compound from the medium.

38. An *in vitro* method for producing kaurenoic acid:

(a) contacting kaurene with a non-naturally occurring kaurene oxidase of any of the preceding claims, capable of converting kaurene to kaurenoic acid, under conditions suitable for forming kaurenoic acid; and

(b) recovering said kaurenoic acid compound from the medium.

39. A fermentation composition comprising:

(a) a genetically modified *Saccharomyces cerevisiae* host cell comprising:

i. a heterologous nucleic acid encoding a non-natural kaurene oxidase of any of the preceding claims, capable of converting kaurene to kaurenoic acid; and

(b) steviol glycosides produced from the genetically modified host cell.

40. The fermentation composition of claim 39, wherein the steviol glycosides comprise RebA, RebD and ReM at a ratio of RebA:RebD:RebM of at least 1:7:50.

41. A non-naturally occurring kaurene oxidase having at least 80%, 85%, 90%, 95% sequence identity to SEQ ID NO:1.

42. A non-naturally occurring kaurene oxidase of claim 41 that is capable of converting kaurene to kaurenoic acid at an efficiency of greater than 30%, 35%, 40%, 45%, 50%, or 55% *in vivo*.

43. A non-naturally occurring nucleic acid encoding the non-naturally occurring kaurene oxidase of claim 41 or 42.

44. A method for producing a steviol glycoside:

(a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making a steviol glycoside.