

Claims

1. An isolated genetically engineered microorganism for producing β -ketoadipic acid from depolymerized lignin, wherein the microorganism has been transformed by at least one polynucleotide molecule; the at least one polynucleotide molecule comprising heterologous β -ketoadipic acid pathway genes, feruloyl-CoA synthetase (*fcs*), enoyl-CoA hydratase (*ech*),
5 β -ketoadipic acid pathway genes, feruloyl-CoA synthetase (*fcs*), enoyl-CoA hydratase (*ech*),
vanillin dehydrogenase (*vdh*), vanillate O-demethylase (*vanAB*; *vanA* and *vanB*), *p*-
hydroxybenzoate hydroxylase (*pobA*), protocatechuate 3,4-dioxygenase (*pcaGH*; *pcaG* and
pcaH), 3-carboxy-cis,cis-muconate cycloisomerase (*pcaB*), 4-carboxymuconolactone
decarboxylase (*pcaC*) and β -ketoadipate enol-lactone hydrolase (*pcaD*) operably linked to at
10 least one promoter, wherein said genetically engineered microorganism can convert
depolymerized lignin to β -ketoadipic acid.
2. The isolated genetically engineered microorganism according to claim 1, further comprising:
15 (a) heterologous β -ketoadipic acid utilization genes, β -ketoadipic acid succinyl-CoA
transferase (*pcaIJ*; *pcaI* and *pcaJ*), 3-hydroxyacyl-CoA dehydrogenase (*paaH1*), enoyl-CoA
hydratase (*ech*), trans-enoyl-CoA reductase (*ter*), phosphate butyryltransferase (*ptb*) and
butyrate kinase 1 (*buk1*) operably linked to at least one promoter, wherein said genetically
engineered microorganism can convert β -ketoadipic acid to adipic acid, and/or
20 (b) a heterologous β -ketoadipic acid utilization gene, acetoacetate decarboxylase (*adc*),
operably linked to at least one promoter, wherein said genetically engineered microorganism
can convert β -ketoadipic acid to levulinic acid.
3. The isolated genetically engineered microorganism according to claim 1 or 2, wherein:
25 the *fcs* gene encodes an amino acid sequence set forth in SEQ ID NO: 2; and/or
the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 4; and/or
the *vdh* gene encodes an amino acid sequence set forth in SEQ ID NO: 6; and/or
the *vanA* gene encodes an amino acid sequence set forth in SEQ ID NO: 8; and/or
the *vanB* gene encodes an amino acid sequence set forth in SEQ ID NO: 10; and/or
30 the *pobA* gene encodes an amino acid sequence set forth in SEQ ID NO: 12; and/or
the *pcaH* gene encodes an amino acid sequence set forth in SEQ ID NO: 14; and/or
the *pcaG* gene encodes an amino acid sequence set forth in SEQ ID NO: 16; and/or
the *pcaB* gene encodes an amino acid sequence set forth in SEQ ID NO: 18; and/or
the *pcaC* gene encodes an amino acid sequence set forth in SEQ ID NO: 20; and/or
35 the *pcaD* gene encodes an amino acid sequence set forth in SEQ ID NO: 22; and/or

- the *ter* gene encodes an amino acid sequence set forth in SEQ ID NO: 24; and/or
the *pcaI* gene encodes an amino acid sequence set forth in SEQ ID NO: 26; and/or
the *pcaJ* gene encodes an amino acid sequence set forth in SEQ ID NO: 28; and/or
the *paaH1* gene encodes an amino acid sequence set forth in SEQ ID NO: 30; and/or
5 the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 32; and/or
the *ptb* gene encodes an amino acid sequence set forth in SEQ ID NO: 34; and/or
the *buk1* gene encodes an amino acid sequence set forth in SEQ ID NO: 36; and/or
the *adc* gene encodes an amino acid sequence set forth in SEQ ID NO: 38.
- 10 4. The isolated genetically engineered microorganism according to claim 3, wherein:
the *fcs* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 1; and/or
the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 3; and/or
15 the *vdh* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 5; and/or
the *vanA* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 7; and/or
the *vanB* gene comprises a nucleic acid sequence at least 80% sequence identity to the
20 polynucleotide sequence set forth in SEQ ID NO: 9; and/or
the *pobA* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 11; and/or
the *pcaH* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 13; and/or
25 the *pcaG* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 15; and/or
the *pcaB* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 17; and/or
the *pcaC* gene comprises a nucleic acid sequence at least 80% sequence identity to the
30 polynucleotide sequence set forth in SEQ ID NO: 19; and/or
the *pcaD* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 21; and/or
the *ter* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 23; and/or
35 the *pcaI* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 25; and/or

- the *pcaJ* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 27; and/or
- the *paaH1* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 29; and/or
- 5 the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 31; and/or
- the *ptb* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 33; and/or
- the *buk1* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 35; and/or
- 10 the *adc* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 37.
5. The isolated genetically engineered microorganism according to any one of claims 1 to 3,
- 15 wherein the at least one promoter is modulated by a heterologous genetic controller.
6. The isolated genetically engineered microorganism according to any one of claims 1 to 5, wherein the at least one promoter is a constitutive promoter, such as T7.
- 20 7. The isolated genetically engineered microorganism according to claim 5 or 6, wherein the heterologous genetic controller is pBAD or hydroxycinnamic acid (HA).
8. The isolated genetically engineered microorganism according to claim 7, wherein the heterologous genetic controller pBAD comprises a nucleic acid sequence set forth in SEQ ID
- 25 NO: 41 or the hydroxycinnamic acid (HA) controller comprises a nucleic acid sequence set forth in SEQ ID NO: 42.
9. The isolated genetically engineered microorganism according to any one of claims 2(a) and 3 to 8, further comprising an inactivated endogenous succinyl CoA synthetase gene,
- 30 such as *sucCD* and/or an inactivated β -ketoadipyl-CoA thiolase gene, such as *paaJ*.
10. The isolated genetically engineered microorganism according to claim 9, wherein the *sucCD* genes encode an amino acid sequence set forth in SEQ ID NO: 54 and SEQ ID NO: 56, respectively, and/or the *paaJ* gene encodes an amino acid sequence set forth in SEQ ID
- 35 NO: 52.

11. The isolated genetically engineered microorganism according to any one of claims 2(b) and 3 to 8, further comprising an inactivated endogenous Acyl CoA:acetate/3-ketoacid CoA transferase gene, such as *atoDA*.
- 5 12. The isolated genetically engineered microorganism according to claim 11, wherein the *atoDA* genes encode an amino acid sequence set forth in SEQ ID NO: 48 and SEQ ID NO: 50, respectively.
13. The isolated genetically engineered microorganism according to any one of claims 1 to 10 12, comprising a bacteria or yeast, preferably a bacteria such as *Escherichia coli*.
14. The isolated genetically engineered microorganism according to claim 13, wherein the bacteria comprises *Escherichia coli* MG1655.
- 15 15. The isolated engineered microorganism according to any one of claims 1 to 14, wherein the depolymerized lignin is from fibrous oil palm empty fruit bunches.
16. Use of the isolated genetically engineered microorganism according to any one of claims 2(a) to 15 for the production of adipic acid, or according to any one of claims 2(b) to 20 15 for the production of levulinic acid.
17. A recombinant vector comprising heterologous β -ketoadipic acid pathway genes, *fcs*, *ech*, *vdh*, *vanAB* (*vanA* and *vanB*), *pobA*, *pcaGH* (*pcaG* and *pcaH*), *pcaB*, *pcaC* and *pcaD* operably linked to at least one promoter, and/or
- 25 heterologous β -ketoadipic acid utilization genes, *pcaIJ* (*pcaI* and *pcaJ*), *paaH1*, *ech*, *ter*, *ptb* and *buk1* operably linked to at least one promoter, and/or
- a heterologous β -ketoadipic acid utilization gene, *adc*, operably linked to at least one promoter.
- 30 18. The recombinant vector of claim 17, wherein:
- the *fcs* gene encodes an amino acid sequence set forth in SEQ ID NO: 2; and/or
- the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 4; and/or
- the *vdh* gene encodes an amino acid sequence set forth in SEQ ID NO: 6; and/or
- the *vanA* gene encodes an amino acid sequence set forth in SEQ ID NO: 8; and/or
- 35 the *vanB* gene encodes an amino acid sequence set forth in SEQ ID NO: 10; and/or
- the *pobA* gene encodes an amino acid sequence set forth in SEQ ID NO: 12; and/or

- the *pcaH* gene encodes an amino acid sequence set forth in SEQ ID NO: 14; and/or
the *pcaG* gene encodes an amino acid sequence set forth in SEQ ID NO: 16; and/or
the *pcaB* gene encodes an amino acid sequence set forth in SEQ ID NO: 18; and/or
the *pcaC* gene encodes an amino acid sequence set forth in SEQ ID NO: 20; and/or
5 the *pcaD* gene encodes an amino acid sequence set forth in SEQ ID NO: 22; and/or
the *ter* gene encodes an amino acid sequence set forth in SEQ ID NO: 24; and/or
the *pcaI* gene encodes an amino acid sequence set forth in SEQ ID NO: 26; and/or
the *pcaJ* gene encodes an amino acid sequence set forth in SEQ ID NO: 28; and/or
the *paaH1* gene encodes an amino acid sequence set forth in SEQ ID NO: 30; and/or
10 the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 32; and/or
the *ptb* gene encodes an amino acid sequence set forth in SEQ ID NO: 34; and/or
the *buk1* gene encodes an amino acid sequence set forth in SEQ ID NO: 36; and/or
the *adc* gene encodes an amino acid sequence set forth in SEQ ID NO: 38.
- 15 19. The recombinant vector of claim 18, wherein:
the *fcs* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 1; and/or
the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 3; and/or
20 the *vdh* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 5; and/or
the *vanA* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 7; and/or
the *vanB* gene comprises a nucleic acid sequence at least 80% sequence identity to the
25 polynucleotide sequence set forth in SEQ ID NO: 9; and/or
the *pobA* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 11; and/or
the *pcaH* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 13; and/or
30 the *pcaG* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 15; and/or
the *pcaB* gene comprises a nucleic acid sequence at least 80% sequence identity to the
polynucleotide sequence set forth in SEQ ID NO: 17; and/or
the *pcaC* gene comprises a nucleic acid sequence at least 80% sequence identity to the
35 polynucleotide sequence set forth in SEQ ID NO: 19; and/or

- the *pcaD* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 21; and/or
- the *ter* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 23; and/or
- 5 the *pcaI* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 25; and/or
- the *pcaJ* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 27; and/or
- the *paaH1* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 29; and/or
- 10 the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 31; and/or
- the *ptb* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 33; and/or
- 15 the *buk1* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 35; and/or
- the *adc* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 37.
- 20 20. A kit comprising an isolated genetically engineered microorganism according to any one of claims 1 to 15, or a recombinant vector of any one of claims 17 to 19.
21. A method of producing β -ketoadipic acid from depolymerized lignin, comprising the step of culturing a plurality of genetically engineered microorganisms of any one of claims 1 to 15 under conditions for production of said β -ketoadipic acid.
- 25 22. A method of producing adipic acid from depolymerized lignin, comprising the step of culturing a plurality of genetically engineered microorganisms of any one of claims 1 to 15 under conditions for production of said adipic acid.
- 30 23. A method of producing levulinic acid from depolymerized lignin, comprising the step of culturing a plurality of genetically engineered microorganisms of any one of claims 1 to 15 under conditions for production of said levulinic acid.
- 35 24. The method of any one of claims 21 to 23, further comprising isolating said product produced by the genetically engineered microorganisms.

25. The method of any one of claims 21 to 24, wherein the microorganism is a bacterium, such as *Escherichia coli*, preferably *Escherichia coli* MG1655.
- 5 26. The method of any one of claims 21 to 25, wherein the depolymerized lignin is from fibrous oil palm empty fruit bunches.