Claims

1. An isolated genetically engineered *Escherichia coli* bacteria for producing β-ketoadipic acid from depolymerized lignin from fibrous oil palm empty fruit bunches, wherein the microorganism has been transformed by at least one polynucleotide molecule; the at least one polynucleotide molecule comprising:

a)(i) heterologous β-ketoadipic acid pathway genes, which are 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 least one promoter, and

a)(ii) heterologous β-ketoadipic acid utilization genes, which are 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, or

b)(i) heterologous β-ketoadipic acid pathway genes, which are 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 least one promoter, and

b)(ii) a heterologous β-ketoadipic acid utilization gene, acetoacetate decarboxylase (*adc*) operably linked to at least one promoter; and

c) a heterologous genetic controller to modulate the at least one promoter, wherein the heterologous genetic controller is pBAD or hydroxycinnamic acid (HA);

wherein said genetically engineered *Escherichia coli* bacteria of a) further comprises an inactivated endogenous succinyl CoA synthetase gene sucCD and/or an inactivated β-ketoadipyl-CoA thiolase gene paaJ;

wherein said genetically engineered *Escherichia coli* bacteria of b) further comprises an inactivated endogenous Acyl CoA:acetate/3-ketoacid CoA transferase gene atoDA;

wherein said genetically engineered *Escherichia coli* bacteria of a) can convert depolymerized lignin to adipic acid, and

wherein said genetically engineered *Escherichia coli* bacteria of b) can convert depolymerized lignin to levulinic acid.

2. The isolated genetically engineered microorganism according to claim 1, wherein:
the *fcs* gene encodes an amino acid sequence set forth in SEQ ID NO: 2;
the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 4;
the *vdh* gene encodes an amino acid sequence set forth in SEQ ID NO: 6;
the *vanA* gene encodes an amino acid sequence set forth in SEQ ID NO: 8;
the *vanB* gene encodes an amino acid sequence set forth in SEQ ID NO: 10;
the *pobA* gene encodes an amino acid sequence set forth in SEQ ID NO: 12;
the *pcaH* gene encodes an amino acid sequence set forth in SEQ ID NO: 14;
the *pcaG* gene encodes an amino acid sequence set forth in SEQ ID NO: 16;
the *pcaB* gene encodes an amino acid sequence set forth in SEQ ID NO: 18;
the *pcaC* gene encodes an amino acid sequence set forth in SEQ ID NO: 20;
the *pcaD* gene encodes an amino acid sequence set forth in SEQ ID NO: 22;
the *ter* gene encodes an amino acid sequence set forth in SEQ ID NO: 24;
the *pcaI* gene encodes an amino acid sequence set forth in SEQ ID NO: 26;
the *pcaJ* gene encodes an amino acid sequence set forth in SEQ ID NO: 28;
the *paaH1* gene encodes an amino acid sequence set forth in SEQ ID NO: 30;
the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 32;
the *ptb* gene encodes an amino acid sequence set forth in SEQ ID NO: 34;
the *buk1* gene encodes an amino acid sequence set forth in SEQ ID NO: 36; and
the *adc* gene encodes an amino acid sequence set forth in SEQ ID NO: 38.

3. The isolated genetically engineered microorganism according to claim 2, 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;
the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 3;
the *vdh* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 5;
the *vanA* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 7;
the *vanB* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 9;
the *pobA* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 11;

the *pcaH* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 13;

the *pcaG* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 15;

the *pcaB* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 17;

the *pcaC* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 19;

the *pcaD* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 21;

the *ter* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 23;

the *pcaI* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 25;

the *pcaJ* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 27;

the *paaH1* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 29;

the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 31;

the *ptb* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 33;

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

the *adc* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 37.

4. The isolated genetically engineered microorganism according to any one of claim 1, wherein the at least one promoter is a constitutive promoter, such as T7.

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5. The isolated genetically engineered microorganism according to claim 1, wherein the heterologous genetic controller pBAD comprises a nucleic acid sequence set forth in SEQ ID NO: 41 or the hydroxycinnamic acid (HA) controller comprises a nucleic acid sequence set forth in SEQ ID NO: 42.

6. The isolated genetically engineered microorganism according to claim 1, 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 NO: 52.

7. The isolated genetically engineered microorganism according to claim 1, wherein the *atoDA* genes encode an amino acid sequence set forth in SEQ ID NO: 48 and SEQ ID NO: 50, respectively.

8. The isolated genetically engineered microorganism according to claim 1, wherein the bacteria is *Escherichia coli* MG1655.

9. Use of the isolated genetically engineered microorganism according to any one of claims 1(a) to 8 for the production of adipic acid, or according to any one of claims 1(b) to 8 for the production of levulinic acid.

10. A recombinant vector comprising:
A) heterologous β-ketoadipic acid pathway genes, which are *fcs*, *ech*, *vdh*, *vanAB* (*vanA* and *vanB*), *pobA*, *pcaGH* (*pcaG* and *pcaH*)*, pcaB, pcaC and pcaD* operably linked to at least one promoter, and heterologous β-ketoadipic acid utilization genes, which are *pcalJ* (*pcaI* and *pcaJ*), *paaH1*, *ech*, *ter*, *ptb* and *buk1* operably linked to at least one promoter; or
B) heterologous β-ketoadipic acid pathway genes, which are *fcs*, *ech*, *vdh*, *vanAB* (*vanA* and *vanB*), *pobA*, *pcaGH* (*pcaG* and *pcaH*)*, pcaB, pcaC and pcaD* operably linked to at least one promoter, and a heterologous β-ketoadipic acid utilization gene, *adc*, operably linked to at least one promoter.

11. The recombinant vector of claim 10, wherein:
the *fcs* gene encodes an amino acid sequence set forth in SEQ ID NO: 2;
the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 4;
the *vdh* gene encodes an amino acid sequence set forth in SEQ ID NO: 6;
the *vanA* gene encodes an amino acid sequence set forth in SEQ ID NO: 8;

the *vanB* gene encodes an amino acid sequence set forth in SEQ ID NO: 10;
the *pobA* gene encodes an amino acid sequence set forth in SEQ ID NO: 12;
the *pcaH* gene encodes an amino acid sequence set forth in SEQ ID NO: 14;
the *pcaG* gene encodes an amino acid sequence set forth in SEQ ID NO: 16;
the *pcaB* gene encodes an amino acid sequence set forth in SEQ ID NO: 18;
the *pcaC* gene encodes an amino acid sequence set forth in SEQ ID NO: 20;
the *pcaD* gene encodes an amino acid sequence set forth in SEQ ID NO: 22;
the *ter* gene encodes an amino acid sequence set forth in SEQ ID NO: 24;
the *pcaI* gene encodes an amino acid sequence set forth in SEQ ID NO: 26;
the *pcaJ* gene encodes an amino acid sequence set forth in SEQ ID NO: 28;
the *paaH1* gene encodes an amino acid sequence set forth in SEQ ID NO: 30;
the *ech* gene encodes an amino acid sequence set forth in SEQ ID NO: 32;
the *ptb* gene encodes an amino acid sequence set forth in SEQ ID NO: 34;
the *buk1* gene encodes an amino acid sequence set forth in SEQ ID NO: 36; and
the *adc* gene encodes an amino acid sequence set forth in SEQ ID NO: 38.

12. The recombinant vector of claim 11, 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;
the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 3;
the *vdh* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 5;
the *vanA* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 7;
the *vanB* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 9;
the *pobA* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 11;
the *pcaH* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 13;
the *pcaG* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 15;
the *pcaB* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 17;

the *pcaC* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 19;

the *pcaD* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 21;

the *ter* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 23;

the *pcaI* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 25;

the *pcaJ* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 27;

the *paaH1* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 29;

the *ech* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 31;

the *ptb* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 33;

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

the *adc* gene comprises a nucleic acid sequence at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO: 37.

13. A kit comprising isolated genetically engineered *Escherichia coli* bacteria according to any one of claims 1 to 8, or a recombinant vector of claim 10.

14. A method of producing β-ketoadipic acid from depolymerized lignin from fibrous oil palm empty fruit bunches, comprising the step of culturing a plurality of genetically engineered *Escherichia coli* bacteria of claim 1 under conditions for production of said β-ketoadipic acid.

15. A method of producing adipic acid from depolymerized lignin from fibrous oil palm empty fruit bunches, comprising the step of culturing a plurality of genetically engineered *E. coli* bacteria of claim 1a under conditions for production of said adipic acid.

16. A method of producing levulinic acid from depolymerized lignin from fibrous oil palm empty fruit bunches, comprising the step of culturing a plurality of genetically engineered *Escherichia coli* bacteria of claim 1b under conditions for production of said levulinic acid.

17. The method of any one of claims 14 to 16, further comprising isolating said product produced by the genetically engineered *Escherichia coli* bacteria.

18. The method of any one of claims 14 to 17, wherein the *Escherichia coli* bacteria is *Escherichia coli* MG1655.