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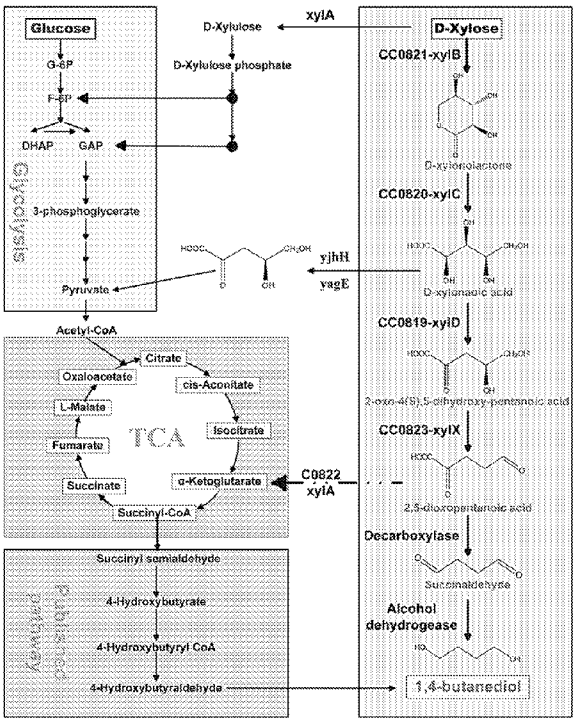
(54) Title: BIOSYNTHETIC PATHWAYS AND METHODS

Figure 1

A.



(57) Abstract: This disclosure describes a recombinant microbial cells and methods of making and using such recombinant microbial cells. Generally, the recombiannt cells may be modified to exhibit increased biosynthesis of a TCA derivative compared to a wild-type control. In some embodiments, the TCA derivative can include 1,4-butanediol. In various embodiments, the microbial cell is a fungal cell or a bacterial cell. In some embodiments, the increased biosynthesis of the TCA derivative can include an increase in xylose dehydrogenase activity, xylonolactonase activity, xylonate dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity.

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BIOSYNTHETIC PATHWAYS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 61/738,752, filed December 18, 2012 and U.S. Provisional Patent Application Serial No. 61/821,490, filed May 9, 2013, each of which is incorporated herein by reference.

SUMMARY

This disclosure describes, in one aspect, a recombinant microbial cell modified to exhibit increased biosynthesis of a TCA derivative compared to a wild-type control. In some embodiments, the TCA derivative can include 1,4-butanediol. In various embodiments, the microbial cell is a fungal cell or a bacterial cell. In some embodiments, the increased biosynthesis of the TCA derivative can include an increase in xylose dehydrogenase activity, xylonolactonase activity, xylonate dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity.

In another aspect, this disclosure describes a method that generally includes incubating any embodiments of the recombinant cell summarized above in medium that includes a carbon source under conditions effective for the recombinant cell to produce a TCA derivative. In some embodiments, the TCA derivative can include 1,4-butanediol. In some embodiments, the carbon source can include xylose, arabinose, glucaric acid, galactaric acid, or hydroxyproline. In some embodiments, the increased biosynthesis of the TCA derivative can include an increase in pentose dehydrogenase activity, pentonolactonase activity, aldonic acid dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity. In other embodiments, the increased biosynthesis of the TCA derivative can include an increase in hexic acid dehydratase activity or 5-dehydro-4-deoxyglucarate dehydratase activity.

In another aspect, this disclousre describes a method that generally includes introducing into a host cell a heterologous polynucleotide encoding at least one polypeptide that catalyzes conversion of a carbon source to a TCA derivative, wherein the at least one polypeptide is operably linked to a promoter so that the modified host cell catalyzes conversion of the carbon source to TCA derivative. In some embodiments, the TCA

derivative can include 1,4-butanediol. In some embodiments, the carbon source can include xylose.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. The 1,4-butanediol synthetic pathway in *E.coli*. (A) The synthetic pathway for 1,4-butanediol from glucose and xylose. Abbreviations: G-6P, glucose-6-phosphate; F-6P, fructose-6-phosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate. (B) Synthetic operon for protein overexpression to drive the xylose towards 2,5-dioxopentanoic acid (left), and then drive 2,5-dioxopentanoic acid towards to 1,4-butanediol (right).

FIG. 2. A, scheme of the organization of conserved genetic clusters involved in the pentose, hexaric acid, and hydroxyproline degradation. Analogous functions are indicated in the same degree of shading. Coding region sizes and distances are not to scale. Protein family numbers are displayed below each coding region according to Clusters of Orthologous Groups of proteins classification system. The coding regions indicated in white or gray encode the following proteins: araA, transcriptional regulator; araF-araH, l-Ara ABC transporter (periplasmic l-Ara binding protein, ATP-binding protein, permease); rrnAC3038, heat shock protein X; ycbE, glucarate/galactarate permease; ycbG, transcriptional regulator; PP1249, hydroxyproline permease. B, schematic representation of the convergence of catabolic pathways for pentoses, hexaric acids, and hydroxyproline at the level of 2,5-dioxopentanoate. Enzymatic activities are indicated by their EC number. Dashed lines indicate proposed spontaneous reactions.

FIG. 3. An engineered 1,4-butanediol synthetic pathway in *E.coli*.

FIG. 4. An exemplary engineered metabolic pathway from 2,5-dioxopentanoic acid to 1,4-butanediol.

FIG. 5. An exemplary engineered metabolic pathway from 2,5-dioxopentanoic acid to 1,4-butanediol.

FIG. 6. An exemplary engineered metabolic pathway from D-arabonose to 2,5-dioxopentanoic acid.

FIG. 7. An exemplary engineered metabolic pathway from D-xylose to 2,5-dioxopentanoic acid.

FIG. 8. An exemplary engineered metabolic pathway from L-arabinose to 2,5-dioxopentanoic acid.

FIG. 9. An exemplary engineered metabolic pathway from D-glucaric acid to 2,5-dioxopentanoic acid.

FIG. 10. An exemplary engineered metabolic pathway from D-galactaric acid to 2,5-dioxopentanoic acid.

FIG. 11. An exemplary engineered metabolic pathway from 4(R)-hydroxy-L-proline to 2,5-dioxopentanoic acid.

FIG. 12. A plasmid map of YEplac195-xylBCDX (13945 bp).

FIG. 13. A plasmid map of YEplac11-KivDyqhD (9687 bp).

FIG. 14. Gas chromatography data showing production of 1,4-butanediol by genetically engineered *S. cerevisiae*.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

This disclosure describes a novel full biosynthetic pathway to biosynthesize high-volume TCA derivatives such as succinate, amino acids, and 1,4-butanediol from xylose by an engineered microbe. The TCA cycle can lead to many commercially important biobased chemicals such as, for example, amino acids (e.g., glutamate, threonine and lysine) and organic acids (e.g., succinate, maleate and fumarate). Here we report the engineering of a shortcut metabolic pathway to TCA cycle. The process from xylose to TCA only involves five steps as compared to conventional published pathways that include more than 20 steps. Because our pathway includes fewer steps from xylose to the TCA cycle, our pathway can produce TCA derivatives with the production of less by-product and, therefore, achieve higher yields than conventional biosynthetic pathways.

We have selected the TCA derivative 1,4-butanediol as a model product to demonstrate the generality of our novel biosynthetic pathway. 1,4-butanediol is a major

commodity chemical; 2.5 million tons of 1,4-butanediol are used per year to make, for example, plastics, polyesters, and spandex fibers. 1,4-butanediol also can react, for example, with dicarboxylic acids to yield polyesters, with diisocyanates to yield polyurethanes, and with phosgene to yield chloroformates. Because our pathway permits the biosynthesis of 1,4-butanediol from, for example, xylose in only six steps from xylose to 1,4-butanediol, 1,4-butanediol may be biosynthesized with less by-product being formed and, therefore, a higher yield. For example, our pathway can produce produce 1.0 g/L 1,4-butanediol from 20 g/L xylose.

1,4-butanediol currently is manufactured from petroleum-based feedstocks such as acetylene, butane, propylene, and butadiene. Given the industrial importance of 1,4-butanediol as a chemical intermediate and the issues associated with petroleum feedstocks, alternative low-cost renewable biosynthetic routes from sugars have been sought. However, the highly reduced nature of 1,4-butanediol relative to carbohydrates has thwarted attempts thus far to develop effective pathways and organisms for direct production.

1,4-butanediol has been reported to be synthesized from glucose and xylose by engineered *E. coli* in which the succinyl-CoA intermediate was converted into succinate semialdehyde, 4-hydroxybutyrate, 4-hydroxybutyryl-CoA, 4-hydroxybutyraldehyde, and 1,4-butanediol by multiple enzymes from various organisms. This process involves around 20 chemical steps that include the pentose phosphate pathway, glycolysis, the TCA cycle, and designed artificial downstream metabolic steps. In contrast, this disclosure describes a shortcut pathway that requires only six steps (FIG.1A).

D-xylose is converted by *Caulobacter crescentus* sequentially to D-xylonolactone, D-xylonate (D-xylonoic acid), 2-keto-3-deoxy-xylonate (2-oxo-4(S),5-dihydroxy-pentanoic acid), then α-ketoglutaric semialdehyde (2,5-dioxopentanoic acid) by, respectively, xylose dehydrogenase (xylB), xylonolactonase (xylC), xylonate dehydrogenase (xylD), Kda dehydratase (xylX). We cloned the coding regions of these enzymes into a single plasmid (pBDO-1), which was then transformed into an *E. coli* host cell. The host cell was then further modified to include a second plasmid that included a decarboxylase and an alcohol dehydrogenase. The decarboxylase converts the α-ketoglutaric semialdehyde to succinaldehyde; the alcohol dehydrogenase reduces the succinaldehyde to 1,4-butanediol (FIG.1A). In some embodiments, the second plasmid was identified as pBDO-3 and included the coding regions of benzoylformate decarboxylase BFD (*Pseudomonas putida*) and an

alcohol dehydrogenase of yqhD (*E. coli*). In other embodiments, the second plasmid was identified as pBDO-4 and included the decarboxylase of KIVD (*Lactococcus lactis*) and alcohol dehydrogenase of yqhD (*E. coli*).

The *E. coli* host cell possesses an endogenous xylose metabolism pathway that includes *xylA*, *yjhH* and *yagE*. To improve the product yield from xylose to 1,4-butanediol, expression of these three coding regions were inhibited. The host cell strain SBDO-1 is based on *E. coli* BW25113 in which *xylA*, *yjhH*, and *yagE* are knocked out so that SBDO-1 cannot metabolize xylose. Strain SBDO-2, carrying plasmid pBDO-1, also cannot metabolize xylose.

The strain SBDO-3, which is based on SBDO-2 but carries plasmid pBDO-2 that expresses α-ketoglutaric semialdehyde dehydrogenase *xylA*, can consume xylose quickly. These results indicate that the endogenous xylose utilization pathway in *E. coli* was blocked fully by the Δ*xylA*, Δ*yjhH*, and Δ*yagE* deletions in SBDO-1. Moreover, these results demonstrate that the *C. crescentus* enzymes function in *E. coli*. Consequently, xylose metabolism observed in SBDO-4 and SBDO-5 is attributable to the xylose pathway from *C. crescentus* that we engineered into the host cell. To produce 1,4-butanediol, plasmids pBDO-3 or pBDO-4, each of which expresses the same alcohol dehydrogenase but a different decarboxylase, were introduced into strain SBDO-2 strain. After two days of fermentation, strain SBDO-4 (carrying pBDO-3) produced 0.25 g/L 1,4-butanediol with 0.1 g/L 1,2,4-butanetriol (a by-product); strain SBDO-5 (carrying pBDP-4) produced 1.0 g/L 1,4-butanediol with 4.0 g/L 1,2,4-butanetriol. Thus, the *kivD* encoded on pBDO-4 and carried by strain SBDO-5 provides better yield of 1,4-butanediol than *BFD*. Other than 1,2,4-butanetriol, no other byproducts were detected in significant amounts in the fermentation broth, suggesting that our new 1,4-butanediol producing pathway has higher 1,4-butanediol yield as compared with the published pathway (Yim et al., 2011. *Nat. Chem. Biol.* 7:445-452).

Thus, in one aspect, the invention provides recombinant microbial cell modified to exhibit increased biosynthesis of a TCA derivative compared to a wild-type control.

While described above in the context of an exemplary embodiment in which the TCA derivative is a 1,4-butanediol, the recombinant cells and methods described herein can provide TCA derivatives other than 1,4-butanediol. Exemplary alternative TCA derivatives include, for example, succinate, fumarate, malate, glutamate, lysine, threonine, 4-hydroxybutyrate, and products synthesizable from a product of the TCA cycle in one, two, three, four, or five enzymatic steps. In some of these embodiments, one or more enzymes

involved in the synthesis of the TCA derivative may be heterologous to the host cell and, therefore, provided recombinantly. Exemplary TCA derivative products and exemplary enzymes involved in the synthesis of the exemplary TCA derivative products are listed in Table 1. For any embodiment in which the identified enzyme is not endogenous to a host cell, the enzyme may be introduced into the host cell to produce a recombinant cell as described herein.

Table 1. Exemplary enzymes, enzyme sources, native substrates, and TCA derivative products

Common Name	Organism	Encoding gene	Accession No. ; GI No.	Native Substrate	TCA derivative product	SEQ ID NO
D-arabinose dehydrogenase						
alcohol dehydrogenase (AraDH)	*Sulfolobus solfataricus*	SSO1300	NP_342747.1; GI:15898142	D-arabinose	D-arabinonic acid from D-arabinose	1
D-arabinonate dehydratase						
arabinonate dehydratase (AraD)	*Sulfolobus solfataricus*	SSO3124	NP_344435.1; GI:15899830	D-arabinonic acid	2-oxo-4(*S*),5-dihydroxy-pentanoic acid	6
2-Keto-3-deoxy-D-arabinonate Dehydratase						
2-keto-4-pentenoate hydratase (KdaD)	*Sulfolobus solfataricus*	*SSO3118*	NP_344431.1; GI:15899826	2-oxo-4(*S*),5-dihydroxy-pentanoic acid	2,5-dioxopentanoic acid	11
2,5-dioxopentanoate dehydrogenase						
aldehyde dehydrogenase (DopDH)	*Sulfolobus solfataricus*	SSO3117	NP_344430.1; GI:15899825	2,5-dioxopentanoic acid	2-oxoglutaric acid	16
2,5-dioxovalerate dehydrogenase						
2,5-dioxovalerate dehydrogenase (YcbD)	Bacillus subtilis	YcbD	NP_388129.1 ; GI:16077316	2,5-dioxopentanoic acid	2-oxoglutaric acid	21
D-xylose dehydrogenase						
D-Xylose dehydrogenase (XylB)	Caulobacter crescentus	CC0821	YP_002516237.1; GI:221233801	D-xylose	D-xylonolactone	26
D-xylonolactonase						
D-xylonolactonase (XylC)	Caulobacter crescentus	*CC0820*	YP_002516236.1; GI:221233800	D-xylonolactone	D-xylonic acid	31
D-xylonate dehydratase						
D-xylonate dehydratase (XylD)	Caulobacter crescentus	*CC0819*	NP_419636.1 GI:16125072	D-xylonic acid	2-oxo-4(*S*),5-dihydroxy-pentanoic acid	36
2-Keto-3-deoxy-D-arabinonate dehydratase						
2-keto-4-pentenoate hydratase (XylX)	Caulobacter crescentus	*CC0823*	NP_419640.1; GI:16125076	2-oxo-4(*S*),5-dihydroxy-pentanoic acid	2,5-dioxopentanoic acid	41
L-arabinose dehydrogenase						
dehydrogenase (AraE)	Burkholderia thailandensis E264	BTH_II1629	YP_439823.1; GI:83716868	L-arabinose	L-arabinonolactone from L-arabinose	46
L-arabinonolactonase						
L-arabinonolactonase (AraI)	Burkholderia thailandensis E264	BTH_II1625	YP_439819.1; GI:83717359	L-arabinonolactone	L-arabinonic acid	51
L-arabinonate dehydratase						
L-arabinonate dehydratase (AraB)	Burkholderia thailandensis E264	BTH_II1632	YP_439826.1; GI:83718062	L-arabinonic acid	2-oxo-4(*R*),5-dihydroxy-pentanoic acid	56

2-Keto-3-deoxy-L-arabinonate Dehydratase						
dihydrodipicolinate synthase (AraD)	Burkholderia thailandensis E264	BTH_II1630	YP_439824.1; GI:83717217	2-oxo-4(*R*),5-dihydroxy-pentanoic acid	2,5-dioxopentanoic acid	61
D-glucarate dehydratase						
D-glucarate dehydratase (YcbF)	Bacillus subtilis	YcbF	NP_388131.2; GI:255767063	D-glucaric acid	4-deoxy-5-keto-D-glucaric acid	66
D-galactarate dehydratase						
D-galactarate dehydratase (YcbH)	Bacillus subtilis	YcbH	NP_388133.2 ; GI:255767065	D-galactaric acid	4-deoxy-5-keto-D-glucaric acid	71
5-dehydro-4-deoxyglucarate dehydratase						
5-dehydro-4-deoxyglucarate dehydratase (YcbC)	Bacillus subtilis	YcbC	NP_388128.2; GI:255767061	4-deoxy-5-keto-D-glucaric acid	2,5-dioxopentanoic acid	76
Amino acid transporter LysE						
Amino acid transporter LysE (HypE)	Pseudomonas putida	PP_1248	NP_743408.1; GI:26987983	4(*R*)-hydroxy-L-proline	4(*R*)-hydroxy-D-proline	81
PP_1245						
Hypothetical protein of PP_1245	Pseudomonas putida	PP_1245	NP_743405.1; GI:26987980	4(*R*)-hydroxy-D-proline	2-carboxy-4(R)-hydroxy-pyrroline	86
PP_1247						
Hypothetical protein of PP_1247	Pseudomonas putida	PP_1247	NP_743407.1; GI:26987982	2-carboxy-4(R)-hydroxy-pyrroline	2,5-dioxopentanoic acid	91
PP_1246						
Hypothetical protein of PP_1246	Pseudomonas putida	PP_1246	NP_743406.1; GI:26987981	2,5-dioxopentanoic acid	2-oxoglutaric acid	93
Alpha-ketoisovalerate decarboxylase						
alpha-ketoisovalerate decarboxylase	Lactococcus lactis	KivD	YP_003353820.1; GI:281491840	2,5-dioxopentanoic acid	Succinaldehyde	98
Alcohol dehydrogenase (YqhD)						
alcohol dehydrogenase	E.coli	yqhD	YP_001459806.1; GI:157162488	Succinaldehyde	1,4-butanediol	103

In addition to the enzymes listed in Table 1, homologs of the listed enzymes may be used. Thus, as an alternative to AraDH (SEQ ID NO:1), one may use, for example, any of the polypeptides depicted in SEQ ID NO:2-5; as an alternative to AraD (SEQ ID NO:6), one may use, for example, any of the polypeptides depicted in SEQ ID NO: 7-10; as an alternative to Kda (SEQ ID NO:11), one may use, for example, any of the polypeptides depicted in SEQ ID NO: 12-15; as an alternative to DopDH (SEQ ID NO:16), one may use, for example, any of the polypeptides depicted in SEQ ID NO:17-20; as an alternative to YcbD (SEQ ID NO:21), one may use, for example, any of the polypeptides depicted in SEQ ID NO:22-25; as an alternative to XylB (SEQ ID NO:26), one may use, for example, any of the polypeptides depicted in SEQ ID NO:27-30; as an alternative to XylC (SEQ ID NO:31), one may use, for example, any of the polypeptides depicted in SEQ ID NO:32-35; as an alternative to XylD (SEQ ID NO:36), one may use, for example, any of the polypeptides depicted in SEQ ID NO:37-40; as an alternative to XylX (SEQ ID NO:41), one may use, for example, any of the polypeptides depicted in SEQ ID NO:42-45; as an alternative to AraE (SEQ ID NO:46), one may use, for example, any of the polypeptides depicted in SEQ ID NO:47-50; as an alternative to AraI (SEQ ID NO:51), one may use, for example, any of the polypeptides depicted in SEQ ID NO:52-55; as an alternative to AraB (SEQ ID NO:56), one may use, for example, any of the polypeptides depicted in SEQ ID NO:57-60; as an alternative to AraD (SEQ ID NO:61), one may use, for example, any of the polypeptides depicted in SEQ ID NO:62-65; as an alternative to YcbF (SEQ ID NO:66), one may use, for example, any of the polypeptides depicted in SEQ ID NO:67-70; as an alternative to YcbH (SEQ ID NO:71), one may use, for example, any of the polypeptides depicted in SEQ ID NO:72-75; as an alternative to YcbC (SEQ ID NO:76), one may use, for example, any of the polypeptides depicted in SEQ ID NO:77-80; as an alternative to HypE (SEQ ID NO:81), one may use, for example, any of the polypeptides depicted in SEQ ID NO:82-85; as an alternative to PP_1245 (SEQ ID NO:86), one may use, for example, any of the polypeptides depicted in SEQ ID NO:87-90; as an alternative to PP_1247 (SEQ ID NO:91), one may use, for example, the polypeptide depicted in SEQ ID NO:92; as an alternative to PP_1246 (SEQ ID NO:93), one may use, for example, any of the polypeptides depicted in SEQ ID NO:94-97; as an alternative to alpha-ketoisovalerate decarboxylase (SEQ ID NO:98), one may use, for example, any of the polypeptides depicted in SEQ ID NO:99-102; as an alternative to YqhD

(SEQ ID NO:103), one may use, for example, any of the polypeptides depicted in SEQ ID NO:104-107.

In some cases, the wild-type control may be unable to produce the TCA derivative and, therefore, an increase in the biosynthesis of a particular product may reflect any measurable biosynthesis of that product. In certain embodiments, an increase in the biosynthesis of a TCA derivative can include biosynthesis sufficient for a culture of the microbial cell to accumulate the TCA derivative to a predetermine concentration.

The predetermined concentration may be any predetermined concentration of the product suitable for a given application. Thus, a predetermined concentration may be, for example, a concentration of at least 0.1 g/L such as, for example, at least 0.25 g/L, at least 0.5 g/L, at least 1.0 g/L, at least 2.0 g/L, at least 3.0 g/L, at least 4.0 g/L, at least 5.0 g/L, at least 6.0 g/L, at least 7.0 g/L, at least 8.0 g/L, at least 9.0 g/L, at least 10 g/L, at least 20 g/L, at least 50 g/L, at least 100 g/L, or at least 200 g/L.

While described above in the context of an exemplary embodiment in which the host cell is *E. coli*, the recombinant cells described herein can be constructed, and the methods of making and using the recombinant cells can be performed, using any suitable host cell.

Thus, the recombinant cell can be, or be derived from, any suitable microbe including, for example, a prokaryotic microbe or a eukaryotic microbe. As used herein, the term "or derived from" in connection with a microbe simply allows for the "host cell" to possess one or more genetic modifications before being modified to exhibit the indicated increased biosynthetic activity. Thus, the term "recombinant cell" encompasses a "host cell" that may contain nucleic acid material from more than one species before being modified to exhibit the indicated biosynthetic activity.

In some embodiments, the host cell may be selected to possess one or more natural physiological activities. For example, the host cell may be photosynthetic (e.g., cyanobacteria) or may be cellulolytic (e.g., *Clostridium cellulolyticum*).

In some embodiments, the recombinant cell may be, or be derived from, a eukaryotic microbe such as, for example, a fungal cell. In some of these embodiments, the fungal cell may be, or be derived from, a member of the Saccharomycetaceae family such as, for example, *Saccharomyces cerevisiae*, *Candida rugosa*, or *Candida albicans*.

In other embodiments, the recombinant cell may be, or be derived from, a prokaryotic microbe such as, for example, a bacterium. In some of these embodiments, the bacterium may

be a member of the phylum Protobacteria. Exemplary members of the phylum Protobacteria include, for example, members of the Enterobacteriaceae family (e.g., *Escherichia coli*) and, for example, members of the Pseudomonaceae family (e.g., *Pseudomonas putida*). In other cases, the bacterium may be a member of the phylum Firmicutes. Exemplary members of the phylum Firmicutes include, for example, members of the Bacillaceae family (e.g., *Bacillus subtilis*), members of the Clostridiaceae family (e.g., *Clostridium cellulolyticum*) and, for example, members of the Streptococcaceae family (e.g., *Lactococcus lactis*). In other cases, the bacterium may be a member of the phylum Cyanobacteria.

In some embodiments, the increased biosynthesis of the TCA derivative compared to a wild-type control can include an increase in activity of one or more enzymes involved in the metabolism of the carbon source (e.g., xylose or arabinose). Such enzymes may be found in the proteome of microbes such as, for example, *Sulfolobus solfataricus*, *Caulobacter crescentus*, *Burkholderia thailandensis*, *Haloarcula marismortui*, *Bacillus subtilis*, and *Pseudomonas putida*. Exemplary enzymes, shown in the context of their native metabolic pathways, are shown in FIG. 2. So, for example, increased biosynthesis of the TCA derivative can include an increase in activity of one or more enzymes involved in the metabolism of D-xylose in *Caulobacter crescentus* such as, for example, xylose dehydrogenase (FIG. 2, CC0821) activity, xylonolactonase (FIG. 2, CC0819) activity, xylonate dehydrogenase (FIG. 2, CC0822) activity, and 2-keto-3-deoxyaldonic acid dehydratase (FIG. 2, CC0823) activity compared to the wild-type control.

In some embodiments, the increased biosynthesis of the TCA derivative compared to a wild-type control can further include an increase in benzoylformate decarboxylase activity and an increase in alcohol dehydrogenase activity. In some of these embodiments, the benzoylformate decarboxylase can include BFD of *Pseudomonas putida*. In some of these embodiments, the alcohol dehydrogenase can include yqhD of *E. coli*.

In some embodiments, the increased biosynthesis of the TCA derivative compared to a wild-type control can further include an increase in decarboxylase activity and an increase in alcohol dehydrogenase activity. In some of these embodiments, the decarboxylase can include KIVD of *Lactococcus lactis*. In some of these embodiments, the alcohol dehydrogenase can include yqhD of *E. coli*. See, e.g., Example 2 and FIGS. 12-14.

In some embodiments, the recombinant cell can include an engineered metabolic pathway designed to permit the recombinant cell to increase its consumption of a particular

carbon source compared to a wild-type control. Exemplary metabolic pathways are illustrated in, for example, FIG. 6 through FIG. 11. Accordingly, exemplary carbon sources include, for example, arabinose, xylose, arabinose, glucaric acid, galactaric acid, or hydroxyproline. In other embodiments, the recombinant cell may be designed to consume a uronic acid such as, for example, galacturonic acid and/or glucuronic acid as a carbon source. In such embodiments, a heterologous polynucleotide that encodes a uronate dehydrogenase enzyme may be introduced into the recombinant cell to confer to the recombinant cell the ability to convert uronic acid to aldonic acid. In still other embodiments, the recombinant cell can utilize a carbon source that includes, for example, glucose, cellulose, galacturonic acid, glucuronic acid, CO_2, or glycerol. In some of these embodiments, the recombinant cell may be further modified to convert the carbon source (e.g., glucose) to one or more of the carbon sources (e.g., xylose and/or a hexaric acid such as, e.g., glucaric acid) that is an entry point to one or more of the engineered pathways described herein.

FIG. 6 shows an exemplary metabolic pathway that permits a recombinant cell to use D-arabinose as a carbon source for the production of 2,5-dioxopentanoic acid. In this example, the recombinant cell can include an enzyme that can convert D-arabinose into D-arabinolactone such as, for example, a pentose dehydrogenase. One example of a suitable pentose dehydrogenase includes AraDH from *Sulfolobus solfataricus*. The pentose dehydrogenase can provide catalytic conversion of D-arabinose into D-arabinolactone that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 6 also includes an enzyme that can convert D-arabinonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid such as, for example, an aldonic acid dehydratase. One example of a suitable aldonic acid dehydratase includes AraD from *Sulfolobus solfataricus*. The aldonic acid dehydratase can provide catalytic conversion of D-arabinonic acid into 2-oxo-4(S),5-dihydroxy-pentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 6 also includes an enzyme that can convert 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid such as, for example, a 2-keto-3-deoxyaldonic acid dehydratase. On example of a suitable 2-keto-3-deoxyaldonic acid dehydratase includes KdaD from *Sulfolobus solfataricus*. The 2-keto-3-deoxyaldonic acid dehydratase can provide catalytic conversion of 2-oxo-4(S),5-dihydroxy-

pentanoic acid to 2,5-dioxopentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

FIG. 7 shows an exemplary metabolic pathway that permits a recombinant cell to use D-xylose as a carbon source for the production of 2,5-dioxopentanoic acid. In this example, the recombinant cell can include an enzyme that can convert D-xylose to D-xylonolactone such as, for example, a pentose dehydrogenase. Exemplary suitable pentose dehydrogenases include XylB from *Caulobacter crescentus* or rrnAC3034 from *Haloarcula marismortui*. The pentose dehydrogenase can provide catalytic conversion of D-xylose to D-xylonolactone that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 7 also includes an enzyme that can convert D-xylonolactone to D-xylonic acid such as, for example, a pentonolactonase. Exemplary suitable pentonolactonases include XylC from *Caulobacter crescentus* or rrnAC3033 from *Haloarcula marismortui*. The pentonolactonase can provide catalytic conversion of D-xylonolactone to D-xylonic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 7 also includes an enzyme that can convert D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid such as, for example, an aldonic acid dehydratase. Exemplary suitable aldonic acid dehydratases include XylD from *Caulobacter crescentus* or rrnAC3032 from *Haloarcula marismortui*. The aldonic acid dehydratase can provide catalytic conversion of D-xylonic acid into 2-oxo-4(S),5-dihydroxy-pentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 7 also includes an enzyme that can convert 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid such as, for example, a 2-keto-3-deoxyaldonic acid dehydratase. Exemplary suitable 2-keto-3-deoxyaldonic acid dehydratases include XylX from *Caulobacter crescentus* or rrnAC3039 from *Haloarcula marismortui*. The 2-keto-3-deoxyaldonic acid dehydratase can provide catalytic conversion of 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

FIG. 8 shows an exemplary metabolic pathway that permits a recombinant cell to use L-arabinose as a carbon source for the production of 2,5-dioxopentanoic acid. In this example, the recombinant cell can include an enzyme that can convert L-arabinose to L-arabinolactone

such as, for example, a pentose dehydrogenase. One example of a suitable pentose dehydrogenase includes AraE from *Burkholderia thailandensis*. The pentose dehydrogenase can provide catalytic conversion of L-arabinose to L-arabinolactone that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 8 also includes an enzyme that can convert L-arabinolactone to L-arabinonic acid such as, for example, a pentonolactonase. One example of a suitable pentonolactonase includes AraI from *Burkholderia thailandensis*. The pentonolactonase can provide catalytic conversion of L-arabinolactone to L-arabinonic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 8 also includes an enzyme that can convert L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid such as, for example, an aldonic acid dehydratase. One example of a suitable aldonic acid dehydratase includes AraB from *Burkholderia thailandensis*. The aldonic acid dehydratase can provide catalytic conversion of L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 8 also includes an enzyme that can convert 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid such as, for example, a 2-keto-3-deoxyaldonic acid dehydratase. One example of a suitable 2-keto-3-deoxyaldonic acid dehydratase includes AraD from *Burkholderia thailandensis*. The 2-keto-3-deoxyaldonic acid dehydratase can provide catalytic conversion of 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

FIG. 9 shows an exemplary metabolic pathway that permits a recombinant cell to use D-glucaric acid as a carbon source for the production of 2,5-dioxopentanoic acid. In this example, the recombinant cell can include an enzyme that can convert D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid such as, for example, an aldonic acid dehydratase. Suitable exemplary aldonic acid dehydratases include YcbF from *Bacillus subtilis*. The aldonic acid dehydratase can provide catalytic conversion of D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 9 also includes an enzyme that can convert 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid such as, for example, a 2-keto-3-deoxyaldonic acid dehydratase. One example of a suitable 2-keto-3-deoxyaldonic

acid dehydratase includes YcbC from *Bacillus subtilis*. The 2-keto-3-deoxyaldonic acid dehydratase can provide catalytic conversion of 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

FIG. 10 shows an exemplary metabolic pathway that permits a recombinant cell to use D-galactaric acid as a carbon source for the production of 2,5-dioxopentanoic acid. In this example, the recombinant cell can include an enzyme that can convert D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid such as, for example, an aldonic acid dehydratase. Suitable exemplary aldonic acid dehydratases include YcbH from *Bacillus subtilis*. The aldonic acid dehydratase can provide catalytic conversion of D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 9 also includes an enzyme that can convert 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid such as, for example, a 2-keto-3-deoxyaldonic acid dehydratase. One example of a suitable 2-keto-3-deoxyaldonic acid dehydratase includes YcbC from *Bacillus subtilis*. The 2-keto-3-deoxyaldonic acid dehydratase can provide catalytic conversion of 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

FIG. 11 shows an exemplary metabolic pathway that permits a recombinant cell to use 4(R)-hydroxy-D-proline as a carbon source for the production of 2,5-dioxopentanoic acid. In this example, the recombinant cell can include an enzyme that can convert 4(R)-hydroxy-D-proline to 4(R)-hydroxy-D-proline. One suitable exemplary enzyme for this embodiment includes, amino acid transporter LysE (HypE) from *Pseudomonas*. The enzyme can provide catalytic conversion of 4(R)-hydroxy-D-proline to 4(R)-hydroxy-D-proline that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 11 also includes an enzyme that can convert 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline. One suitable exemplary enzyme for this embodiment includes, for example, HypOX from *Pseudomonas*. The enzyme can provide catalytic conversion of 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline that is, for example, at least 110% greater than that exhibited by a wild-type control.

The exemplary metabolic pathway illustrated in FIG. 11 also includes an enzyme that can convert 2-oxo-4(R)-5-aminopentanoic acid to 2,5-dioxopentanoic acid such as, for example, a 2-keto-3-deoxyaldonic acid dehydratase. One exemplary 2-keto-3-deoxyaldonic acid dehydratase includes PP1247 from *Pseudomonas*. The 2-keto-3-deoxyaldonic acid dehydratase can provide catalytic conversion of 2-oxo-4(R)-5-aminopentanoic acid to 2,5-dioxopentanoic acid that is, for example, at least 110% greater than that exhibited by a wild-type control.

The recombinant cell can be engineered to convert the 2,5-dioxopentanoic acid to any desirable TCA derivative. In some embodiments, the recombinant cell can include an α-ketoglutaric semialdehyde dehydrogenase to shunt the 2,5-dioxopentanoic acid into the TCA cycle. In this manner, TCA cycle derivatives such as, for example, succinate, fumarate, malate, glutamate, lysine, threonine, 4-hydroxybutyrate may be produced.

In some embodiments, however, the recombinant cell may be further modified to possess a metabolic pathway for the conversion of 2,5-dioxopentanoic acid to 1,4-butanediol. Exemplary metabolic pathways are illustrated, for example, in FIG. 4 and FIG. 5. The exemplary pathway illustrated in FIG. 4 includes an enzyme that can convert 2,5-dioxopentonoic acid to succinaldehyde such as, for example, a 2-ketoacid decarboxylase or a 2-oxoglutarate decarboxylase. Suitable exemplary enzymes include, for example, Kivd, BFD, and IPDC. The exemplary pathway illustrated in FIG. 4 also includes an enzyme that can convert succinaldehyde to 1,4-butanediol such as, for example, an alcohol dehydrogenase. Suitable exemplary alcohol dehydrogenases include YqhD, ADH6, YjgB, and YahK.

The exemplary pathway illustrated in FIG. 5 includes an enzyme that can convert 2,5-dioxopentonoic acid to 2-keto-5-hydroxy-pentonate such as, for example, an alcohol dehydrogenase. Here again, suitable exemplary alcohol dehydrogenases include, for example, YqhD, ADH6, YjgB, and YahK. The exemplary pathway illustrated in FIG. 5 includes an enzyme that can convert 2-keto-5-hydroxy-pentonate to 4-hydroxy-1-butyraldehyde such as, for example, a 2-ketoacid decarboxylase or a 2-oxoglutarate decarboxylase. Suitable exemplary enzymes include, for example, Kivd, BFD, and IPDC. The exemplary pathway illustrated in FIG. 5 includes conversion of 4-hydroxy-1-butyraldehyde into 1,4-butanediol. This conversion may be catalyzed by an alcohol dehydrogenase such as, for example, YqhD, ADH6, YjgB, and YahK. For the metabolic pathway illustrated in FIG. 5, therefore, the recombinant cell can include one or more alcohol dehydrogenases.

In some embodiments, the host cell can include one or more genetic modifications to reduce endogenous metabolism of the carbon source so that metabolism of the carbon source is directed toward the production of the TCA derivative. For example, in embodiments in which the carbon source is xylose and the host cell is *E. coli*, the host cell can include one or more modifications to decrease endogenous metabolism of xylose. In the case of *E. coli*, such modifications can include for example, a decrease in α-ketoglutaric semialdehyde dehydrogenase activity, aldolase activity, and/or 2-keto-3-deoxy gluconate aldolase activity. Such modifications can include modifications to coding regions of, or regulatory regions that control expression of, *xylA*, *yjhH*, and/or *yagE*. Such modifications can include, for example, a deletion of a sufficient amount of one or more coding regions that the enzymatic activity is reduced.

As used herein, the terms "activity" with regard to particular enzyme refers to the ability of a polypeptide, regardless of its common name or native function, to catalyze the conversion of the enzyme's substrate to a product, regardless of whether the "activity" as less than, equal to, or greater than the native activity of the identified enzyme. Methods for measuring the biosynthetic activities of cells are routine and well known to those of ordinary skill in the art.

As used herein, an increase in catalytic activity can be quantitatively measured and described as a percentage of the catalytic activity of an appropriate wild-type control. The catalytic activity exhibited by a genetically-modified polypeptide can be, for example, at least 110%, at least 125%, at least 150%, at least 175%, at least 200% (two-fold), at least 250%, at least 300% (three-fold), at least 400% (four-fold), at least 500% (five-fold), at least 600% (six-fold), at least 700% (seven-fold), at least 800% (eight-fold), at least 900% (nine-fold), at least 1000% (10-fold), at least 2000% (20-fold), at least 3000% (30-fold), at least 4000% (40-fold), at least 5000% (50-fold), at least 6000% (60-fold), at least 7000% (70-fold), at least 8000% (80-fold), at least 9000% (90-fold), at least 10,000% (100-fold), or at least 100,000% (1000-fold) of the activity of an appropriate wild-type control.

Alternatively, an increase in catalytic activity may be expressed as at an increase in k_{cat} such as, for example, at least a two-fold increase, at least a three-fold increase, at least a four-fold increase, at least a five-fold increase, at least a six-fold increase, at least a seven-fold increase, at least an eight-fold increase, at least a nine-fold increase, at least a 10-fold

increase, at least a 15-fold increase, or at least a 20-fold increase in the k_{cat} value of the enzymatic conversion.

An increase in catalytic activity also may be expressed in terms of a decrease in K_m such as, for example, at least a two-fold decrease, at least a three-fold decrease, at least a four-fold decrease, at least a five-fold decrease, at least a six-fold decrease, at least a seven-fold decrease, at least an eight-fold decrease, at least a nine-fold decrease, at least a 10-fold decrease, at least a 15-fold decrease, or at least a 20-fold decrease in the K_m value of the enzymatic conversion.

A decrease in catalytic activity can be quantitatively measured and described as a percentage of the catalytic activity of an appropriate wild-type control. The catalytic activity exhibited by a genetically-modified polypeptide can be, for example, no more than 95%, no more than 90%, no more than 85%, no more than 80%, no more than 75%, no more than 70%, no more than 65%, no more than 60%, no more than 55%, no more than 50%, no more than 45%, no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, no more than 5%, no more than 4%, no more than 3%, no more than 2%, no more than 1% of the activity, or 0% of the activity of a suitable wild-type control.

Alternatively, a decrease in catalytic activity can be expressed as an appropriate change in a catalytic constant. For example, a decrease in catalytic activity may be expressed as at a decrease in k_{cat} such as, for example, at least a two-fold decrease, at least a three-fold decrease, at least a four-fold decrease, at least a five-fold decrease, at least a six-fold decrease, at least a seven-fold decrease, at least an eight-fold decrease, at least a nine-fold decrease, at least a 10-fold decrease, at least a 15-fold decrease, or at least a 20-fold decrease in the k_{cat} value of the enzymatic conversion.

A decrease in catalytic activity also may be expressed in terms of an increase in K_m such as, for example, an increase in K_m of at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least six-fold, at least seven-fold, at least an eight-fold, at least nine-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 75-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 230-fold, at least 250-fold, at least 300-fold, at least 350-fold, or at least 400-fold.

Thus, in another aspect, we describe herein methods for biosynthesis of a TCA derivative. Generally, the methods includes incubating a recombinant cell as described herein in medium that includes a carbon source under conditions effective for the recombinant cell to produce the TCA derivative. The carbon source can include, for example, saccharides (e.g., xylose, arabinose, glucose, cellulose), a uronic acid (e.g., galacturonic acid or glucuronic acid), CO_2, glycerol, or a native substrate of an enzyme that is part of the engineered metabolic pathway. Exemplary native substrates of exemplary enzymes are shown in Table 1 and include, for example, glucaric acid, galactaric acid, hydroxyproline, arabinonic acid, 2-oxo-4(*S*),5-dihydroxy-pentanoic acid, 2-oxo-4(*R*),5-dihydroxy-pentanoic acid, 2,5-dioxopentanoic acid, xylonolactone, xylonic acid, arabinonolactone, 4-deoxy-5-keto-D-glucaric acid, 4(*R*)-hydroxy-L-proline , 4(*R*)-hydroxy-D-proline, 2-carboxy-4(R)-hydroxy-pyrroline, 2,5-dioxopentanoic acid, succinaldehyde.

In yet another aspect, we describe herein methods for introducing a heterologous polynucleotide into cell so that the host cell exhibits an increased ability to convert a carbon source to a TCA derivative. The heterologous polynucleotide can encode a polypeptide operably linked to a promoter so that modified cell catalyzes conversion of the carbon source to the TCA derivative. In some of these embodiments, the carbon source can include xylose. The host cells for such methods can include, for example, any of the microbial species identified above with regard to the recombinant cells described herein.

In some embodiments, the heterologous polynucleotide may be inserted into a vector. A vector is a replicating polynucleotide such as, for example, a plasmid, phage, or cosmid, to which another polynucleotide may be inserted so as to bring about the replication of the inserted polynucleotide. Construction of vectors containing a polynucleotide of the invention employs standard ligation techniques known in the art. See, e.g., Sambrook et al, *Molecular Cloning: A Laboratory Manual.*, Cold Spring Harbor Laboratory Press (1989). A vector can permit, for example, further cloning—i.e., a cloning vector—or expression of the polypeptide encoded by the coding region—i.e., an expression vector. The term vector includes, but is not limited to, plasmid vectors, viral vectors, cosmid vectors, or artificial chromosome vectors. In one embodiment, the vector is a plasmid. Selection of a vector can depend upon a variety of desired characteristics in the resulting construct, such as a selection marker, vector replication rate, and the like.

An expression vector optionally includes regulatory sequences operably linked to the coding region. The polynucleotides described herein are not limited by the use of any particular promoter, and a wide variety of promoters are known. Promoters act as regulatory signals that bind RNA polymerase in a cell to initiate transcription of a downstream (3' direction) coding region. The promoter used can be a constitutive or an inducible promoter. It can be, but need not be, heterologous with respect to the host cell. Exemplary promoters include, for example, trp, tac, and T7.

"Coding sequence" or "coding region" refers to a nucleotide sequence that encodes a polypeptide and, when placed under the control of appropriate regulatory sequences, expresses the encoded polypeptide. The boundaries of a coding region are generally determined by a translation start codon at its 5' end and a translation stop codon at its 3' end. As used herein, the term "polypeptide" refers broadly to a polymer of two or more amino acids joined together by peptide bonds. The term "polypeptide" also includes molecules that contain more than one polypeptide joined by disulfide bonds, ionic bonds, or hydrophobic interactions, or complexes of polypeptides that are joined together, covalently or noncovalently, as multimers (e.g., dimers, tetramers). Thus, the terms peptide, oligopeptide, and protein are all included within the definition of polypeptide and these terms are used interchangeably. The term "polypeptide" does not connote a specific length of a polymer of amino acids, nor does it imply or distinguish whether the polypeptide is produced using recombinant techniques, chemical or enzymatic synthesis, or is naturally occurring.

"Regulatory sequence" refers to a nucleotide sequence that regulates expression of a coding region to which it is operably linked. Nonlimiting examples of regulatory sequences include, for example, promoters, transcription initiation sites, translation start sites, translation stop sites, and terminators. "Operably linked" refers to a juxtaposition wherein the components are in a relationship permitting them to function in their intended manner. A regulatory sequence is "operably linked" to a coding region when it is joined in such a way that expression of the coding region is achieved under conditions compatible with the regulatory sequence.

As used in the preceding description, the term "and/or" means one or all of the listed elements or a combination of any two or more of the listed elements; the term "comprises" and variations thereof do not have a limiting meaning where these terms appear in the description and claims; unless otherwise specified, "a," "an," "the," and "at least one" are

used interchangeably and mean one or more than one; and the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

In the preceding description, particular embodiments may be described in isolation for clarity. Unless otherwise expressly specified that the features of a particular embodiment are incompatible with the features of another embodiment, certain embodiments can include a combination of compatible features described herein in connection with one or more embodiments.

For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

EXAMPLES

Example 1

Bacterial strains and plasmids

All the primers were ordered from Eurofins MWG Operon and are listed in Table 1. The *E.coli* strains used in this study are listed in Table 2, which were all derived from *E. coli* K-12 strain BW25113.

Table 2. Strains, plasmids and primers used in this study

Name	Relevant genotype	Reference
Strains		
BW25113	*rrnB*$_{T14}$ ΔlacZ$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	A
SBDO-1	BW25113 ΔxylA ΔyjhH ΔyagE	This work
SBDO-2	SBDO-1 + pBDO-1	This work
SBDO-3	SBDO-1 + pBDO-1 and pBDO-2	This work
SBDO-4	SBDO-1 + pBDO-1 and pBDO-3	This work
SBDO-5	SBDO-1 + pBDO-1 and pBDO-4	This work
Plasmids		
pIBA7	ColE1 ori, Amp^R, P$_L$lacO$_1$::*kivD padA*	B
pBDO-1	p15A ori, Kan^R, P$_L$lacO$_1$::*xylBCDX*	This work
pBDO-2	ColE1 ori, Amp^R, P$_L$lacO$_1$::*xylA*	This work
pBDO-3	ColE1 ori, Amp^R, P$_L$lacO$_1$::*BFD- yqhD*	This work
pBDO-4	ColE1 ori, Amp^R, P$_L$lacO$_1$::*kivD- yqhD*	This work
Primers		SEQ ID NO:
xylBAcc-F	GGGCCCggtaccatgtcctcagccatctatcccagcct	108
xylBHinNheBa-R	GGGCCCGCTCAGCAAGCTTGCTAGCggatcctTaacgccagccggcgtcgatccagt	109
xylCBamHI-F	GGGCCCggatccAGGAGAAATTAACTatgaccgctcaagtcacttgcgtatg	110
xylCHindNhe-R	GGGCCCAAGCTTgctagcttagacaaggcggacctcatgctggg	111
xylDNheI-F	GGGCCCgctagcAGGAGAAATTAACTatgaggtccgccttgtctaaccgcac	112
xylDHind-R	GGGCCCaagctttTagtggttgtggcggggcagcttgg	113
xylXHind-F	GGGCCCaagcttAGGAGAAATTAACTAtggtttgtcggcggcttctagcatg	114
xylXBIpRem-R	gcgcagctggcgttgttgtccttggccttTctgagcagcagggccgaacgaccttcgaa	115
XylXBIpI-R	GGGCCCGCTCAGCttagaggaggccgcggccggccaggt	116
pZEkivD-F	actgaccgaattcattaaagaggagaaaggtaccatgtatacagtaggagattacctatt	117
kivD-R	ttatgatttattttgttcagcaaata	118
YqhDkivD-F	ctgaacaaaataaatcataaAGGAGAAATTAACTATGAACAACTTTAATCTGCACACCCC	119
BFDpZE-F	actgaccgaattcattaaagaggagaaaggtaccatggcttcggtacacggcaccacata	120
BFD-R	tTacttcaccgggcttacggtgctta	121
CC0822Acc-F	GGGCCCggtaccatgaccgacaccctgcgccattacat	122
CC0822Xba-R	GGGCCCtctagattacgaccacgagtaggaggttttgg	123

A. Datsenko et al., 2000 *Proc. Natl. Acad. Sci. U.S.A.* 97:6640-5.

B. Zhang et al., 2011 *ChemSusChem* 4:1068-1070.

All cloning procedures were carried out in the *E. coli* strain XL10-gold (Stratagene, Agilent Technologies, Santa Clara, CA). To build the plasmid pBDO-1, the coding regions of *xylB*, *xylC*, *xylD*, and *xylX* were amplified by PCR with oligos of xylBAcc-F and xylBHinNheBa-R, xylCBamHI-F and xylCHindNhe-R, xylDNheI-F and xylDHind-R, xylXHind-F and xylXBlpRem-R, using genomic DNA of *Caulobacter crescentus* strain as template, and then these four coding regions of *xylB*, *xylC*, *xylD*, and *xylX* were inserted into the corresponding restriction sites of pZA vector after digestion.

To make the plasmid pBDO-2, the coding region of *xylA* was PCR amplified by oligos of CC0822Acc-F and CC0822Xba-R using genomic DNA of *C. crescentus* strain as template, and then this coding region was inserted into the site between *Acc65*I and *Xba*I of vector pZE after digestion.

To construct the plasmids pBDO-3 and pBDO-4, four coding regions of BFD (using *Pseudomonas putida* genomic DNA as template), yqhD-1 (using *E. coli* genomic DNA as template), KIVD (from *Lactococcus lactis*, using plasmid pIBA7 as template) and yqhD-2 (using *E. coli* genomic DNA as template), were PCR amplified with oligos of BFDpZE-F and BFD-R, yqhDBFD-F and yqhDpZE-R, pZEkivD-F and kivD-R, yqhDkivD-F and yqhDpZE-R, and then pBDO-3 and pBDO-4 were completed by Gibson cloning method (Gibson et al., 2009. *Nat. Meth.* 6:343-345). P1 phages of *xylA*, *yjhH* and *yagE* and were obtained from the Keio collection (Baba et al., 2006 *Mol. Syst. Biol.* 2:10.1038). The phages were used to transfect the BW25113 strain to construct triple knockout strains. All the knockout strains were then transformed with pCP20 plasmid to remove the kanamycin marker. The correct knockouts were verified by PCR.

Cell cultivation and shake flask fermentation

Unless otherwise stated, cells were grown in test tubes at 37°C in 2X YT rich medium (16 g/L Bacto-tryptone, 10 g/L yeast extract, and 5 g/L NaCl) supplemented with 100 mg/L ampicillin and 50 mg/L kanamycin. 200 μL of overnight cultures incubated in 2X YT medium were transferred into 5 mL M9 minimal medium supplemented with 5 g/L yeast extract, 5 g/L glucose, 40 g/L xylose, 100 mg/L ampicillin, and 50 mg/L kanamycin in 125 mL conical flasks. Isopropyl-β-D-thiogalactoside (IPTG) was added at a concentration of 0.1 mM to induce protein expression. The fermentation broth was buffered by the presence of 0.5 g $CaCO_3$.

Metabolite analysis and dry cell weight determination

Fermentation products were analyzed using an Agilent 1260 Infinity HPLC equipped with an Aminex HPX 87H column (Bio-Rad Laboratories, Inc., Hercules, CA) and a refractive-index detector. The mobile phase was 5 mM H_2SO_4 with a flow rate 0.6 mL/min. The column temperature and detection temperature were 35°C and 50°C, respectively. Cell dry weight was determined by filtering 5 mL culture through a 0.45 μm glass fiber filter (Pall Life Sciences, Ann Arbor, MI). After removal of medium, the filter was washed with 15 mL of MilliQ water (EMD Millipore Corp., Billerica, MA), dried in an oven and then weighed. Cell dry weight was determined in triplicate.

Example 2

To produce 1,4-butanediol from xylose in yeast, one artificial synthetic pathway was introduced into the wild type *Saccharomyces cerevisiae* strain W303. To generate the artificial pathway we cloned a polynucleotide that encodes enzymes that convert xylose into 2,5-dioxopentanoic acid into the host yeast cell. We also cloned a polynucleotide that encoded enzymes that convert 2,5-dioxopentanoic acid into 1,4-butanediol. These enzymes were cloned into plasmids YEplac195-xylBCDX and YEplac112-KivdDyqhD as described in more detail below.

The transformed yeast were grown under fermentation conditions as described in more detail below for two day. After the fermentation, 1,4-butanediol was accumulated to a concentration of 20 mg/L. (FIG. 14).

Plasmid construction in the yeast 1,4-butanediol synthetic pathway

The construction of plasmid YEplac195-xylBCDX was finished by Gibson assembly. All of the primers are listed in Table 3.

The coding region for HXT7p was PCR amplified with the primer pair Hxt7p195-1F and Hxt7pXylB-R, using *S. cerevisiae* W303 genomic DNA as a template. Similarly, the PGK1p coding region was PCR amplified with the primer pair PGK1Phxt7t-F and PGK1PxylC-R; the ADH1p coding region was PCR amplified with the primer pair ADH1Ppgk1t-F and ADH1PxylD-R; the PDC1p coding region was PCR amplified with the primer pair PDC1PADH1T-F and PDC1PxylX-R; the HXT7t coding region was PCR

amplified with the primer pair Hxt7tXylB-F and Hxt7tPGK1P-R; the PGK1t coding region was PCR amplified with the primer pair PGK1tXylC-F and PGK1tADH1p-R; the ADH1t coding region was PCR amplified with the primer pair ADH1TxylD-F and ADH1TPDC1P-R; and the PDC1t coding region was PCR amplified with primer pairs PDC1TxylX-F and PDC1T195-R, each by using *S. cerevisiae* W303 genomic DNA as template.

Caulobacter crescentus xylB coding region was PCR amplified with primer pair xylBhxt7p-F and xylBhxt7t-R using *C. crescentus* genomic DNA as template. Similarly, the *xylC* coding region was PCR amplified with primer pair xylCPGK1P-F and xylCPGK1t-R; the *xylD* coding region was PCR amplified with primer pair xylDADH1P-F/xylDADH1T-R; and the *xylX* coding region was PCR amplified with primer pair xylXPDC1P-F/xylXPDC1T-R; each using *C. crescentus* genomic DNA as template.

The combined fragment of HXT7p-xylB-HXT7t was amplified by overlapping PCR with the primer pair Hxt7p195-1F and Hxt7tPGK1P-R using the HXT7p/xylB/HXT7t DNA as a PCR template. The combined fragment PGK1p-xylC-PGK1t was amplified by overlapping PCR with the primer pair PGK1Phxt7t-F and PGK1tADH1p-R using the PGK1p/xylC/PGK1t DNA as a PCR template. The combined fragment ADH1p-xylD-ADH1t was amplified by overlapping PCR with the primer pair ADH1Ppgk1t-F and ADH1TPDC1P-R using the fragment ADH1p/xylD/ADH1t DNA as a PCR template. The combined fragment PDC1p-xylX-PDC1t was amplified by overlapping PCR with the primer pair PDC1PADH1T-F and PDC1T195-R using the PDC1p/xylX/PDC1t DNA as a PCR template.

The vector fragment YEp195v was amplified with primer pair 195HindIII-2F and 195EcoRI-2R by using YEplac195 as template. The fragments of YEp195v, HXT7p-xylB-HXT7t, PGK1p-xylC-PGK1t, ADH1p-xylD-ADH1t, and PDC1p-xylX-PDC1t were assembled by Gibson method to form the plasmid of YEplac195-xylBCDX (FIG. 12).

To build the plasmid of YEplac112-KivD-yqhD, the fragments of HXT7P2, HXT7T2, PGK1P2 and PGK1T2 were PCR amplified using *S. cerevisiae* W303 genmic DNA as a template. The HXT7P2 fragment was PCR amplified using the primer pair Hxt7p195-1F and HXT7PkivD-R; the HXT7T2 fragment was PCR amplified using the primer pair HXT7TKIVD-F and HXT7TPGK1P-R; the PGK1P2 fragment was PCR amplified using the primer pair PGK1PHXT7T-F/PGK1PyqhD-R; and the PGK1T2 fragment was PCR amplified using the primer pair PGK1TyqhD-F and PGK1T112-R.

The KIVD coding region from *Lactococcus lactis* was amplified with the primer pair KIVDHXT7P-F and KIVDHXT7T-R using *L. lactis* genomic DNA as template. The *E. coli* YqhD coding region was amplified with the primer pair yqhDPGK1P-F and yqhDPGK1T-R using *E. coli* genomic DNA as template.

The vector fragment YEp112v was amplified with primer pair 195HindIII-2F and 195EcoRI-2R by using YEplac112 as template. The fragments of YEp112v, HXT7P2, KIVD, HXT7T2, PGK1P2, yqhD and PGK1T2 were assembled by Gibson method to generate the plasmid of YEplac112-KivDyqhD. (FIG. 13).

Table 3. The used primers in this study

Primer		SEQ ID NO:
195HindIII-2F	attgtgagcggataacaatttcacacaggaaacagctatgaccatgattacgccaagctt	124
Hxt7p195-1F	cagctatgaccatgattacgccaagcttGGTACCtcgtaggaacaatttcgggcccctgc	125
Hxt7pXylB-R	cttcaggctgggatagatggctgaggacatttttgattaaaattaaaaaaacttttgt	126
XylBhxt7p-F	acaaaaagttttttttaattttaatcaaaaaatgtcctcagccatctatcccagcctgaag	127
XylBhxt7t-R	tgatcatgaattaataaaagtgttcgcaaatTaacgccagccggcgtcgatccagtattc	128
Hxt7tXylB-F	gaatactggatcgacgccggctggcgttAatttgcgaacacttttattaattcatgatca	129
Hxt7tPGK1P-R	actcacgagtaattcttgcaaatgcctCCTAGGagacacttttgaagcgggatacagaa	130
PGK1Phxt7t-F	ttctgtatcccgcttcaaaaagtgtctCCTAGGaggcatttgcaagaattactcgtgagt	131
PGK1PxylC-R	atcccatacgcaagtgacttgagcggtcattgttttatatttgttgtaaaaagtagataa	132
xylCPGK1P-F	ttatctacttttttacaacaaatataaaacaatgaccgctcaagtcacttgcgtatgggat	133
XylCPGK1t-R	attgatctatcgatttcaattcaattcaatttagacaaggcggacctcatgctggggttg	134
PGK1tXylC-F	caaccccagcatgaggtccgccttgtctaaattgaattgaattgaaatcgatagatcaat	135
PGK1tADH1p-R	ccgatgtatgggtttggttgccagaaGCtgagcttggagcaggaagaatacactatactg	136
ADH1Ppgk1t-F	cagtatagtgtattcttcctgctccaagctcaGCttctggcaaccaaacccatacatcgg	137
ADH1PxylD-R	gggcgtgcggttagacaaggcggacctcattgtatatgagatagttgattgtatgcttgg	138
xylDADH1P-F	ccaagcatacaatcaactatctcatatacaatgaggtccgccttgtctaaccgcacgccc	139
xylDADH1T-R	aataaaaatcataaatcataagaaattcgctTagtggttgtggcggggcagcttggccgc	140
ADH1TxylD-F	gcggccaagctgccccgccacaaccactAagcgaatttcttatgatttatgatttttatt	141
ADH1TPDC1P-R	gaaggtatgggtgcagtgtgcttatctACTAGTtgtggaagaacgattacaacaggtgtt	142
PDC1PADH1T-F	aacacctgttgtaatcgttcttccacaACTAGTagataagcacactgcacccataccttc	143
PDC1PxylX-R	ggtccatgctagaagccgccgacaaaccaTtttgattgatttgactgtgttattttgcgt	144
xylXPDC1P-F	acgcaaaataacacagtcaaatcaatcaaaAtggtttgtcggcggcttctagcatggacc	145
xylXPDC1T-R	actttaactaataattagagattaaatcgcttagaggaggccgcggccggccaggttgcg	146
PDC1TxylX-F	cgcaacctggccggccgcggcctcctctaagcgatttaatctctaattattagttaaagt	147
PDC1T195-R	acgttgtaaaacgacggccagtgaattcTCTAGAgcttgtcttgagcaattgcagagtcg	148
195EcoRI-2R	agttgggtaacgccagggttttcccagtcacgacgttgtaaaacgacggccagtgaattc	149
HXT7PkivD-R	ctaataggtaatctcctactgtatacatGGATCCtttttgattaaaattaaaaaaacttt	150
KIVDHXT7P-F	aaagttttttttaattttaatcaaaaaGGATCCatgtatacagtaggagattacctattag	151
KIVDHXT7T-R	tcatgaattaataaaagtgttcgcaaaGGTACCttatgatttattttgttcagcaaatag	152
HXT7TKIVD-F	ctatttgctgaacaaaataaatcataaGGTACCtttgcgaacacttttattaattcatga	153
HXT7TPGK1P-R	cttactcacgagtaattcttgcaaatgcctagacacttttgaagcgggatacagaaaaa	154
PGK1PHXT7T-F	tttttctgtatcccgcttcaaaaagtgtctaggcatttgcaagaattactcgtgagtaag	155
PGK1PyqhD-R	TGGGGTGTGCAGATTAAAGTTGTTCATTCTAGAtgttttatatttgttgtaaaaagtaga	156
yqhDPGK1P-F	tctacttttttacaacaaatataaaacaTCTAGAATGAACAACTTTAATCTGCACACCCCA	157
yqhDPGK1T-R	gatctatcgatttcaattcaattcaatCTCGAGTTAGCGGGCGGCTTCGTATATACGGCG	158
PGK1TyqhD-F	CGCCGTATATACGAAGCCGCCCGCTAACTCGAGattgaattgaattgaaatcgatagatc	159
PGK1T181-R	gtcacgacgttgtaaaacgacggccagtgaattctgagcttggagcaggaagaatacact	160

1,4-butanediol fermentation by yeast in shake flask

The W303 yeast strain carrying plasmids of YEplac195-xylBCDX and YEplac112-KivDyqhD was cultured overnight in the Complete Minimal medium without uracil and tryptophan supplements at 30°C with shaking at 200 rpm. The yeast cells were harvested and washed in the next day, and then inoculated into 10 mL fresh medium identical to the overnight culture medium except that it further contained 20 g/L xylose. The shake flask was

then sealed with parafilm, and cultured for two days at 30°C with shaking at 200 rpm. The fermentation broth was analyzed by gas chromatography to measure the amount of 1,4-butanediol. Results are shown in FIG. 14.

EXEMPLARY EMBODIMENTS

Embodiment 1. A recombinant microbial cell modified to exhibit increased biosynthesis of a TCA derivative compared to a wild-type control.

Embodiment 2. The recombinant cell of Embodiment 1 wherein the TCA derivative comprises 1,4-butanediol.

Embodiment 3. The recombinant microbial cell any preceding Embodiment wherein the microbial cell is a fungal cell.

Embodiment 4. The recombinant cell of Embodiment 3 wherein the fungal cell is a member of the Saccharomycetaceae family.

Embodiment 5. The recombinant cell of Embodiment 3 wherein the fungal cell is *Saccharomyces cerevisiae*, *Candida rugosa*, or *Candida albicans*.

Embodiment 6. The recombinant cell of Embodiment 1 or Embodiment 2 wherein the microbial cell is a bacterial cell.

Embodiment 7. The recombinant cell of Embodiment 6 wherein the bacterial cell is a member of the phylum Protobacteria.

Embodiment 8. The recombinant cell of Embodiment 7 wherein the bacterial cell is a member of the Enterobacteriaceae family.

Embodiment 9. The recombinant cell of Embodiment 8 wherein the bacterial cell is *Escherichia coli*.

Embodiment 10. The recombinant cell of Embodiment 7 wherein the bacterial cell is a member of the Pseudomonaceae family.

Embodiment 11. The recombinant cell of Embodiment 10 wherein the bacterial cell is *Pseudomonas putida*.

Embodiment 12. The recombinant cell of Embodiment 6 wherein the bacterial cell is a member of the phylum Firmicutes.

Embodiment 13. The recombinant cell of Embodiment 12 wherein the bacterial cell is a member of the Bacillaceae family.

Embodiment 14. The recombinant cell of Embodiment 13 wherein the bacterial cell is *Bacillus subtilis*.

Embodiment 15. The recombinant cell of Embodiment 12 wherein the bacterial cell is a member of the Streptococcaceae family.

Embodiment 16. The recombinant cell of Embodiment 15 wherein the bacterial cell is *Lactococcus lactis*.

Embodiment 17. The recombinant cell of Embodiment 12 wherein the bacterial cell is a member of the Clostridiaceae family.

Embodiment 18. The recombinant cell of Embodiment 17 wherein the bacterial cell is *Clostridium cellulolyticum*.

Embodiment 19. The recombinant cell of Embodiment 6 wherein the bacterial cell is a member of the phylum Cyanobacteria.

Embodiment 20. The recombinant cell of any preceding Embodiment wherein the microbial cell is photosynthetic.

Embodiment 21. The recombinant cell of any preceding Embodiment wherein the microbial cell is cellulolytic.

Embodiment 22. The recombinant cell of any preceding Embodiment wherein the increased biosynthesis of the TCA derivative comprises an increase in xylose dehydrogenase activity, xylonolactonase activity, xylonate dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity.

Embodiment 23. The recombinant cell of Embodiment 22 wherein the increased biosynthesis of the TCA derivative further comprises an increase in benzoylformate decarboxylase activity and an increase in alcohol dehydrogenase activity.

Embodiment 24. The recombinant cell of Embodiment 23 wherein the benzoylformate decarboxylase comprises BFD of *Pseudomonas putida*.

Embodiment 25. The recombinant cell of Embodiment 23 wherein the alcohol dehydrogenase comprises yqhD of *E. coli*.

Embodiment 26. The recombinant cell of Embodiment 22 wherein the increased biosynthesis of the TCA derivative further comprises an increase in decarboxylase activity and an increase in alcohol dehydrogenase activity.

Embodiment 27. The recombinant cell of Embodiment 26 wherein the decarboxylase comprises KIVD of *Lactococcus lactis*.

Embodiment 28. The recombinant cell of Embodiment 26 wherein the alcohol dehydrogenase comprises yqhD of *E. coli*.

Embodiment 29. The recombinant cell of preceding Embodiment wherein the increased biosynthesis of the TCA derivative comprises a decrease in α-ketoglutaric semialdehyde dehydrogenase activity.

Embodiment 30. The recombinant cell of preceding Embodiment wherein the increased biosynthesis of the TCA derivative comprises a decrease in aldolase activity.

Embodiment 31. The recombinant cell of preceding Embodiment wherein the increased biosynthesis of the TCA derivative comprises a decrease in 2-keto-3-deoxy gluconate aldolase activity.

Embodiment 32. The recombinant cell of any preceding Embodiment comprising an engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol.

Embodiment 33. The recombinant cell of Embodiment 32 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 2,5-dioxopentonoic acid into succinaldehyde.

Embodiment 34. The recombinant cell of Embodiment 33 wherein the enzyme that converts 2,5-dioxopentonoic acid into succinaldehyde comprises a 2-ketoacid decareboxylase or a 2-oxoglutarate decarboxylase.

Embodiment 35. The recombinant cell of Embodiment 33 or 34 wherein the enzyme that converts 2,5-dioxopentonoic acid into succinaldehyde comprises KIVD, BFD, or IPDC.

Embodiment 36. The recombinant cell of any one of Embodiments 32-35 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts succinaldehyde to 1,4-butanediol.

Embodiment 37. The recombinant cell of Embodiment 36 wherein the enzyme that converts succinaldehyde to 1,4-butanediol comprises an alcohol dehydrogenase.

Embodiment 38. The recombinant cell of Embodiment 36 or Embodiment 37 wherein the enzyme that converts succinaldehyde to 1,4-butanediol comprises YqhD, ADH6, YjgB, or YahK.

Embodiment 39. The recombinant cell of Embodiment 32 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 2,5-dioxopentonoic acid into 2-keto-5-hydroxy-pentanoic acid.

Embodiment 40. The recombinant cell of Embodiment 39 wherein the enzyme that converts 2,5-dioxopentonoic acid into 2-keto-5-hydroxy-pentanoic acid comprises an alcohol dehydrogenase.

Embodiment 41. The recombinant cell of Embodiment 39 or Embodiment 40 wherein the enzyme that converts 2,5-dioxopentonoic acid into 2-keto-5-hydroxy-pentanoic acid comprises YqhD, ADH6, YjgB, or YahK.

Embodiment 42. The recombinant cell of any one of Embodiments 39-41 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 2-keto-5-hydroxy-pentanoic acid to 4-hydroxy-1-butyraldehyde.

Embodiment 43. The recombinant cell of Embodiment 42 wherein the enzyme that converts 2-keto-5-hydroxy-pentanoic acid to 4-hydroxy-1-butyraldehyde comprises a 2-ketoacid decareboxylase or a 2-oxoglutarate decarboxylase.

Embodiment 44. The recombainant cell of Embodiment 42 or Embodiment 43 wherein the enzyme that converts 2-keto-5-hydroxy-pentanoic acid to 4-hydroxy-1-butyraldehyde comprises Kivd, BFD, or IPDC.

Embodiment 45. The recombinant cell of any one of Embodiments 42-44 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 4-hydroxy-1-butyraldehyde to 1,4-butanediol.

Embodiment 46. The recombinant cell of Embodiment 45 wherein the enzyme that converts 4-hydroxy-1-butyraldehyde to 1,4-butanediol comprises an alcohol dehydrogenase.

Embodiment 47. The recombinant cell of Embodiment 45 or Embodiment 46 wherein the enzyme that converts 4-hydroxy-1-butyraldehyde to 1,4-butanediol comprises YqhD, ADH6, YjgB, or YahK.

Embodiment 48. The recombinant cell of any preceding Embodiment comprising an engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid.

Embodiment 49. The recombinant cell of Embodiment 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-arabinose into D-arabinolactone.

Embodiment 50. The recombinant cell of Embodiment 49 wherein the enzyme that can convert D-arabinose into D-arabinolactone comprises a pentose dehydrogenase.

Embodiment 51. The recombinant cell of Embodiment 49 or Embodiment 50 wherein the enzyme that can convert D-arabinose into D-arabinonolactone comprises AraDH.

Embodiment 52. The recombinant cell of any one of Embodiments 49-51 wherein the recombinant cell exhibits conversion of D-arabinose into D-arabinonolactone at a level at least 110% of a wild-type control cell.

Embodiment 53. The recombinant cell of any one of Embodiments 49-52 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid.

Embodiment 54. The recombanant cell of Embodiment 53 wherein the enzyme that converts D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid comprises an aldonic acid dehydratase.

Embodiment 55. The recombanant cell of Embodiment 53 or Embodiment 54 wherein the enzyme that converts D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid comprises AraD.

Embodiment 56. The recombinant cell of any one of Embodiments 53-55 wherein the recombinant cell exhibits conversion of D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid at a level at least 110% of a wild-type control cell.

Embodiment 57. The recombinant cell of any one of Embodiments 49-56 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid.

Embodiment 58. The recombinant cell of Embodiment 57 wherein the enzyme that converts 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

Embodiment 59. The recombinant cell of Embodiment 57 or Embodiment 58 wherein the enzyme that converts 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises KdaD.

Embodiment 60. The recombinant cell of any one of Embodiments 57-59 wherein the recombinant cell exhibits conversion of 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid at a level at least 110% of a wild-type control cell.

Embodiment 61. The recombinant cell of Embodiment 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-xylose to D-xylonolactone.

Embodiment 62. The recombinant cell of Embodiment 61 wherein the enzyme that converts D-xylose to D-xylonolactone comprises a pentose dehydrogenase.

Embodiment 63. The recombinant cell of Embodiment 61 or Embodiment 62 wherein enzyme that converts D-xylose to D-xylonolactone comprises XylB or rrnAC3034.

Embodiment 64. The recombinant cell of any one of Embodiments 61-63 wherein the recombinant cell exhibits conversion of D-xylose to D-xylonolactone at a level at least 110% of a wild-type control.

Embodiment 65. The recombinant cell of any one of Embodiments 61-64 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-xylonolactone to D-xylonic acid.

Embodiment 66. The recombinant cell of Embodiment 65 wherein the enzyme that converts D-xylonolactone to D-xylonic acid comprises a pentonolactonase.

Embodiment 67. The recombinant cell of Embodiment 65 or Embodiment 66 wherein the enzyme that converts D-xylonolactone to D-xylonic acid comprises XylC or rrnAC3033.

Embodiment 68. The recombinant cell of any one of Embodiments 65-67 wherein the recombinant cell exhibits conversion of D-xylonolactone to D-xylonic acid at a level at least 110% of a wild-type control.

Embodiment 69. The recombinant cell of any one of Embodiments 61-68 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid.

Embodiment 70. The recombinant cell of Embodiment 69 wherein the enzyme that converts D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid comprises an aldonic acid dehydratase.

Embodiment 71. The recombinant cell of Embodiment 69 or Embodiment 70 wherein the enzyme that converts D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid comprises XylD or rrnAC3032.

Embodiment 72. The recombinant cell of any one of Embodiments 69-71 wherein the recombinant cell exhibits conversion of D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid at a level at least 110% of a wild-type control.

Embodiment 73. The recombinant cell of any one of Embodiments 61-72 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid.

Embodiment 74. The recombinant cell of Embodiment 73 wherein the enzyme that converts 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

Embodiment 75. The recombinant cell of Embodiment 73 or Embodiment 74 wherein the enzyme that converts 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid comprises XylX or rrnAC3039.

Embodiment 76. The recombinant cell of any one of Embodiments 73-75 wherein the recombinant cell exhibits conversion of 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid at a level at least 110% of a wild-type control.

Embodiment 77. The recombinant cell of Embodiment 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts L-arabinose to L-arabinolactone.

Embodiment 78. The recombinant cell of Embodiment 77 wherein the enzyme that converts L-arabinose to L-arabinolactone comprises a pentose dehydrogenase.

Embodiment 79. The recombinant cell of Embodiment 77 or Embodiment 78 wherein the enzyme that converts L-arabinose to L-arabinolactone comprises AraE.

Embodiment 80. The recombinant cell of any one of Embodiments 77-79 wherein the recombinant cell exhibits conversion of L-arabinose to L-arabinolactone at a level at least 110% of a wild-type control.

Embodiment 81. The recombinant cell of any one of Embodiments 77-80 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts L-arabinolactone to L-arabinonic acid.

Embodiment 82. The recombinant cell of Embodiment 81 wherein the enzyme that converts L-arabinolactone to L-arabinonic acid comprises a pentonolactonase.

Embodiment 83. The recombinant cell of Embodiment 81 or Embodiment 82 wherein the enzyme that converts L-arabinolactone to L-arabinonic acid comprises AraI.

Embodiment 84. The recombinant cell of any one of Embodiments 81-83 wherein the recombinant cell exhibits conversion of L-arabinolactone to L-arabinonic acid at a level at least 110% of a wild-type control.

Embodiment 85. The recombinant cell of any one of Embodiments 77-84 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid.

Embodiment 86. The recombinant cell of Embodiment 85 wherein the enzyme that converts L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid comprises an aldonic acid dehydratase.

Embodiment 87. The recombinant cell of Embodiment 85 or Embodiment 86 wherein the enzyme that converts L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid comprises AraB.

Embodiment 88. The recombinant cell of any one of Embodiments 81-87 wherein the recombinant cell exhibits conversion of L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid at a level at least 110% of a wild-type control.

Embodiment 89. The recombinant cell of any one of Embodiments 77-88 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid.

Embodiment 90. The recombinant cell of Embodiments 89 wherein the enzyme that converts 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

Embodiment 91. The recombinant cell of Embodiments 89 wherein the enzyme that converts 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises AraD.

Embodiment 92. The recombinant cell of any one of Embodiments 89-90 wherein the recombinant cell exhibits conversion of 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid at a level at least 110% of a wild-type control.

Embodiment 93. The recombinant cell of Embodiment 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid.

Embodiment 94. The recombinant cell of Embodiment 93 wherein the enzyme that converts D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid comprises an aldonic acid dehydratase.

Embodiment 95. The recombinant cell of Embodiment 93 or Embodiment 94 wherein the enzyme that converts D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid comprises YcbF.

Embodiment 96. The recombinant cell of any one of Embodiments 93-95 wherein the recombinant cell exhibits conversion of D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid at a level at least 110% of a wild-type control.

Embodiment 97. The recombinant cell of any one of Embodiments 93-96 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid.

Embodiment 98. The recombinant cell of Embodiment 97 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

Embodiment 99. The recombinant cell of Embodiment 97 or Embodiment 98 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises YcbC.

Embodiment 100. The recombinant cell of Embodiment 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid.

Embodiment 101. The recombinant cell of Embodiment 100 wherein the enzyme that converts D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid comprises an aldonic acid dehydratase.

Embodiment 102. The recombinant cell of Embodiment 100 or Embodiment 101 wherein the enzyme that converts D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid comprises YcbH.

Embodiment 103. The recombinant cell of any one of Embodiments 100-102 wherein the recombinant cell exhibits conversion of D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid at a level at least 110% of a wild-type control.

Embodiment 104. The recombinant cell of any one of Embodiments 100-103 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid

comprises an enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid.

Embodiment 105. The recombinant cell of Embodiment 104 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

Embodiment 106. The recombinant cell of Embodiment 104 or Embodiment 105 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises YcbC.

Embodiment 107. The recombinant cell of Embodiment 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline.

Embodiment 108. The recombinant cell of Embodiment 107 wherein the enzyme that converts 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline comprises an amino acid transporter.

Embodiment 109. The recombinant cell of Embodiment 107 or Embodiment 108 wherein the enzyme that converts 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline comprises LysE or HypE.

Embodiment 110. The recombinant cell of any one of Embodiments 107-109 wherein the recombinant cell exhibits conversion of 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline at a level at least 110% of a wild-type control.

Embodiment 111. The recombinant cell of any one of Embodiments 107-110 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline.

Embodiment 112. The recombinant cell of Embodiment 111 wherein the enzyme that converts 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline comprises HypOX.

Embodiment 113. The recombinant cell of Embodiment 111 or Embodiment 112 wherein the recombinant cell exhibits conversion of 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline at a level at least 110% of a wild-type control.

Embodiment 114. The recombinant cell of any one of Embodiments 107-113 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid

comprises an enzyme that converts 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid.

Embodiment 115. The recombinant cell of Embodiment 114 wherein the enzyme that converts 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

Embodiment 116. The recombinant cell of Embodiment 114 or Embodiment 115 wherein the enzyme that converts 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid comprises PP1247.

Embodiment 117. The recombinant cell of any one of Embodiments 114-116 wherein the recombinant cell exhibits conversion of 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid at a level at least 110% of a wild-type control.

Embodiment 118. The recombinant cell of any one of Embodiments 48-117 modified to exhibit increased α-ketoglutaric semialdehyde dehydrogenase activity compared to a wild-type control.

Embodiment 119. The recombinant cell of Embodiment 118 exhibiting increased conversion of 2,5-dioxopentanoic acid to a TCA derivative compared to a wild-typ control.

Embodiment 120. The recombinant cell of Embodiment 119 wherein the TCA derivative comprises succinate, fumarate, malate, glutamate, lysine, threonine, or 4-hydroxybutyrate.

Embodiment 121. The recombinant cell of any preceding Embodiment genetically modified to increase consumption of xylose, arabinose, glucaric acid, galactaric acid, or hydroxyproline compared to a wild-type control.

Embodiment 122. The recombinant cell of any preceding Embodiment genetically modified to in crease consumption of a uronic acid compared to a wild-type control.

Embodiment 123. The recombinant cell of Embodiment 122 wherein the urnic acid comprises galacturonic acid or glucuronic acid.

Embodiment 124. The recombinant cell of Embodiment 122 or Embodiment 123 genetically modified to increase conversion of the uronic acid to an aldonic acid compared to a wild-type control.

Embodiment 125. The recombinant cell of any one of Embodiments 122-124 wherein the recombinant cell comprises an exogenous urinate dehydrogenase.

Embodiment 126. A method comprising:

incubating a recombinant cell of any preceding Embodiment in medium that comprises a carbon source under conditions effective for the recombinant cell to produce a TCA derivative.

Embodiment 127. The method of Embodiment 126 wherein the TCA derivative comprises 1,4-butanediol.

Embodiment 128. The method of Embodiment 126 wherein the carbon source comprises xylose, arabinose, glucaric acid, galactaric acid, or hydroxyproline.

Embodiment 129. The method of any one of Embodiments 126-128 wherein the increased biosynthesis of the TCA derivative comprises an increase in pentose dehydrogenase activity, pentonolactonase activity, aldonic acid dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity.

Embodiment 130. The method of any one of Embodiments 126-129 wherein the increased biosynthesis of the TCA derivative comprises an increase in hexic acid dehydratase activity or 5-dehydro-4-deoxyglucarate dehydratase activity.

Embodiment 131. A method comprising:

introducing into a host cell a heterologous polynucleotide encoding at least one polypeptide that catalyzes conversion of a carbon source to a TCA derivative, wherein the at least one polypeptide is operably linked to a promoter so that the modified host cell catalyzes conversion of the carbon source to TCA derivative.

Embodiment 132. The method of Embodiment 131 wherein the TCA derivative comprises 1,4-butanediol.

Embodiment 133. The method of Embodiment 131 wherein the carbon source comprises xylose.

Embodiment 134. The method of Embodiment 131 wherein the TCA derivative comprises succinate, fumarate, malate, glutamate, lysine, threonine, 4-hydroxybutyrate.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference in their entirety. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document

incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

Sequence Listing Free Text

D-arabinose dehydrogenase (AraDH)

SEQ ID NO:1 (NP_342747.1; GI:15898142; Zinc-containing alcohol dehydrogenase (*Sulfolobus solfataricus* P2))

```
  1 menvnmvksk aallkkfsep lsiedvnipe pqgeevliri ggagvcrtdl
rvwkgveakq
 61 gfrlpiilgh enagtivevg elakvkkgdn vvvyatwgdl tcrycregkf
nicknqiipg
121 qttnggfsey mlvkssrwlv klnslspvea apladagtts mgairqalpf
iskfaepvvi
181 vngigglavy tiqilkalmk nitivgisrs kkhrdfalel gadyvsemkd
aeslinkltd
241 glgasiaidl vgteettynl gkllaqegai ilvgmegkrv sleafdtavw
nkkllgsnyg
301 slndledvvr lsesgkikpy iikvplddin kaftnldegr vdgrqvit
```

SEQ ID NO:2 (Chain A, D-arabinose dehydrogenase (*Sulfolobus solfataricus*))

```
  1 mvkskaallk kfseplsied vnipepqgee vliriggagv crtdlrvwkg
veakqgfrlp
 61 iilghenagt ivevgelakv kkgdnvvvya twgdltcryc regkfnickn
qiipgqttng
121 gfseymlvks srwlvklnsl spveaaplad agttsmgair qalpfiskfa
epvvivngig
181 glavytiqil kalmknitiv gisrskkhrd falelgadyv semkdaesli
nkltdglgas
241 iaidlvgtee ttynlgklla qegaiilvgm egkrvsleaf dtavwnkkll
gsnygslndl
301 edvvrlsesg kikpyiikvp lddinkaftn ldegrvdgrq vitp
```

SEQ ID NO:3 (Alcohol dehydrogenase GroES domain-containing protein (*Sulfolobus islandicus* M.14.25))

```
  1 mfgitfysam rknismvksk aallkkfsep lsiedveipe pkgeevlvri
ggagvcrtdl
 61 rvwkgveakq gfrlpiilgh enagtvvevg elakakkgdn vvvyatwgdm
tcrycregkf
121 nicknqvipg qttnggfsey mlvksyrwlv kldslspvda spladagtts
mgairqalpf
181 mnkfaepvvi vngigglavy tiqilkalmk nivivgisrs kkhrdlalel
gadyavemke
241 aesliskltd glgasvaidl vgteetsynl gkllaqegai ilvgmegkrv
sleafdtavw
301 nkkllgsnyg slndledvvr lsesgkikpy vvkipldein kafkdldegr vegrqvitp
```

SEQ ID NO:4 (Alcohol dehydrogenase GroES domain-containing protein (*Sulfolobus islandicus* M.16.27))

```
  1 mfgitfysam rknismvksk aallkkfsep lsiedveipe pkgeevlvri
ggagvcrtdl
```

```
 61 rvwkgveakq gfrlpiilgh enagtvvevg elakakkgdn vvvyatwgdm
tcrycregkf
121 nicknqvipg qttnggfsey mlvksyrwlv kldslspvda spladagtts
mgairqalpf
181 mnkfaepvvi vngigglavy tiqilkalmk nivivgisrs rkhrdlalel
gadyavemke
241 aesliskltd glgasvaidl vgteetsynl gkllaqegai ilvgmegkrv
sleafdtavw
301 nkkllgsnyg slndledvvr lsesgkikpy vvkipldein kafkdldegr vegrqvitp
```

SEQ ID NO:5 (Alcohol dehydrogenase GroES domain-containing protein (*Sulfolobus islandicus* L.S.2.15))

```
  1 mfgitfysam rknismvksk aallkkfsep lsiedveipe pkgeevlvri
ggagvcrtdl
 61 rvwkgveakq gfrlpiilgh enagtvvevg elakakkgdn vvvyatwgdm
tcrycregkf
121 nicknqvipg qttnggfsey mlvksyrwlv kldslspvda spladagtts
mgairqalpf
181 mnkfaepvvi vngigglavy tiqilkalmk nivivgisrs kkhrdlalel
gadhavemke
241 aesliskltd glgasvaidl vgteetsynl gkllaqegai ilvgmegkrv
sleafdtavw
301 nkkllgsnyg slndledvvr lsesgkikpy vvkipldein kafkdldegr vegrqvitp
```

Arabinonate dehydratase (AraD)

SEQ ID NO:6 (NP_344435.1; GI:15899830; Mandelate racemase/muconate lactonizing family protein (*Sulfolobus solfataricus* P2))

```
  1 mikdirtykl cyeginderd alaikglaeh pmeivateie tsdgyvgyge
slaygcsdav
 61 qvtiekilkp lllkedeeli eylwdkmyka tlrfgrrgia iagisgvdta
lwdimgkkak 121 kpiykllggs krkvrayitg gyysekkdle klrdeeayyv
kmgfkgikvk igaksmeedi
181 erlkairevv gedvkiavda nnvytfeeal emgrrleklg iwffeepiqt
dyldlsarla
241 eelevpiagy etaytrwefy eimrkravdi vqtdvmwtgg isemmkignm
akvmgyplip
301 hysaggisli gnlhvaaaln spwiemhlrk ndlrdkifke sieidnghlv
vpdrpglgyt
361 irdgvfeeyk cks
```

SEQ ID NO:7 (Mandelate racemase/muconate lactonizing protein (*Sulfolobus islandicus* Y.G.57.14))

```
  1 mikdirtykl cyeginderd alaikglaeh pmeivvteie tsdgyvgyge
slaygcsdav
 61 qvtiekilkp lllkedeeli eylwdkmyka tlrfgrrgia iagisgvdta
lwdimgkkak
121 kpiykllggs krkvrayitg gyysekkdle klrdeeayyv kmgfkgikvk
igaksmeedi
181 erlkairevv gedvkiavda nnvytfeeal emgrrleklg iwffeepiqt
dyldlsarla
```

241 eelevpiagy etaytrwefy eimrkravdi vqtdvmwtgg isemmkignm akvmgyplip
301 hysaggisli gnlhvaaaln spwiemhlrk ndlrdkifke sieidnghlv vpdrpglgyt
361 irdgvfeeyk cks

SEQ ID NO:8 (Mandelate racemase/muconate lactonizing domain-containing protein (*Sulfolobus islandicus* L.D.8.5))

1 mikdirtykl cyeginderd alaikglaeh pmeivvteie tsdgyvgyge slaygcsdav
61 qvtiekilkp lllkedeefi eylwdkmyka tlrfgrrgia iagisgvdta lwdimgkkak
121 kpiykllggs krkvrayitg gyysekkdle klrdeeayyv kmgfkgikvk igaksmeedi
181 erlkairevv gedvkiavda nnvytfeeal emgrrleklg iwffeepiqt dyldlsarla
241 eelevpiagy etaytrwefy eimrkravdi vqtdvmwtgg isemmkignm akvmgyslip
301 hysaggisli gnlhvaaaln spwiemhlrk ndlrdkifke sieidnghlv vpdrpglgyt
361 irdgvfeeyk cks

SEQ ID NO:9 (Mandelate racemase/muconate lactonizing protein (*Sulfolobus islandicus* M.14.25))

1 mikdirtykl cyeginderd alaikglaeh pmeivvteie tsdgyvgyge slaygcsdav
61 qvtiekilkp lllkedeeli eylwdkmyka tlrfgrrgia iagisgvdtg lwdimgkkak
121 kpiykllggs krkvrayitg gyysekkdle klrdeeayyv kmgfkgikvk igaksmeedi
181 erlkairevv gedvkiavda nnvytfeeal emgrrleklg iwffeepiqt dyldlsarla
241 eelevpiagy etaytrwefy eimrkravdi vqtdvmwtgg isemmkignm akvmgyplip
301 hysaggisli gnlhvaaaln spwiemhlrk ndlrdkifke sieidnghlv vpdrpglgyt
361 irdgvfeeyk cks

SEQ ID NO:10 (Mandelate racemase/muconate lactonizing protein (*Sulfolobus islandicus* L.S.2.15))

1 mikdirtykl cyeginderd alaikglaeh pmeivvteie tsdgyvgyge slaygcsdav
61 qvtiekilkp lllkedeeli eylwdkmyka tlrfgrrgia iagisgvdta lwdimgkkak
121 kpiykllggs krkvrayitg gyysekkdle klrdeeayyv kmgfkgikik igaksmeedi
181 erlkairevv gedvkiavda nnvytfeeal emgrrleklg iwffeepiqt dyldlsarla
241 eelevpiagy etaytrwefy eimrkravdi vqtdvmwtgg isemmkignm akvmgyplip
301 hysaggisli gnlhvaaaln spwiemhlrk ndlrdkifke sieidnghlv vpdrpglgyt
361 irdgvfeeyk cks

2-Keto-3-deoxy-D-arabinonate Dehydratase (KdaD)

SEQ ID NO:11 (NP_344431.1; GI:15899826; Hypothetical protein SSO3118 (*Sulfolobus solfataricus* P2)

```
  1 mhfimmklfr vvkrgyyisy aildnstiir ldedpikalm rysenkevlg
drvtgidyqs
 61 llksfqindi ritkpidppe vwgsgisyem areryseenv akilgktiye
kvydavrpei
121 ffkatpnrcv ghgeaiavrs dsewtlpepe lavvldsngk ilgytimddv
sardleaenp
181 lylpqskiya gccafgpviv tsdeiknpys lditlkivre grvffegsvn
tnkmrrkiee
241 qiqylirdnp ipdgtilttg taivpgrdkg lkdediveit isnigtlitp vkkrrkit
```

SEQ ID NO:12 (Fumarylacetoacetate (FAA) hydrolase (*Sulfolobus islandicus* Y.N.15.51))

```
  1 mltcllptll yakcifimmk lfrvvkrgyy isyaildnst iirldedpik
almrysenke
 61 vlgdrvtgid yqsllksfqi ndiritkpid ppevwgsgis yemareryse
envakilgkt
121 iyekvydavr peiffkatpn rcvghgeaia vrsdsewtlp epelavvlds
ngkilgytim
181 ddvsardlea enplylpqsk iyagccafgp vivtsdeikn pyslditlki
vregrvffeg
241 svntnkmrrk ieeqiqylir dnpipdgtil ttgtaivpgr dkglkdediv
eitisnigtl
301 itpvkkrrki t
```

SEQ ID NO:13 (Fumarylacetoacetate (FAA) hydrolase (*Sulfolobus solfataricus* 98/2))

```
  1 mmklfrvvkr gyyisyaild nstiirlded pikalmryse nkevlgdrvt
gidyqsllks
 61 fqindiritk pidppevwgs gisyemarer yseenvakil gktiyekvyd
avrpeiffka
121 tpnrcvghge aiavrsdsew tlpepelavv ldsngkilgy timddvsard
leaenplylp
181 qskiyagcca fgpvivtsde iknpysldit lkivregrvf fegsvntnkm
rrkieeqiqy
241 lirdnpipdg tilttgtaiv pgrdkglkde diveitisni gtlitpvkkr rkit
```

SEQ ID NO:14 (Chain X, 2-keto-3-deoxy-D-arabinonate dehydratase)

```
  1 mklfrvvkrg yyisyaildn stiirldedp ikalmrysen kevlgdrvtg
idyqsllksf
 61 qindiritkp idppevwgsg isyemarery seenvakilg ktiyekvyda
vrpeiffkat
121 pnrcvghgea iavrsdsewt lpepelavvl dsngkilgyt imddvsardl
eaenplylpq
181 skiyagccaf gpvivtsdei knpyslditl kivregrvff egsvntnkmr
rkieeqiqyl
241 irdnpipdgt ilttgtaivp grdkglkded iveitisnig tlitpvkkrr kit
```

SEQ ID NO:15 (Fumarylacetoacetate (FAA) hydrolase (*Sulfolobus islandicus* HVE10/4))

```
  1 mmklfrvvkr gyyisyaild nstiirlded pikalmryse nkevlgdrvt
gidyqsllks
```

```
 61 fqindiritk pidppevwgs gisyemarer yseenvakil gktiyekvyd
avrpeiffka
121 tpnrcvghge aiavrsdsew tlpepelavv ldsngkilgy timddvsard
leaenplylp
181 qskiyagcca fgpvivtsde iknpysldit lkivrkdrvf fegsvntnkm
rrkieeqiqy
241 lirdnpipdg tilttgtaiv pgrdkglkde diveitisni gtlitpvkkr rkit
```

2,5-dioxopentanoate dehydrogenase (DopDH)

SEQ ID NO:16 (NP_344430.1; GI:15899825; Aldehyde dehydrogenase (*Sulfolobus solfataricus* P2)

```
  1 mksyqgladk wikgsgeeyl dinpadkdhv lakirlytkd dvkeainkav
akfdewsrtp
 61 apkrgsillk agelmeqeaq efallmtlee gktlkdsmfe vtrsynllkf
ygalafkisg
121 ktlpsadpnt riftvkeplg vvalitpwnf plsipvwkla palaagntav
ikpatktplm
181 vaklvevlsk aglpegvvnl vvgkgsevgd tivsddniaa vsftgstevg
kriyklvgnk
241 nrmtriqlel ggknalyvdk sadltlaael avrggfgltg qsctatsrli
inkdvytqfk
301 qrllervkkw rvgpgtedvd mgpvvdegqf kkdleyieyg knvgakliyg
gniipgkgyf
361 leptifegvt sdmrlfkeei fgpvlsvtea kdldeairlv navdyghtag
ivasdikain
421 efvsrveagv ikvnkptvgl elqapfggfk nsgattwkem gedalefylk ektvyegw
```

SEQ ID NO:17 (Aldehyde dehydrogenase (*Sulfolobus islandicus* HVE10/4))

```
  1 mksyqgladk wikgsgeeyl dinpadkdhv lakirlytkd dvkeainkav
akfdewsrtp
 61 apkrgsillk agelmeqeaq efallmtlee gktlkdsmfe vtrsynllkf
ygalgfkisg
121 ktlpsadpnt riftvkeplg vvalitpwnf plsipvwkla palaagntav
ikpatktplm
181 vaklvevlsk aglpegvvnl vvgkgsevgd tivsddniaa vsftgstevg
kriyklvgnk
241 nrmtriqlel ggknalyvdk sadltlaael avrggfgltg qsctatsrli
ihkdvytqfk
301 qrllervkkw rvgpgtedvd mgpvvdegqf kkdleyieyg knagakliyg
gniipgkgyf
361 leptifegvt shmrlfkeei fgpvlsvtea kdldeairlv navdyghtag
ivasdikain
421 efvsrveagv ikvnkptvgl elqapfggfk nsgattwkem gedalefylk ektvyegw
```

SEQ ID NO:18 (Aldehyde dehydrogenase (*Sulfolobus islandicus* Y.G.57.14))

```
  1 mksyqgladk wikgsgeeyl dinpadkdhv lakirlytkd dvkeainkav
akfdewsrtp
 61 apkrgsillk agelmeqeaq efallmtlee gktlkdsmfe vtrsynllkf
ygalafkisg
121 ktlpsadpnt riftvkeplg vvalitpwnf plsipvwkla palaagntav
ikpatktplm
```

181 vaklvevlsk aglpegvvnl vvgkgsevgd tivsddniaa vsftgstevg kriyklvgnk
241 nrmtriqlel ggknalyvdk sadltlaael avrggfgltg qsctatsrli inkdvytqfk
301 qrllervkkw rvgpgtedvd mgpvvdegqf kkdleyieyg knvgakliyg gniipgkgyf
361 leptifegvt sdmrlfkeei fgpvlsvtea kdldeairlv navdyghtag ivasdinain
421 efvsrveagv ikvnkptvgl elqapfggfk nsgattwkem gedalefylk ektvyegw

SEQ ID NO:19 (Aldehyde dehydrogenase (*Sulfolobus islandicus* Y.N.15.51))
1 mksyqgladk wikgsgeeyl dinpadkdhv lakirlytkd dvkeainkav akfdewsrtp
61 apkrgsillk agelmeqeaq efallmtlee gktlkdsmfe vtrsynllkf ygalafkisg
121 ktlpsadpnt riftvkeplg vvalitpwnf plsipvwkla palaagntai ikpatktplm
181 vaklvevlsk aglpegvvnl vvgkgsevgd tivsddniaa vsftgstevg kriyklvgnk
241 nrmtriqlel ggknalyvdk sadltlaael avrggfgltg qsctatsrli inkdvytqfk
301 qrllervkkw rvgpgtedvd mgpvvdegqf kkdleyieyg knvgakliyg gniipgkgyf
361 leptifegvt sdmrlfkeei fgpvlsvtea kdldeairlv navdyghtag ivasdikain
421 efvsrveagv ikvnkptvgl elqapfggfk nsgattwkem gedalefylk ektvyegw

SEQ ID NO:20 (Aldehyde dehydrogenase (*Sulfolobus islandicus* L.S.2.15))
1 mksyqgladk wikgsgeeyl dinpadkdhv lakirlytkd dvkeainkav akfdewsrtp
61 apkrgsillk agelmeqeaq efallmtlee gktlkdsmfe vtrsynllkf ygalafkisg
121 ktlpsadpnt riftvkeplg vvalitpwnf plsipvwkla palaagntav ikpatktplm
181 vaklvevlsk aglpegvvnl vvgkgsevgd tivsddniaa vsftgstevg kriyklvgnk
241 nrmtriqlel ggknalyvdk sadltlaael airggfgltg qsctatsrli inkdvytqfk
301 qrllervkkw rvgpgtedvd mgpvvdegqf kkdleyieyg knvgakliyg gniipgkgyf
361 leptifegvt sdmrlfkeei fgpvlsvtea kdldeairlv navdyghtag ivasdikain
421 efvsrveagv ikvnkptvgl elqapfggfk nsgattwkem gedalefylk ektvyegw

2,5-dioxovalerate dehydrogenase (YcbD)

SEQ ID NO:21 (NP_388129.1; GI:16077316; 2,5-dioxovalerate dehydrogenase (*Bacillus subtilis* subsp. subtilis str. 168))
1 msviteqnty lnfingewvk sqsgdmvkve npadvndivg yvqnstaedv eravtaanea
61 ktawrkltga ergqylykta dimeqrleei aacatremgk tlpeakgeta rgiailryya

121 gegmrktgdv ipstdkdalm fttrvplgvv gvispwnfpv aipiwkmapa lvygntvvik
181 patetavtca kiiacfeeag lpagvinlvt gpgsvvgqgl aehdgvnavt ftgsnqvgki
241 igqaalarga kyqlemggkn pvivaddadl eaaaeavitg afrstgqkct atsrvivqsg
301 iyerfkekll qrtkditigd slkedvwmgp iasknqldnc lsyiekgkqe gaslliggek
361 lengkyqngy yvqpaifdnv tsemtiaqee ifgpvialik vdsieealni andvkfglsa
421 siftenigrm lsfideidag lvrinaesag velqapfggm kqssshsreq geaakdffta
481 iktvfvkp

SEQ ID NO:22 (Aldehyde dehydrogenase, thermostable (*Bacillus subtilis* subsp. subtilis str. RO-NN-1))

1 msviteqnty lnfingewvk sqsgdmvkve npadvndivg yvqnstaedv eravaaanea
61 ktawrkltga ergqylykta dimeqrleei aacatremgk tlpeakgeta rgiailryya
121 gegmrktgdv ipstdkdalm fttrvplgvv gvispwnfpv aipiwkmapa lvygntvvik
181 patetavtca kiiacfeeag lpagvinlvt gpgsvvgqgl aehegvnavt ftgsnqvgki
241 igqaalarga kyqlemggkn pvivaddadl eaaaeavitg afrstgqkct atsraivqsg
301 iyerfkekll qrtkditigd slkedvwmgp iasknqldnc lsyiekgkqe gaslliggek
361 lengkyqngy yvqpaifdnv tsemtiaqee ifgpvialik vdsmeealni andvkfglsa
421 siftenigrm lsfideidag lvrinaesag velqapfggm kqssshsreq geaakdffta
481 iktvfvkp

SEQ ID NO:23 (Hypothetical protein BSNT_00439 (*Bacillus subtilis* subsp. natto BEST195))

1 msviteqnty lnfikgewvk sqsgdmvkve npadvndivg yvqnstaedv eravaaanea
61 ktawrkltga ergqylykta dimeqrleei aacatremgk tlpeakgeta rgiailryya
121 gegmrktgdv ipstdkaalm fttrvplgvv gvispwnfpv aipiwkmapa lvygntvvik
181 patetavtca kiiacfeeag lpagvinlvt gpgsvvgqgl aehdgvnavt ftgsnqvgki
241 igqaalarga kyqlemggkn pvivaddadl eaaaeavitg afrstgqkct atsrvivqse
301 iyerfkekll qrtkditigd slkedvwmgp iasknqldnc lsyiekgkqe gaslliggek
361 lengkyqngy yvqpaifdnv tsemtiaqee ifgpvialik vdsmeealni andvkfglsa
421 siftenigrm lsfideidag lvrinaesag velqapfggm kqssshsreq geaakdffta
481 iktvfvkp

SEQ ID NO:24 (Aldehyde dehydrogenase (*Bacillus subtilis* subsp. spizizenii TU-B-10))

```
  1 msviteqnty lnfingewvk sqsgdmvkve npadvndivg yvqnstaddv
eravaaanea
 61 ktawrkltga ergqylykta dimeqrleei aacatremgk tlpeakgeta
rgiailryya
121 gegmrktgdv ipstdkdalm fttrvplgvv gvispwnfpv aipiwkmapa
lvygntvvik
181 patetavtca kiiacfeeag lpagvinlvt gpgsvvgqgl aehegvnait
ftgsnqvgki
241 igqaalarga kyqlemggkn pvivaddadl eaaaeavitg afrstgqkct
atsrvivqsg
301 iydrfkekll qrtkdikigd slkedvwmgp iasknqldnc lsyiekgkqe
gaslliggek
361 ledgkyqngy yvqpaifdnv tsemtiaqee ifgpvialik vdsmeealdi
andvkfglsa
421 siftqnigrm lsfvdeidag lvrinaesag velqapfggm kqssshsreq
geaakdffta
481 iktvfvkp
```

SEQ ID NO:25 (Aldehyde dehydrogenase (Bacillus sp. JS))

```
  1 msviteqnty lnfingewvq sqsgdmvkve npadvndivg yvqnstaedv
eravaaanka
 61 ktawrkltga ergqylykta dimerrleei aacatremgk tlpeakgeta
rgiailryya
121 gegmrktgdv ipstdkdalm fttrvplgvv gvispwnfpv aipiwkmapa
lvygntvvik
181 patetavtca kiiacfeeag lpagvinlvt gpgsvvgqgl aehdsvnavt
ftgsnqvgki
241 igqaalarga kyqlemggkn pvivaddadl eaaaeavitg afrstgqkct
atsrvivqsg
301 iyerfkekll qrtkditigd slkedvwmgp iasknqldnc lsyiekgkre
gasllmggek
361 lenekyqngy yvqpaifdnv tsemtiaqee ifgpvialik vdsmeealdi
andvkfglsa
421 siftenigkm lsfideidag lvrvnaesag velqapfggm kqssshsreq
geaakdffta
481 iktvfvkp
```

Xylose dehydrogenase (xylB)

SEQ ID NO:26 (YP_002516237.1; GI:221233801; Xylose dehydrogenase xylB (*Caulobacter crescentus* NA1000))

```
  1 mssaiypslk gkrvvitggg sgigagltag farqgaevif ldiadedsra
leaelagspi
 61 ppvykrcdlm nleaikavfa eigdvdvlvn nagnddrhkl advtgaywde
rinvnlrhml
121 fctqavapgm kkrgggavin fgsiswhlgl edlvlyetak agiegmtral
arelgpddir
181 vtcvvpgnvk tkrqekwytp egeaqivaaq clkgrivpen vaalvlflas
ddaslctghe
241 ywidagwr
```

SEQ ID NO:27 (Oxidoreductase, short-chain dehydrogenase/reductase (Phenylobacterium zucineum HLK1))

```
  1 mgvtsaiyps lkgkrvvvtg ggsgigaglv eafvrqgaev hfldvletes
rvletslaga
 61 evppvfhrcd ltdagaiegc fakigpvqvl vnnagnddrh tldevtpayf
ddriavnlrh
121 mvfcakavvp amkaagegai infgsiswhl glpdlvlyet akagiegmtr
alarelgpfg
181 irvtcvapgn vktlrqmkwy tpegeaeiva qqclksriep advaalvlfl
asddarmctg
241 heywidagwr
```

SEQ ID NO:28 (Dehydrogenase of unknown specificity, short-chain alcohol dehydrogenase (*Caulobacter* sp. AP07))

```
  1 mssaiypslq gkrvvvtggg sgigagivaa farqgaevif ldvvdadsea
laaklsdspi
 61 aptymrcdlt dleamaetfa rigpidvlvn nagnddrhgl aeitpaywdq
rmavnlrhml
121 fatqavapgm kargggavin fgsiswhlgl pdlvlyetak agiegmtral
arelgpddir
181 vtcvvpgnvk tkrqekwytp egeaeivaaq alkgrlvpdh vaslvlflas
ddaalctghe
241 ywidagwr
```

SEQ ID NO:29 (Short-chain dehydrogenase/reductase SDR (*Caulobacter* sp. K31))

```
  1 mnievkrpqv stssaiypsl kgkrvvvtgg gsgigagiva gfarqgsevi
fldvadqdsk
 61 alaeqlsgae iapvylrcdl tdldavaktf adigpvdvlv nnagnddrhg
laqitpaywd
121 ermsvnlrhm lfatqavapg mkargggaii nfgsiswhlg lpdlvlyeta
kagiegmtra
181 larelgpddi rvtcvvpgni ktkrqekwyt pegeaeivaa qalkgrlvpd
hvaslvmfla
241 sddaslctgh eywidagwr
```

SEQ ID NO:30 (Short-chain dehydrogenase/reductase SDR (*Caulobacter* segnis ATCC 21756))

```
  1 mssaiypslk gkrvvitggg sgigaglvag fvrqgaevif ldivdadsqa
lvaelskdav
 61 iapvykrcdl mdidalkatf aeigdvdvlv nnagnddrhs ladltpaywd
nrigvnlrhm
121 vfaaqavagg mkkrgggaii nfgsiswhlg ledlvlyeta kagiegmtra
larelgpddi
181 rvtcvvpgnv ktkrqekwyt pegeaeivka qclkgrilpd hvaslvlfla
sddaslctgh
241 eywidagwr
```

Xylonolactonase (xylC)

SEQ ID NO:31 (YP_002516236.1; GI:221233800; Xylonolactonase xylC (*Caulobacter crescentus* NA1000))

```
  1 mtaqvtcvwd lkatlgegpi whgdtlwfvd ikqrkihnyh patgerfsfd
apdqvtflap
 61 ivgatgfvvg lktgihrfhp atgfslllev edaalnnrpn datvdaqgrl
wfgtmhdgee
121 nnsgslyrmd ltgvarmdrd icitngpcvs pdgktfyhtd tlektiyafd
laedgllsnk
181 rvfvqfalgd dvypdgsvvd segylwtalw ggfgavrfsp qgdavtriel
papnvtkpcf
241 ggpdlktlyf ttarkglsde tlaqyplagg vfavpvdvag qpqhevrlv
```

SEQ ID NO:32 (SMP-30/gluconolaconase/LRE domain-containing protein (*Caulobacter segnis* ATCC 21756))

```
  1 mtaevtcvwd lkatlgegpi whgdalwfvd ikqrkihnyk pttgehfsfd
apdqvtflap
 61 iadaggfvvg lktgihrfhp itgfrlliev edsaldnrpn datvdangrl
wfgtmhdgee
121 aksgslyrmd aegvarmdkd icitngpcvs pdgktfyhtd tlektvwayd
laedgtlsnk
181 rafvhvklgd diypdgtvvd segclwialw ggfgvirvsp ageivgriev
papnvtkvcf
241 ggpdlktlfl ttarkglsde tlaqyplagg lfaigvniag qpqhevrlv
```

SEQ ID NO:33 (Gluconolactonase (*Caulobacter* sp. AP07))

```
  1 mpepicvwdl katlgegpiw iaaeqalwfv dikshkvhrf hpesgetksf
dapdqvtfla
 61 pragggfvag lksglhhfhp etgfaylgei epadlnnrpn datvdaegrl
wfgtmhdgee
121 tptgalyrlg adgqpvqqdq gvcitngpcv spdgktfyht dtlekviway
dlgadgelsn
181 krqffrleid dawpdgsvvd aegyvwaalw gghgairisp agelvdrvtl
painvtkpcf
241 ggpdlktlyf ttarkglgde qlaayplcgg vfalpvavag qpqyevrldl p
```

SEQ ID NO:34 (SMP-30/gluconolaconase/LRE domain-containing protein (*Caulobacter* sp. K31))

```
  1 mpepicvwdl katlgegpiw saeeqavwfv dikghkvhrf hpasgatasf
dapdqvtfla
 61 phaggggfva glksglhrfd pttgafvfla qieppelnnr pndatvdaeg
rlwfgtmhdg
121 emtptgalyr lsadgkpiqq degvcitngp caspdgktfy htdtlekviw
aydlgadgsl
181 snkreffrle iadawpdgsv vdsegfvwta lwgghgalrl spageivdrv
ilpainvtkp
241 cfggpdlktv yftsarkgls deqlaaypqc gglfalpvav agqpqyevrl dlr
```

SEQ ID NO:35 (Gluconolactonase (Phenylobacterium zucineum HLK1))

```
  1 mkvlsepdcv lradaelgeg pvwradddav wfvdikgrri hryepvtgaa
wswaapaqpg
 61 fiapvagggw vaglktglhr feprggrfel itavedpsld nrlndgfvda
kgrlwfgsmh
121 dgetaltgal yrlderglqr cdtgycitng paaspdgrtl yhtdtlqkti
yafdlspage
```

181 lsnkrvfari eegggypdgp avdaegcvwt glfagwhvrr yspkgellak
vgfpvanitk
241 lafggddlts vyattawkgl saderekqpl agglfrfevd vpglpqnqma ha

D-xylonate dehydratase (xylD)

SEQ ID NO:36 (NP_419636.1; GI:16125072; Dihydroxy-acid dehydratase)Caulobacter crescentus CB15))

1 mrsalsnrtp rrfrsrdwfd npdhidmtal ylerfmnygi tpeelrsgkp
iigiaqtgsd
61 ispcnrihld lvqrvrdgir daggipmefp vhpifencrr ptaaldrnls
ylglvetlhg
121 ypidavvltt gcdkttpagi maattvnipa ivlsggpmld gwhenelvgs
gtviwrsrrk
181 laageiteee fidraassap saghcntmgt astmnavaea lglsltgcaa
ipapyrergq
241 mayktgqriv dlayddvkpl diltkqafen aialvaaagg stnaqphiva
marhagveit
301 addwraaydi plivnmqpag kylgerfhra ggapavlwel lqqgrlhgdv
ltvtgktmse
361 nlqgretsdr evifpyhepl aekagflvlk gnlfdfaimk ssvigeefrk
rylsqpgqeg
421 vfearaivfd gsddyhkrin dpaleiderc ilvirgagpi gwpgsaevvn
mqppdhllkk
481 gimslptlgd grqsgtadsp silnaspesa iggglswlrt gdtiridlnt
grcdalvdea
541 tiaarkqdgi pavpatmtpw qeiyrahasq ldtggvlefa vkyqdlaakl prhnh

SEQ ID NO:37 (Dihydroxy-acid dehydratase (*Caulobacter* sp. K31))

1 mtsantpsgr pprrfrsrdw fdnpdhidmt alylerfmny gitpeelrsg
kpiigiaqtg
61 sdispcnrih ldlvtrirdg irdaggipme fpvhpifenc rrptaaldrn
lsylglvevl
121 hgypidavvl ttgcdkttpa gimaattvni paivlsggpm ldgwhdgelv
gsgtviwrsr
181 rklaageine eefiqrasds apsaghcntm gtastmnava ealglsltgc
aaipapyrer
241 gqmayktgqr ivdlayedvk pldiltkkaf enaialvaaa ggstnaqphi
vamarhagld
301 itaddwraay diplilnmqp agkylgerfh raggapavlw ellqagrlhg
dvmtvtgktm
361 genlegretk drevvfpygq pmseragflv lkgnlfdfai mktsvisqef
rqrylsepgk
421 edsfearavv fdgsddyhar indpslnide rtilvirgag pigwpgsaev
vnmqppdall
481 krgimslptl gdgrqsgtad spsilnaspe saiggglswl rtgdmiridl
ntgrcdalvd
541 eatiaerrke gvppvpatmt pwqeiyraht gqletggvle favkyqdlas klprhnh

SEQ ID NO:38 (Dihydroxyacid dehydratase/phosphogluconate dehydratase (*Caulobacter* sp. AP07))

1 mtspnrtprr frsrdwfdnp dhidmtalyl erfmnygitp eelrsgkpii
giaqtgsdis

```
 61 pcnrihldlv trirdgirda ggipmefpvh pifencrrpt aaldrnlsyl
glvetlhgyp
121 idavvlttgc dkttpagima attvnipaiv lsggpmldgw hdgelvgsgt
viwrsrrkla
181 ageiteeefi qrasdsapsa ghcntmgtas tmnavaealg lsltgcaaip
apyrergqma
241 yrtgqrivdl ayedikpkdi ltkqafenai alvaaaggst naqphivama
rhagldvtad
301 dwraaydipl ilnmqpagky lgerfhragg apavlwellq agrlhgdamt
vtgktmaenl
361 egretrdrev vfpyaapmse ragflvlkgn lfdfaimkts visqefrdry
lsepgqegaf
421 earavvfdgs gdyharindp slgidertil virgagpigw pgsaevvnmq
ppdallkkgi
481 mslptlgdgr qsgtadspsi lnaspesavg gglswlrtgd viridlntgr
cdalvdeati
541 aarkleglpp vpetmtpwqe iyrahtgqle tggvlefavk yqdlaaklpr hnh
```

SEQ ID NO:39 (Dihydroxy-acid dehydratase (*Caulobacter segnis* ATCC 21756))

```
  1 msertprrfr srdwfdnpdh idmtalyler fmnygitpee lrsgkpiigi
aqtgsdispc
 61 nrihldlvtr irdgirdagg ipmefpvhpi fencrrptaa ldrnlsylgl
vetlhgypid
121 avvlttgcdk ttpagimaat tvnipaivls ggpmldgwhe gelvgsgtvi
wrsrrklaag
181 eiteeefidr aassapsagh cntmgtastm navaealgls ltgcaaipap
yrergqmayk
241 tgqrivdlay edvkpldilt kkafqnaial vaaaggstna qphivamarh
agveitaddw
301 raaydipliv nmqpagkylg erfhraggap avlwellqqg rlhgdvltvt
gktmgenlqg
361 retsdrevif pyhqplaeka gflvlkgnlf dfaimkssvi geefrkryls
epgkegvfea
421 raivfdgsdd yhkrindpal eidercilvi rgagpigwpg saevvnmqpp
dhllkkgims
481 lptlgdgrqs gtadspsiln aspesaiggg lswlrtgdti ridintgrcd
alvdeatiae
541 rkkegipavp atmtpwqeiy rahtgqlesg gvlefavkyq dlasklprhn h
```

SEQ ID NO:40 (Dihydroxy-acid dehydratase (*Caulobacter crescentus* NA1000))

```
  1 msnrtprrfr srdwfdnpdh idmtalyler fmnygitpee lrsgkpiigi
aqtgsdispc
 61 nrihldlvqr vrdgirdagg ipmefpvhpi fencrrptaa ldrnlsylgl
vetlhgypid
121 avvlttgcdk ttpagimaat tvnipaivls ggpmldgwhe nelvgsgtvi
wrsrrklaag
181 eiteeefidr aassapsagh cntmgtastm navaealgls ltgcaaipap
yrergqmayk
241 tgqrivdlay ddvkpldilt kqafenaial vaaaggstna qphivamarh
agveitaddw
301 raaydipliv nmqpagkylg erfhraggap avlwellqqg rlhgdvltvt
gktmsenlqg
```

361 retsdrevif pyheplaeka gflvlkgnlf dfaimkssvi geefrkryls qpgqegvfea
421 raivfdgsdd yhkrindpal eidercilvi rgagpigwpg saevvnmqpp dhllkkgims
481 lptlgdgrqs gtadspsiln aspesaiggg lswlrtgdti ridlntgrcd alvdeatiaa
541 rkqdgipavp atmtpwqeiy rahasqldtg gvlefavkyq dlaaklprhn h

2-Keto-3-deoxy-D-arabinonate Dehydratase (xylX)

SEQ ID NO:41 (NP_419640.1; GI:16125076; Hypothetical protein CC_0823 (*Caulobacter crescentus* CB15))

1 mvcrrllawt arareaedfa lvrqptcrph mlalpsader apptvsalqt lefwgddavg
61 vseflpedwk aatllgridf gegptpvlvr ggrvedvski aptvadlmna fqpgaviprg
121 edkgpleald irpvwedpdg aapvkllapv dlqclkaagv tfavstlerv ieerargdag
181 ealkirtlla ermggdlksv epgsqgaqrl kdaliadglw sqylevaigp daeiftkgpt
241 lssmgwgdqv gvrydshwnn pepevvllcd gsglirgaal gndvnlrdfe grsalllska
301 kdnnascaig pffrlfdetf glddvrsaev elkitgrdnf vldgksnmsl isrdpavlag
361 qaygkqhqyp dgfalflgtm fapiqdrdtp gqgfthkvgd rvrvstpklg vlenevttcd
421 kakpwtfgis alirnlagrg ll

SEQ ID NO:42 (Fumarylacetoacetate hydrolase family protein (*Caulobacter crescentus* NA1000))

1 mgvseflped wkaatllgri dfgegptpvl vrggrvedvs kiaptvadlm nafqpgavip
61 rgedkgplea ldirpvwedp dgaapvklla pvdlqclkaa gvtfavstle rvieerargd
121 agealkirtl laermggdlk svepgsqgaq rlkdaliadg lwsqylevai gpdaeiftkg
181 ptlssmgwgd qvgvrydshw nnpepevvll cdgsglirga algndvnlrd fegrsallls
241 kakdnnasca igpffrlfde tfglddvrsa evelkitgrd nfvldgksnm slisrdpavl
301 agqaygkqhq ypdgfalflg tmfapiqdrd tpgqgfthkv gdrvrvstpk lgvlenevtt
361 cdkakpwtfg isalirnlag rgll

SEQ ID NO:43 (Fumarylacetoacetate (FAA) hydrolase (*Caulobacter segnis* ATCC 21756))

1 mgvseflpdd wknatllgri dfgegptpvl vrggrvedms kvaptvadlm nafgpgaaip
61 rgedkgples ldirpvwedp dgaapvklla pvdlqclkaa gvtfavstle rvieerargd
121 aaaalkireq lsasmggdlr svnpgsegae rlkqtlikdg lwsqylevai gpdaeiftkg
181 ptlssmgwgd hvgvrydshw nnpepevvll cdgagqirga slgndvnlrd fegrsallls

241 kakdnnasca igpffrlfde tfalddvrsa evelkitgrd nfvldgksnm slisrdpavl
301 agqaygkqhq ypdgfalflg tmfapiqdrd tpgqgfthkv gdrvrvstpk lgvlenevtt
361 cdkakpwtfg isalirnlag rgll

SEQ ID NO:44 (Hypothetical protein Caul_4000 (*Caulobacter* sp. K31))
1 malsdflpdd wrdatllgri dfgqgptpvl irggriedvs kiapttsdlm nafapgaaip
61 rgedlgplea ldvravwenp qgaaakllap vdlqvlkaag vtfavstler vieerargda
121 aealkiraql adsmggdlrs vnpgsdgaer lkqtlikdgl wsqylevaig pdaeiftkgp
181 tlssmgwgdh vgvrsdshwn npepevvllc dgsgqirgaa lgndvnlrdf egrsalllsk
241 akdnnascai gpffrlfddg fslddvrsae vtlkitgrdn fvldghsnms lisrdpavla
301 gqafgkqhqy pdgfalflgt mfapiqdrda agqgfthkvg drvrvatpkl gvlenevttc
361 dlaapwtfgv salirnlagr gll

SEQ ID NO:45 (Fumarylacetoacetate (FAA) hydrolase family protein (*Caulobacter* sp. AP07))
1 malsdflpdd wrdatllgrv dfgdgptpvl vrggriedvs riapttsdlm nafapgaaip
61 agadlgplea ldvrpvwenp dgaaakllap vdlqvlkaag vtfavstler vieerargda
121 aealkiraql adsmggdlrg vnpgsegaar lketlikggl wsqylevaig pdaeiftkgp
181 tlssmgwgdq vgvrsdshwn npepevvllc dgsgrirgas lgndvnlrdf egrsalllsk
241 akdnnascai gpffrlfddg fglddvrsae vtlkitgrdn fvldghsnms lisrdpavla
301 gqafgkqhqy pdgfvlflgt mfapiqdrdt agqgfthkvg drvrvatpkl gvlenevttc
361 dvappwtfgv salirnlagr gll

L-arabinose dehydrogenase (AraE)

SEQ ID NO:46 (YP_439823.1; GI:83716868; Dehydrogenase (*Burkholderia thailandensis* E264))
1 mnsvytlglv gigkiardqh lpaiaaepgf dllacasrha qvrgvrnypd idallaaepa
61 ldavslaapp qvryaqaraa lgagkhvmle kppgatagei aalralarer grtlfaawhs
121 rhasavepar awlatrtira vqarwkedvr rwhpgqqwiw epgglgvfdp ginalsivtr
181 ilprelvlra atlvvpanah tpiaaeldcv dtagvpvrae fdwrhgpveq wdiavdtdgg
241 vlsigaggar lsiagepval ppereypsly arfraligeg asdvddrplr lvadafmigr
301 riaadpfqr

SEQ ID NO:47 (Dehydrogenase (*Burkholderia thailandensis* TXDOH))

```
  1 mnsvytlglv gigkiardqh lpaiaaepgf dllacasrha qvrgvrnypd
idallaaepa
 61 ldavslaapp qvryaqaraa lgagkhvmle kppgatagei aalhalarer
grtlfaawhs
121 rhasavepar awlatrtira vqvrwkedvr rwhpgqqwiw epgglgvfdp
ginalsivtr
181 ilprelvlra atlvvpanah tpiaaeldcv dtagvpvrae fdwrhgpveq
wdiavdtdgg
241 vlsigaggar lsiagepval ppereypsly arfraligeg asdvddrplr
lvadafmigr
301 riaadpfqr
```

SEQ ID NO:48 (Galactose 1-dehydrogenase (*Burkholderia ambifaria* IOP40-10))

```
  1 mskvislgvi gigkiardqh lpaiaaepgf altacasrha evngvrnype
lgallaaepe
 61 leavslcapp qvryaqaraa leagkhvmle kppgatlgev aaldalarer
gltlfatwhs
121 rcasavepar awlatrtira vqvrwkedvr rwhpgqqwiw epgglgvfdp
ginalsivtr
181 ilprelvlre atlyvpsdvq tpiaaeldca dtdgvpvhae fdwrhgpveq
weiavdtsdg
241 vlaisrggaq lsiggepvei gpqreypaly ahfraliarg esdvdvrplr
lvadaflfgr
301 rvgtdafgr
```

SEQ ID NO:49 (Galactose 1-dehydrogenase (*Burkholderia ambifaria* MC40-6))

```
  1 mskvislgvi gigkiardqh lpaiaaepgf altacasrha evngvrnype
lgallaaepe
 61 leavslcapp qvryaqaraa leagkhvmle kppgatlgev aaldalarer
gltlfatwhs
121 rcasavepar awlatrtira vqvrwkedvr rwhpgqqwiw epgglgvfdp
ginalsivtr
181 ilprelvlre atlyvpsdvq tpiaaeldca dtdgvpvhae fdwrhgpveq
weiavdtsdg
241 vlaisrggaq lsiagepvei gpqreypaly ahfraliarg esdvdvrplr
lvadaflfgr
301 rvgtdafgr
```

SEQ ID NO:50 (Dehydrogenase (Burkholderia thailandensis MSMB43))

```
  1 mntvytlglv gigkiardqh lpaiaaepgf dlracasrha evrgvrnhpd
igallaaepa
 61 ldavslaapp qvryaqaraa ldagkhvmle kppgatvgei aalralarer
grtlfaswhs
121 rharavepar awlatrtira vqvrwkedvr rwhpgqqwiw epgglgvfdp
ginalsivtr
181 ilprelvlra atlvvpanvh tpiaaefdcv dtagvpvrae fdwrhgpveq
wdiavdtdgg
241 vlaigaggar lsiagepval ppeceypsly arfhaliaar esdvddrplr
lvadafmvgr
301 riaadpfhr
```

L-arabinonolactonase (AraI)

SEQ ID NO:51 (YP_439819.1; GI:83717359; Senescence marker protein-30 family protein (*Burkholderia thailandensis* E264))

```
  1 messnrpart gaasaatlrv dcrnalgega twcdatraly wvdiegarlw
rwraagaqgg
 61 aatdswempe rigcfaltdd pdvllvglas rlaffdarrr aftpivdvep
dlptrlndgr
121 cdragafvfg mkdegggspr avggyyrlnp dlslqrlalp laaiangitf
spdgsamyfc
181 dsptreiqvc dyrpggdvdr irsfvrladd cgepdgsavd adggvwnaqw
ggarivryda
241 qgveteriav ptpqpscval ddggrlyvts arvglddgal arspgaggvf
vadtrhagla
301 tsrfalarna
```

SEQ ID NO:52 (Senescence marker protein-30 family protein (*Burkholderia thailandensis* TXDOH))

```
  1 messsrpart gaasaatlrv dcrnalgega twcdatraly wvdiegarlw
rwraagaqgg
 61 aatdswempe rigcfaltdd pdvllvglas rlaffdarrr aftpivdvep
dlptrlndgr
121 cdragafvfg mkdegggspr avggyyrlnp dlslqrlalp paaiangiaf
spdgsamyfc
181 dsptreiqvc dyrpggdvdr irpfvrladd cgepdgstvd adggvwsaqw
ggarivryda
241 qgveteriav ptpqpscval ddggrlyvts arvglddgal arspgaggvf
vadtrhagla
301 tsrfalarna
```

SEQ ID NO:53 (Senescence marker protein-30 family protein (Burkholderia thailandensis Bt4)

```
  1 messnrpart gaasaatlrv dcrnalgega twcdatraly wvdiegarlw
rwraagaqgg
 61 aatdswempe rigcfaltdd pdvllvglas rlaffdarrr aftpivdvep
dlptrlndgr
121 cdragafvfg mkdegggspr avggyyrlnp dlslqrlalp laaiangiaf
spdgsamyfc
181 dsptreiqvc dyrpggdvdr irsfvrladd cgepdgsavd adggvwnaqw
ggarivryda
241 qgveteriav ptpqpscval ddggrlyvts arvglddgal arspgaggvf
vadtrhagla
```

SEQ ID NO:54 (Hypothetical protein BPSS0776 (*Burkholderia pseudomallei* K96243))

```
  1 messnrpart heasaatllv dcrnalgega twcdaahaly wvdiegarlw
rwraagahgg
 61 ercdswempe riacfaltgd pdvllvglas rlaffdtrrr altpivdvep
drptrlndgr
121 cdragafvfg tkdesggasp raiggyyrln adlslqrlal ppaaiangia
fspdgsamyf
181 cdsptreiqv cdyrpggdvd rvrsfvrlad ahgepdgstv dasggvwnaq
wggarvvryd
241 aqgvetdria vptpqpscvt ldaagrlyvt sarvglddga lagnpgaggv
fvahtrhsgs
```

301 atprfalarh a

SEQ ID NO:55 (Gluconolactonase (*Burkholderia pseudomallei* NCTC 13177))

```
  1 messnrpart heasaatllv dcrnalgega twcdaahaly wvdiegarlw
rwraagahgg
 61 ercdswempe riacfaltgd pdvllvglas rlaffdtrrr altpivdvep
drptrlndgr
121 cdragafvfg tkdesggasp raiggyyrln adlslqrlal ppaaiangia
fspdgsamyf
181 cdsptreiqv cdyrpggdvd rvrsfvrlad ehgepdgstv dasggvwnaq
wggarvvryd
241 aqgvetdria vptpqpscvt ldaagrlyvt sarvglddga lagnpgaggv
fvahtrhpgg
301 atprfalarh a
```

L-arabinonate dehydratase (AraB)

SEQ ID NO:56 (YP_439826.1; GI:83718062; Dihydroxy-acid dehydratase (*Burkholderia thailandensis* E264))

```
  1 msaskpklrs aqwfgthdkn gfmyrswmkn qgipdhefdg rpivgicntw
seltpcnahf
 61 rklaehvkrg vyeaggfpve fpvfsngesn lrpsamltrn lasmdveeai
rgnpidavvl
121 lagcdkttpa llmgaascdv paivvsggpm lngkldgkni gsgtavwqlh
ealkageidl
181 hrflsaeagm srsagtcntm gtastmacla ealgvalphn aaipavdarr
yvlahmsgmr
241 ivgmaheglv lskiltraaf enairvnaai ggstnavihl kaiagrlgvp
leledwlrlg
301 rgtptivdlm psgrflmeef yyagglpavl rrlgeanllp hpgaltvngq
slwdnvrdap
361 shddevirpl drpliadggi rilrgnlapr gavlkpsaas pellkhrgra
vvfenfehyk
421 atiddealdv dansvlvlkn cgprgypgma evgnmglppk llrqgvkdmv
risdarmsgt
481 aygtvvlhva peaaaggpla avrngdwiel dgeagtltld vsddelarrl
sdhdpasapg
541 vaehaagggy arlyvdhvlq adegcdldfl vgrrgaavpr hsh
```

SEQ ID NO:57 (Dihydroxy-acid dehydratase (*Burkholderia thailandensis* TXDOH))

```
  1 msaskpklrs aqwfgthdkn gfmyrswmkn qgipdhefdg rpivgicntw
seltpcnahf
 61 rklaehvkrg vyeaggfpve fpvfsngesn lrpsamltrn lasmdveeai
rgnpidavvl
121 lagcdkttpa llmgaascdv paivvsggpm lngkldgrni gsgtavwqlh
ealkageidl
181 hrflsaeagm srsagtcntm gtastmacla ealgvalphn aaipavdarr
yvlahmsgmr
241 ivgmaheglv lskiltraaf enairvnaai ggstnavihl kaiagrlgvp
leledwlrlg
301 rgtptivdlm psgrflmeef yyagglpavl rrlgeanllp hpgaltvngq
slwdnvrdap
```

361 shddevirpl drpliadggi rilrgnlapr gavlkpsaas pellkhrgra vvfenfehyk
421 atiddealev dansvlvlkn cgprgypgma evgnmglppk llrqgvkdmv risdarmsgt
481 aygtvvlhva peaaaggpla avrngdwiel dceagtltld vsddelarrl sdhdpasapg
541 vaehaagggy arlyvdhvlq adegcdldfl vgrrgaavpr hsh

SEQ ID NO:58 (Dihydroxy-acid dehydratase (*Burkholderia multivorans* ATCC 17616))
1 msatkprlrs aqwfgtndkn gfmyrswmkn qgipdhefdg rpiigicntw seltpcnahf
61 rklaehvkrg ifeaggfpve fpvfsngesn lrpsamltrn lasmdveeai rgnpidavvl
121 lagcdkttpa llmgaascdv paivvsggpm lngklegkni gsgtavwqlh ealkageidl
181 hhflsaeagm srsagtcntm gtastmacma ealgvalphn aaipavdsrr yvlahmsgir
241 ivemaleglv lskvltraaf enairvnaai ggstnavihl kaiagrigvp leledwmrig
301 rdtptivdlm psgrflmeef yyagglpavl rrlgeggllp hpdaltvngk tlwdnvreap
361 nyddevirpl drpliadggi rilrgnlapr gavlkpsaas pellkhrgra vvfenfdhyk
421 atindesldv dansvlvlkn cgprgypgma evgnmglppk llrqgvkdmv risdarmsgt
481 aygtvvlhva peaaaggpla avrngdwiel dceagtlhld ipddelqrrl sdvdpaaapg
541 vagqagkggy arlyldhvlq adegcdldfl vgtrgaevps hsh

SEQ ID NO:59 (Dihydroxy-acid dehydratase (*Burkholderia multivorans* CGD2M))
1 msatkprlrs aqwfgtndkn gfmyrswmkn qgipdhefdg rpiigicntw seltpcnahf
61 rklaehvkrg ifeaggfpve fpvfsngesn lrpsamltrn lasmdveeai rgnpidavvl
121 lagcdkttpa llmgaascdv paivvsggpm lngklegkni gsgtavwqlh ealkageidl
181 hhflsaeagm srsagtcntm gtastmacma ealgvalphn aaipavdsrr yvlahmsgir
241 ivemaleglv lskvltraaf enairvnaai ggstnavihl kaiagrigvp leledwmrig
301 rdtptivdlm psgrflmeef yyagglpavl rrlgeggllp hpdaltvngk tlwdnvrdap
361 nyddevirpl drpliadggi rilrgnlapr gavlkpsaas pellkhrgra vvfenfdhyk
421 atindealdv dansvlvlkn cgprgypgma evgnmglppk llrqgvkdmv risdarmsgt
481 aygtvvlhva peaaaggpla avrngdwiel dceagtlhld ipddelqrrl sdvdpaaapg
541 vagqagkggy arlyldhvlq adegcdldfl vgtrgaevps hsh

SEQ ID NO:60 (Dihydroxy-acid dehydratase (*Burkholderia thailandensis* MSMB43))
1 msaskpklrs aqwfgthdkn gfmyrswmkn qgipdhefdg rpivgicntw seltpcnahf

```
 61 rklaehvkrg vyeaggfpve fpvfsngesn lrpsamltrn lasmdveeai
rgnpidavvl
121 lagcdkttpa llmgaascdv paivvsggpm lngkldgkni gsgtavwqlh
ealkageidl
181 hrflsaeagm srsagtcntm gtastmacla ealgvalphn aaipavdarr
yvlahlsgar
241 ivemahegla lstiltraaf enairanaai ggstnavihl kaiagrlgvp
leledwmrig
301 rdtptivdlm psgrflmeef yyagglpavl rrlgeanllp hpgaltvngk
slwenvrdap
361 nhddevirpl arpliadggi rvlrgnlapr gavlkpsaas pellrhrgra
vvfenfehyk
421 atiddealdv dassvlvlkn cgprgypgma evgnmglppk llrqgvkdmv
risdarmsgt
481 aygtvvlhva peaaaggpla avrngdwial dceagtltld vsddelarrl
sdldpasapg
541 aagqagsggy arlyvdhvlq adegcdldfl vgrrgaavpr hsh
```

2-Keto-3-deoxy-L-arabinonate Dehydratase (AraD)

SEQ ID NO:61 (YP_439824.1; GI:83717217; Dihydrodipicolinate synthase (*Burkholderia thailandensis* E264))

```
  1 mntsrspryr gvfpvvpttf aeageldlps qkravdfmid agseglcila
nfseqfalad
 61 derdvltrti lehvagrvpv ivttthystq vcaarsrraq elgaamvmam
ppyhgatfrv
121 pdtqihafya rlsdaldipi miqdapasgt vlsapflarm areieqvsyf
kietpgaank
181 lrelirlggd aiegpwdgee aitlladlna gatgamtgga ypdgirpive
ahregradda
241 falyqrwlpl inhenrqtgl laakalmreg gviacerprh plppihpdsr
aeliaiarrl
301 dplvlrwar
```

SEQ ID NO:62 (Dihydrodipicolinate synthase, putative (*Burkholderia thailandensis* TXDOH))

```
  1 mntsrspryr gvfpvvpttf teageldlps qkravdfmid agseglcila
nfseqfalad
 61 derdvltrti lehvagrvpv ivttthystq vcaarsrraq elgaamvmam
ppyhgatfrv
121 pdtqihafya rlsdaldipi miqdapasgt vlsapflarm areieqvsyf
kietpgaank
181 lrelirlggd aiegpwdgee aitlladlna gatgamtgga ypdgirpive
ahregradda
241 falyqrwlpl inhenrqtgl laakalmreg gviacerprh plppihpdsr
aeliaiarrl
301 dplvlrwar
```

SEQ ID NO:63 (Dihydrodipicolinate synthase, putative (*Burkholderia thailandensis* MSMB43))

```
  1 mntsrspryr gvfpvvpttf tetgeldlps qmravdfmid agseglcila
nfseqfalad
```

61 derdvltrti lehvagrvpv ivttthystr vcaarsrraq elgaamvmam
ppyhgatfrv
121 pdtqihafya rlsdaldipi miqdapasgt vlsapflarm areieqvsyf
kietpgaank
181 lrelirlggd aiegpwdgee aitlladlna gatgamtgga ypdgirpivd
ahrdgradda
241 falyqrwlpl inhenrqtgl vaakalmreg gviacerprh plppihpdsr
aelieiarrl
301 dplvlrwar

SEQ ID NO:64 (Dihydrodipicolinate synthase/N-acetylneuraminate lyase (*Burkholderia dolosa* AUO158))

1 mtssrtpryr gifpvvpttf tdtgeldlas qkravdfmid agsdglcila
nfseqfaitd
61 derdvltrti lehvagrvpv ivttthystq vcaarslraq qlgaamvmam
ppyhgatfrv
121 peaqiydfya rvsdaidipi miqdapasgt vlsapllarm areieqvsyf
kietpgaank
181 lrelirlggd avegpwdgee aitlladlna gatgamtgga ypdgirpile
ahregrhdda
241 fahyqrwlpl inhenrqsgi lsakalmreg gviacerprh pmpelhpdtr
aeliaiarrl
301 dplvlrwar

SEQ ID NO:65 (Dihydrodipicolinate synthetase family protein (*Burkholderia multivorans* ATCC BAA-247))

1 mtssrtpryr gifpvvpttf tetgeldlas qkravdfmid agsdglcila
nfseqfalad
61 derdvltrti lehvagrvpv ivttshystq tciarsvraq qlgaamvmvm
ppyhgatfrv
121 peaqihafya rlsdalsipi miqdapasgt vlsapflaql areiehvayf
kietpgaank
181 lrelirlggd aiegpwdgee aitlladlha gatgamtgga ypdgirpile
ahregrhdda
241 faryqtwlpl inhenrqsgi ltakalmreg gviaceaprh pmpalhpdtr
aeliaiarrl
301 dplvlrwar

D-glucarate dehydratase (YcbF)

SEQ ID NO:66 (NP_388131.2; GI:255767063; Glucarate dehydratase (*Bacillus subtilis* subsp. subtilis str.168))

1 msspiqeqvq kekrsnipsi semkvipvag hdsmllnlsg ahspfftrni
viltdssgnq
61 gvgevpggeh irrtlelsep lvvgksigay qailqtvrkq fgdqdrggrg
nqtfdlrttv
121 havtaleaal ldllgkflqe pvaallgegk qrdevkmlgy lfyigdrnrt
tlpyqsdeqs
181 dcawfrlrhe ealtpeaivr laesaqeryg fqdfklkggv lrgeeeieav
talskrfpea
241 ritldpngaw sleeaialck gkqdvlayae dpcgdengys arevmaefrr
atglptatnm

301 iatdwremgh aiqlhavdip ladphfwtmq gsvrvaqmch dwgltwgshs nnhfdislam
361 fthvaaaapg ritaidthwi wqdgqrltkq pfeissgcvk vpdkpglgvd idmeqvekah
421 eiyrkmnlga rndaipmqfl isnwefdrkr pclvr

SEQ ID NO:67 (Glucarate dehydratase (*Bacillus subtilis*))
1 msspiqeqvq kekrsnipsi semkvipvag hdsmllnlsg ahspfftrni viltdssgnq
61 gvgevpggeh irrtlelsep lvvgksigay qailqtvrkq fgdqdrggrg nqtfdlrttv
121 havtaleaal ldflgkflqe pvaallgegk qrdevkmlgy lfyigdrnrt tlpyqsdeqs
181 dcawfrlrhe ealtpeaivr laesaqeryg fqdfklkggv lrgeeeieav talskrfpea
241 ritldpngaw sleeaialck gkqdvlayae dpcgdengys arevmaefrr atglptatnm
301 iatdwremgh aiqlhavdip ladphfwtmq gsvrvaqmch dwgltwgshs nnhfdislam
361 fthvaaaapg ritaidthwi wqdgqrltkq pfeissgcvk vpdkpglgvd idmeqvekah
421 eiyrkmnlga rndaipmqfl isnwefdrkr pclvr

SEQ ID NO:68 (Hypothetical protein BSNT_00441 (*Bacillus subtilis* subsp. natto BEST195))
1 msspiqeqvq kekrsnipsi temkvipvag hdsmllnlsg ahspfftrni viltdssgnq
61 gvgevpggeh irrtlelsep lvvgksigay qailqtvrkq fgdqdrggrg nqtfdlrttv
121 havtaleaal ldllgkflqe pvaallgegk qrdevkmlgy lfyigdrkrt tlpyqsdeqs
181 dcawfrlrhe ealtpeaivr laesaqeryg fqdfklkggv lqgeeeieav talskrfpea
241 ritldpngaw sleeaialck gkqdvlayae dpcgdengys arevmaefrr atglptatnm
301 iatdwremgh aiqlhavdip ladphfwtmq gsvrvaqmch dwgltwgshs nnhfdislam
361 fthvaaaapg ritaidthwi wqdgqrltkq pfeissgcvk vpdkpglgid idmeqvekah
421 eiyrkmnlga rndaipmqfl isnwefdrkr pclvr

SEQ ID NO:69 (Glucarate dehydratase (*Bacillus subtilis* subsp. spizizenii TU-B-10))
1 msspiqeqvq kekrsnipsi cemkvipvag hdsmllnlsg ahspfftrni viltdssgnq
61 gvgevpggeq irrtlelaep lvvgksigay qsilqtvrkq fadqdrggrg iqtfdlrttv
121 havtaleaal ldllgkflqe pvaallgegk qrdevkmlgy lfyigdrkqt tlpyqsdeqs
181 dcgwfrlrhe ealtpeaivr laesaqeryg fqdfklkggv lrgedeieav talakrfpea
241 ritldpngaw sleeaialck gkhdvlayae dpcgdengys arevmaefrr atglptatnm
301 iatdwremgh aiqlhavdip ladphfwtmq gsvrvaqmch dwgltwgshs nnhfdislam

361 fthvaaaapg ritaidthwi wqdgqrltkq pfeisegcvk vpnkpglgid idmeqvekah
421 elyrkmnlga rndavpmqfl isnwefdrkr pclvr

SEQ ID NO:70 (Glucarate dehydratase (*Bacillus subtilis* subsp. subtilis str. RO-NN-1))
1 msspmqeqiq kekrsnvpsi semkvipvag hdsmllnlsg ahspfftrni viltdssgnq
61 gvgevpggeh irrtlelsep lvvgksigay qailqtvrkq fgdqdrggrg nqtfdlrttv
121 havtaleaal ldllgkflqe pvaallgegk qrdevkmlgy lfyigdrkrt tlpyqsdeqs
181 ycawfrlrhe ealtpeaivr laesaqeryg fqdfklkggv lrgeeeieav talskrfpea
241 ritldpngaw sleeaialck gkqdvlayae dpcgdengys arevmaefrr atglptatnm
301 iatdwremgh aiqlhavdip ladphfwtmq gsvrvaqmcn dwgltwgshs nnhfdislam
361 fthvaaaapg ritaidthwi wqdgqrltkq pfeissgcvk vpdkpglgvd idmeqvekah
421 eiyrkmnlga rndaipmqsl isnwefdrkr pclvr

D-galactarate dehydratase (YcbH)

SEQ ID NO:71 (NP_388133.2; GI:255767065; D-galactarate dehydratase (*Bacillus subtilis* subsp. subtilis str.168))
1 mamnlrknqa plyikvheid ntaiivndgg lpkgtvfscg lvleedvpqg hkvaltdlnq
61 gdeivrygev igfadetikr gswirealvr mpappalddl planrvpqpr pplegytfeg
121 yrnadgsagt knilgittsv qcvvgvldya vkrikeellp kypnvddvvp lhhqygcgva
181 inapdavipi rtiqnlakhp nfggevmvig lgcekllper iasendddil slqdhrgfaa
241 miqsilemae erlirlnsrt rvscpvsdlv iglqcggsda fsgvtanpav gyaadllvra
301 gatvlfsevt evrdaihllt pravseevgq slikemkwyd sylrrgdadr sanpspgnkk
361 gglsnvveka lgsvaksgts pisgvlgpge rakqkgllfa atpasdfvcg tlqlaagmnl
421 qvfttgrgtp yglaaapvlk vstrhslseh wadlidinag riatgeasie dvgweifrti
481 ldvasgrkqt wadrwglhnd lclfnpapvt

SEQ ID NO:72 (Hypothetical protein BSNT_00443 (Bacillus subtilis subsp. natto BEST195))
1 mamnlrknqa plyikvheid ntaiivndgg lpkgtvfscg lvleedvpqg hkvaltdlnq
61 gdeivrygev igfadetikr gswirealvr mpappalddl planrvpqpr pplegytfeg
121 yrnadgsagt knilgittsv qcvvgvldya vkrikeellp kypnvddvvp lhhqygcgva
181 inapdavipi rtiqnlakhp nfggevmvig lgcekllper iasendddil slqdhrgfaa

241 miqsilemae erlirlnsrt rvscpvsdlv iglqcggsda fsgvtanpav gyaadllvra
301 gatvlfsevt evrdaihllt pravseevgq slikemkwyd sylrrgdadr sanpspgnkk
361 gglsnvveka lgsvaksgts pisgvlgpge raeqkgllfa atpasdfvcg tlqlaagmnl
421 qvfttgrgtp yglaaapvlk vstrhslseh wadlidinag riatgeasie dvgweifrti
481 ldvasgrkqt wadrwglhnd lclfnpapvt

SEQ ID NO:73 (D-galactarate dehydratase (*Bacillus subtilis* subsp. subtilis str. RO-NN-1))

1 mamnlrknqa plyikvheid ntaiivndgg lpkgtvfscg lvleedvpqg hkvaltdlnq
61 gdeivrygev igfadetikr gswirealvr mpappalddl planrvpqpr pplegytfeg
121 yrnadgsagt knilgittsv qcvvgvldya vkrikeellp kypnvddvvp lhhqygcgva
181 inapdavipi rtiqnlakhp nfggevmvig lgcekllper iasendddil slqdhrgfaa
241 miqsilemae erlirlnsrt rvscpvsdlv iglqcggsda fsgvtanpav gyaadllvra
301 gatvlfsevt evrdaihllt pravseevgq slieemkwyd sylrrgdadr sanpspgnkk
361 gglsnvveka lgsvaksgts pisgvlgpge raeqkgllfa atpasdfvcg tlqlaagmnl
421 qvfttgrgtp yglaaapvlk vstrhslseh wadlidinag riatgeasie dvgweifrti
481 ldvasgrkqt wadrwglhnd lclfnpapvt

SEQ ID NO:74 (Hypothetical protein BSSC8_40810 (*Bacillus subtilis* subsp. subtilis str. SC-8))

1 mamnlrknqa plyikvheid ntaiivnegg lpkgtvfscg lvleedvpqg hkvaltdlnq
61 gdeivrygev igfadetikr gswirealvr mpappalddl plenrvpqpr pplegytfeg
121 yrnadgsagt knilgittsv qcvvgvldya vkrikeellp kypnvddvvp lhhqygcgva
181 inapdavipi rtiqnlakhp nfggevmvig lgcekllper iasendddil slqdhrgfaa
241 miqsilemae erlirlnsrt rvscpvsdlv iglqcggsda fsgvtanpav gyaadllvra
301 gatvlfsevt evrdaihllt pravseevgq slikemkwyd sylrrgdadr sanpspgnkk
361 gglsnvveka lgsvaksgts pisgvlgpge raeqkgllfa atpasdfvcg tlqlaagmnl
421 qvfttgrgtp yglaaapvlk vstrhslseh wadlidinag qiatgeasie dvgweifrti
481 ldvasgrkqt wadrwglhnd lclfnpapvt

SEQ ID NO:75 (Galactarate dehydratase (*Bacillus subtilis* BSn5))

1 mamnlrknqa plyikvheid ntaiivndgg lpkgtvfscg lvleedvpqg hkvaltdlnq

```
 61 gdeivrygev igfadetikr gswiredlvr mpappalddl planrvpqpr
pslegytfeg
121 yrnadgstgt knilgittsv qcvvgvldya vkrikeellp kypnvddvvp
lhhqygcgva
181 inapdavipi rtiqnlakhp nfggevmvig lgcekllper iasengddil
slqdhrgfaa
241 miqsilemae erlirlnsrt rvscpvsdlv iglqcggsda fsgvtanpav
gyaadllvra
301 gatvlfsevt evrdaihllt pravseevgq slikemkwyd sylrrgdadr
sanpspgnkk
361 gglsnvveka lgsvaksgts pisgvlgpge rakqkgllfa atpasdfvcg
tlqlaagmnl
421 qvfttgrgtp yglaaapvlk vstrhslseh wadlidinag riatgeasie
dvgweifrti
481 ldvasgrkqt wadrwglhnd lclfnpapvt
```

5-dehydro-4-deoxyglucarate dehydratase (YcbC)

SEQ ID NO:76 (NP_388128.2; GI:255767061; 5-dehydro-4-deoxyglucarate dehydratase (*Bacillus subtilis* subsp. subtilis str.168))

```
  1 msrirkapag ilgfpvapfn tqgkleeeal fqniefllne gleaifiacg
sgefqslsqk
 61 eyeqmvevav saaggkvpvy tgvggnlsta ldwaqlsekk gadgylilpp
ylvhgeqegl
121 yqyaktiies tdlnailyqr dnavlsveqi krlteceqlv gvkdgvgnmd
lninlvytig
181 drlgwlngmp maevtmpayl pigfhsyssa isnyiphisr mfydalkngn
delvkelyrh
241 vilpindirk qrkgyavsli kagmeimgln vrntarppvg pvekdhyqql
eailkqaadr
301 fpkkaatv
```

SEQ ID NO:77 (Putative 5-dehydro-4-deoxyglucarate dehydratase (*Bacillus subtilis* subsp. subtilis str. RO-NN-1))

```
  1 msrirkapag ilgfpvapfn tqgkleeeal fqniefllne gleaifiacg
sgefqslsqk
 61 eyeqmvevav saaggkvpvy tgvggnlsta lewaqlsekk gadgylilpp
ylvhgeqegl
121 yqyaktiies tdlnailyqr dnavlsveqi krlteceqlv gvkdgvgnmd
lninlvytig
181 drlgwlngmp maevtmpayl pigfhsyssa isnyiphisr mfydalkngn
delvkelyrh
241 vilpindirk qrkgyavsli kagmeimgln vrntarppvg pvekdhyqql
eailkqaadr
301 fpkkaatv
```

SEQ ID NO:78 (5-dehydro-4-deoxyglucarate dehydratase (*Bacillus vallismortis* DV1-F-3))

```
  1 mnrirkaptg ilgfpvapfn tqgqleeeal fqniefllee gleaifiacg
sgefqslsqk
 61 eyeqmvevav saaegkvpvy tgvggnlsta lewarlsekk gadgylilpp
ylvhgeqegl
121 yqyaktiies tdlnailyqr dnavlsleqi krlteceqlv gvkdgvgnmd
lninlvytlg
```

181 drlgwlngmp maevtmpayl pigfhsyssa isnyiphisr mfydalkngn
delvkelyqh
241 vilpindirk qrkgyavsli kagmeimgln vrntarppvg pvekehyrql
eailkqaadr
301 fpkkaatv

SEQ ID NO:79 (5-dehydro-4-deoxyglucarate dehydratase (Bacillus subtilis subsp. spizizenii TU-B-10))

1 msrirkapag ilgfpvapfn tqgkleeeal fqniefllee gleaifiacg
sgefqslsqk
61 eyeqmvevai saaggkvpvy tgvggnlsta lewaqlsekk gadgylilpp
ylvhgeqegl
121 yqyaktiies tdlnailyqr dnavlsveqi krltefeqlv gvkdgvgnmd
lninlvytlg
181 drlgwlngmp maevtmpayl pigfhsyssa isnyiphisr mfydalkngd
delvkelyqh
241 vilpindirk qrkgyavsli kagmeimgln vrntarppvg pvekdhyqql
eailkqaadr
301 fpkkaatv

SEQ ID NO:80 (ycbC (*Bacillus subtilis*))

1 msrirkapag ilgfpvapfn tqgtleeeal fqniefllne gleaifiacg
sgefqslsqk
61 eyeqmvevav saaggkvpvy tgvggnlsta ldwaqlsekk gadgylilpp
ylvhgeqegl
121 yqyaktiies tdlnailyqr dnavlsveqi krlteceqlv gvkdgvgnmd
lninlvytig
181 drlgwlngmp maevtmpayl pigfhsyssa isnyiphisr mfydalkngn
delvkelyrh
241 vilpindirk qrkgyavsli kagmeimgln vrntarppvg pvekdhyqql
eailkqpadr
301 fpkkaatv

Amino acid transporter LysE (HypE)

SEQ ID NO:81 (NP_743408.1; GI:26987983; Amino acid transporter LysE (*Pseudomonas putida* KT2440))

1 maaesyrlqa ldpsrawhrf fatvqqqvek rafgddsseh clrnaqqelt
mlgvtdygaf
61 viaflillai pgpgnfalit atgkggikag laatcgvivg dqvllwlava
gvatllatyp
121 aafhmvqwag aaylaylglr mllskpggaa htcrmdngqy lrqtmmitll
npkaimfyma
181 ffplfvdpvk hqglvtfgfm aatvavvtfl ygliavvlth qlaermrasp
rianmferla
241 gaclvgfgik laamr

SEQ ID NO:82 (Amino acid transporter LysE (*Pseudomonas putida* BIRD-1))

1 mqqqvekraf gddssahclr naqqeltmlg vtdygafvia flillaipgp
gnfalitatg
61 kggikaglaa tcgvivgdqv llwlavagva tllatypaaf hvvqwagaay
laylglrmll

```
121 skpggaahtc rmdngqylrq tmmitllnpk aimfymaffp lfvdpvkhqg
lvtfgfmaat
181 vavvtflygl iavvlthqla ermraspria nmferlagac lvgfgiklaa mr
```

SEQ ID NO:83 (Amino acid transporter LysE (*Pseudomonas putida* ND6))

```
  1 mqqqvekrav gddssahclr naqqeltmlg vtdygafvia flillaipgp
gnfalitatg
 61 kggikaglaa tcgvivgdqv llwlavagva tllatypaaf hmvqwagaay
laylglrmll
121 skpggaahtc rmdngqylrq tmmitllnpk aimfymaffp lfvdpvkhqg
lvtfgfmaat
181 vavvtflygl iavvlthqla ermranpria nmferlagac lvgfgiklaa mr
```

SEQ ID NO:84 (Lysine exporter protein LysE/YggA (*Pseudomonas putida* F1))

```
  1 mlgvtdygaf viaflillai pgpgnfalit atgkggikag laatcgvivg
dqvllwlava
 61 gvatllatyp aafhmvqwag aaylaylglr mllskpggaa htcrmdngqy
lrqtmmitll
121 npkaimfyma ffplfvdpvk hqglvtfgfm aatvavvtfl ygliavvlth
qlaermranp
181 rianmferla gaclvgfgik laamr
```

SEQ ID NO:85 (Unknown (*Pseudomonas putida*))

```
  1 mlgvtdygaf viafiillai pgpgnfalit atgkggikag laatcgvivg
dqvllwlava
 61 gvatllatyp aafhivqwag aaylaylglr mllskpgdap rtsrmdngqy
lrqtmlitll
121 npkaimfyma ffplfidpvk hqglvtfgfm aatvavitfl ygliavvlth
rlaermranp
181 ritnmferla gaclvgfgik laamr
```

PP_1245

SEQ ID NO:86 (NP_743405.1; GI:26987980; Hypothetical protein PP_1245 (*Pseudomonas putida* KT2440))

```
  1 mrptengvlh lrkkfvasll avaiasttac aqlgiskeqa gtvigglagv
aigstmgsgn
 61 gkiaaaliag gigayvgnri ghmldekdqq alalrtqevl sqqqttasaq
pvtwksdhsg
121 ataqivpgke ytktkqvevk rapkiqavps mklinepyvt isdnlnvraa
pnqagekvgs
181 lknhteftav gstgdwilvg rkgvtvgyvh knyvepkaqa vakrvtpavn
ldeldvaask
241 etqgfdldsv qslptqtvaa eaacrpvtvs lksqsgqteq eqntfckqan gtweli
```

SEQ ID NO:87 (SH3 type 3 domain-containing protein (*Pseudomonas putida* W619))

```
  1 mrkkfvasll avaiatttac aqlgiskeqa gtvigglagv aigstmgsgn
gkiaaaliag
 61 gigayvgnri ghmldekdqq alalrtqevl sqsatasaqp vtwksdhsga
taqitpgkey
121 tqtkkvevkr apkiqavpsm klinepyvti sdnlnvraap nttgekvgsl
kshteftavg
```

181 stgdwilvgr kgvtvgyvhk nyvepkaqai akraapavnl ddldvaanke tqgfdldsiq
241 slptetvaae aacrpvtvsl ksqsgqteqe qntfckqang tweli

SEQ ID NO:88 (Hypothetical protein G1E_03180 (*Pseudomonas* sp. TJI-51))
1 mrkkfvasll avaiasttac aqlgiskeqa gtvigglagv aigstlgsgn gkiaaaliag
61 gigayvgnri gnmldekdqq alalrtqevl sqqqatasaq pvtwksdhsg asaqivpgke
121 ytktkqvevk rapkiqavps mklinepyvt tsdnlnvraa pnasgekvgs lknhteftav
181 gatgdwilvg rkgvtvgyvh kdyvepkaqa vakrvtpavn ldeldvaask etqafdldsl
241 qslptqtvaa eaacrpvtvs lkaqngkteq eqntfckqan gtweli

SEQ ID NO:89 (SH3 type 3 domain-containing protein (*Pseudomonas putida* GB-1))
1 mrkkfvasll avaiasttac aqlgiskeqa gtvigglagv aigstmgsgn gkiaaaliag
61 gigayvgnri ghmldekdqq alalrtqevl sqqqatasaq pvtwksdhsg ataqivpgke
121 ytqtkkvevk rapkiqavps mklinepyvt vsdnlnvraa pnqsgekvgs lknhteftav
181 gstgdwilvg rkgvtvgyvh knyvepkaqa vakrvtpavn ldeldvaask etqgfdldsv
241 qslptetvaa eaacrpvtvs lksqsgqteq eqntfckqan gtweli

SEQ ID NO:90 (SH3 type 3 domain-containing protein (*Pseudomonas putida* F1))
1 mrkkfvasll avaiasttac aqlgiskeqa gtvigglagv aigstmgsgn gkiaaaliag
61 gigayvgnri ghmldekdqq alalrtqevl sqqqttasaq pvtwksdhsg ataqivpgke
121 ytktkqvevk rapkiqavps mklinepyvt isdnlnvraa pnqagekvgs lknhteftav
181 gstgdwilvg rkgvtvgyvh knyvepkaqa vakrvtpavn ldeldvaask etqgfdldsv
241 qslptqtvaa eaacrpvtvs lksqsgqteq eqntfckqan gtweli

PP_1247

SEQ ID NO:91 (NP_743407.1; GI:26987982; Hypothetical protein PP_1247 (*Pseudomonas putida* KT2440))
1 mpicssgwrg lawwdsasnw rrcadpkpds vrarltatlk kppathgsrg lvhsaitqsi
61 gfqliglahe qrrkqalafl egvllferav fdqllpdgaf rvavvlglga kvtaprrqpn
121 llaegcelcl gdlllvfaes lfqrfeaava hrvvldlgla gkaahrfsqh rlagvravra
181 nqhraqgtle lgfdivqfrq rlevglahdf phlgavvavg dherhrafai agaldgevqv
241 drgtkvtgaa dqkragywla hrhvgapgev rrggptiggq lgtwldfvad irhqhdfgpl
301 ggnvrvahlh aqqldmnaai lavsvmgqlq rislqvhpgh iaadielvlg parqaffsrt

361 tlyglhqarq aahellgaig lrrrhadlrv gyrqvagkrr vgnvplrqhi
lkeiallevv
421 vvgqrsllar agdhriatte hqhrcghtan qqlllvhlfd hgvcltgpwr
krcssrsrtv
481 grprgss

SEQ ID NO:92 (Uncharacterized protein LOC100789425 (*Glycine max*))

1 msniafrsti vfllfsavls tppedpikca tsenttctit nsygafpdrs
ickaaqvlyp
61 tteqelvsvv asatrnktkm kvatrfshsi pklvcpegen gllistkyln
kilkvdvetr
121 tmtvesgvtl qqlineaakv glalpyapyw wgltigglmg tgahgstlrg
kgsavhdyvv
181 elrivrpagp edgyamvenl neqhedlnaa kvslgvlgvi sqitlklepl
fkrsityvak
241 ddsdlggqvv afgdahefad itwypsqhka iyrvddrvpi ntsgnglydf
ipfrptpsla
301 svfirtteei qestndangk civastasnt litaaygltn ngiifagypi
igfqnrlqss
361 gscldslqda littcawdpr mkglffhqtt fsirlsfvks fiedvqklve
lepkglcvlg
421 lyngmlmryv tassaylghq enaldidity yrskdpmtpr lyedileeve
qlgifkyggl
481 phwgknrnla fegaikkyks aeyflkvkek ydldglfsst wtdqvlglkd
gvtilkdgca
541 leglciclqd shcnpskgyy crpgkvykea rvctnlk

PP_1246

SEQ ID NO:93 (NP_743406.1; GI:26987981; Hypothetical protein PP_1246 (*Pseudomonas putida* KT2440))

1 mkkhalalav igacglvpqa fahelafskk dnikvevpgd atswckpqvd
ltitrpawdn
61 qellaglltk lpfvfakdcs takvswkavd akgnlyasgs gnasnlglvt
laaapataap
121 apaaaptptp apapapapap aaaaapavve aapaqakpap apapapapav
aaepapapea
181 paaapvvppa papatavaaa ptsdfgrsvv lenrnlmqvt dgtgckwvls
tsiigdgdtl
241 sfgttpampc pasgfgegsf dkiswkavgt yrgdnwtrvy ahpsglifnk
nlepavkdka
301 vsyltpqadq aaflvgeipg rqmkvyltft rssygvlrpf ssdpyyvavt
pdesfaldat
361 kykeaaleif dlikttsptt tdvanlfivk dlsaisnniw gndaqkitrn
riginrqglf
421 fdvrdganwa vqreqqrvre qrqrqqelar vhtrvleryq qlqdgmsdfk
gretealaqm
481 agikvrfasp leqqnpatsa svvpmmvhvt gkkgdfysid fpsngrlvad
eeysegwyvt
541 qvanatpyyp lddgravpty raysagepea ckqdhcadrv sfgavlakef
pnagidfswt
601 pevsqqyvnd wnnasamvq

SEQ ID NO:94 (Hypothetical protein T1E_4663 (*Pseudomonas putida* DOT-T1E))

```
  1 mvlenrnlmq vtdgtgckwv lstsiigdgd tlsfgttpam pcpasgfgeg
sfdkiswkav
 61 gtyrgdnwtr vyahpsglif nkhlepavkd kavsyltpqa dqaaflvgei
pgrqmkvylt
121 ftrssygvlr pfgsdpyyva vtpdesfald atkykeaale ifdlikttsp
tttdvanlfi
181 vkdlsaisnn iwgndaqkit rnriginrqg lffdvrdgan wavqreqqrv
reqrqrqqel
241 arvhtrvler yqqlqdgmsd fkgreteala qmagikvrfa spleqqnpat
sasvvpmmvh
301 vtgkkgdfys idfpsngrlv adeeysegwy vtqvanatpy yplddgravp
tyraysagep
361 eackqdhcad rvsfgavlak efpnagidfs wtpevsqqyv ndwnnasamv q
```

SEQ ID NO:95 (Hypothetical protein YSA_07676 (*Pseudomonas putida* ND6))

```
  1 mkkhalalav igacglvpqa fahelafskk dnikvevpgd attwckpqvd
ltitrpawdn
 61 qellsglltk lpfvfakdcs takvswkavd akgnlyasgs gnasnlglvt
laaapataap
121 apaaavapap apaqpeapaa aaptpapapa papapaaaaa pavveaapaq
akpapapapa
181 pavaaepapt peapaaapvv ppapapatav aaaptsdfgr svvlenrnlm
qvtdgtgckw
241 vlstsiigdg dtlsfgttpa mpcpasgfge gsfdkiswka vgtyrgdnwt
rvyahpsgli
301 fnkhlepavk dkavsyltpq adqaaflvge ipgrqmkvyl tftrssygvl
rpfgsdpyyv
361 avtpdesfal datkykeaal eifdliktts ptttdvanlf ivkdlsaisn
niwgndaqki
421 trnriginrq glffdvrdga nwavqreqqr vreqrqrqqe larvhtrvle
ryqqlqdgms
481 dfkgreteal aqmagikvrf aspleqqnpa tsasvvpmmv hvtgkkgdfy
sidfpsngrl
541 vadeeysegw yvtqvanatp yyplddgrav ptyraysage peackqdhca
drvsfgavla
601 kefpnagidf swtpevsqqy vndwnnasam vq
```

SEQ ID NO:96 (Hypothetical protein Pput_1275 (*Pseudomonas putida* F1))

```
  1 mkkhalalav igacglvpqa fahelafskk dnikvevpgd attwckpqvd
ltitrpawdn
 61 qellsglltk lpfvfakdcs takvswkavd akgnlyasgs gnasnlglvt
laaapapapa
121 papapapaaa apapaaavap apapaqpeap aaaaptpapa papapaaaaa
pavveaaaaq
181 akpapapapa pavaaepapt peapaaapvv ppapapatav aaaptsdfgr
svvlenrnlm
241 qvtdgtgckw vlstsiigdg dtlsfgttpa mpcpasgfge gsfdkiswka
vgtyrgdnwt
301 rvyahpsgli fnknlepavk dkavsyltpq adqaaflvge ipgrqmkvyl
tftrssygvl
361 rpfgsdpyyv avtpdesfal datkykeaal eifdliktts ptttdvanlf
ivkdlsaisn
```

421 niwgndaqki trnriginrq glffdvrdga nwavqreqqr vreqrqrqqe larvhtrvle
481 ryqqlqdgms dfkgreteal aqmagikvrf aspleqqnpa tsasvvpmmv hvtgkkgdfy
541 sidfpsngrl vadeeysegw yvtqvanatp yyplddgrav ptyraysage peackqdhca
601 drvsfgavla kefpnagidf swtpevsqqy vndwnnasam vq

SEQ ID NO:97 (Hypothetical protein PputGB1_4145 (*Pseudomonas putida* GB-1))

1 mkkhalalav vgacglvpqa fahelafskk enikvevpgd aatwckpeve ltitrpawdk
61 qellsglltk lpfvfakdca takvswkavd akgnlyasgs gnatnlglvt lavapaaasa
121 apapapapap apapapapap avaalapaap avpapaeapa avaaapapav vepapakaev
181 apapvvaaep apapvaetpv aapvappvpa padavaaapt sdfgravvlq nrnlmqvtdg
241 tgckwvlsts iisdgdtlsf gttpvmpcpa sgfgegsfek iswkavgtyr gdnwtrvyah
301 psglifnknl esavkdkavs yltadadqaa flvgeipsrq mkvyltftrs sygvlrpfss
361 dpyyvavtpd esfaldaaky keaaleifdl ikatsptttd vanlfivkdi saitnsmwgn
421 daqkitrnri gitrqglffd vreganwavq reqqrvreer qrqqelarvh trvleryqql
481 qdgmsdfkgr etealaqmag ikvrfaspla qqdpatsarv apmmvhvtgk kgdfytldfp
541 skgrlvadee ysegwyvtqv anatpyypld dgravptyra ysagepeacq qdhcadrvsf
601 gavlakefpn agidfswtpe vsqkyvndwn nasamvq

Alpha-ketoisovalerate decarboxylase

SEQ ID NO:98 (YP_003353820.1; GI:281491840; Alpha-ketoisovalerate decarboxylase (*Lactococcus lactis* subsp. lactis KF147))

1 mytvgdylld rlhelgieei fgvpgdynlq fldqiisrkd mkwvgnanel nasymadgya
61 rtkkaaaflt tfgvgelsav nglagsyaen lpvveivgsp tskvqnegkf vhhtladgdf
121 khfmkmhepv taartlltae natveidrvl sallkerkpv yinlpvdvaa akaekpslpl
181 kkenptsnts dqeilnkiqe slknakkpiv itgheiisfg lentvtqfis ktklpittln
241 fgkssvdetl psflgiyngk lsepnlkefv esadfilmlg vkltdsstga fthhlnenkm
301 islnidegki fnesiqnfdf eslisslldl sgieykgkyi dkkqedfvps nallsqdrlw
361 qavenltqsn etivaeqgts ffgassiflk pkshfigqpl wgsigytfpa algsqiadke
421 srhllfigdg slqltvqelg lairekinpi cfiinndgyt vereihgpnq syndipmwny
481 sklpesfgat eervvskivr tenefvsvmk eaqadpnrmy wielvlaked apkvlkkmgk

541 lfaeqnks

SEQ ID NO:99 (Indole-3-pyruvate decarboxylase (*Lactococcus lactis* subsp. lactis IO-1))

1 mytvgdylld rlhelgieei fgvpgdynlq fldqiisrkd mkwvgnanel nasymadgya
61 rtkkaaaflt tfgvgelsav nglagsyaen lpvveivgsp tskvqnegkf vhhtladgdf
121 khfvkmhepv taartlltae natveidrvl svllkerkpv yinlpvdvaa akaekpslpl
181 kkenpnsnts dqeilnkiqe slknakkpiv itgheiisfg lektvtqfis ktklpittln
241 fgkssvdeal psflgiyngk lsepnlkefv esadfilmlg vkltdsstga fthhlnenkm
301 islninegki fsesiqnfdf eslisslldl sgieykgkyi dkkqenfvps nallsqdrlw
361 qavenltqsn etivaeqgts ffgassiflk pkshfigqpl wgsigftfpa algsqiadke
421 srhllfigdg slqltvqelg lairekinpi cfiinndgyt vereihgpnq syndipmwny
481 sklpesfgat edrvvskivr tenefvsvmk eaqadpnrmy wielvlaked apkvlkkmgk
541 lfaeqnks

SEQ ID NO:100 (Branched-chain alpha-ketoacid decarboxylase (*Lactococcus lactis*))

1 mytvgdylld rlhelgieei fgvpgdynlq fldqiisred mkwignanel nasymadgya
61 rtkkaaaflt tfgvgelsai nglagsyaen lpvveivgsp tskvqndgkf vhhtladgdf
121 khfmkmhepv taartlltae natyeidrvl sqllkerkpv yinlpvdvaa akaekpalsl
181 ekessttntt eqvilskiee slknaqkpvv iaghevisfg lektvtqfvs etklpittln
241 fgksavdesl psflgiyngk lseislknfv esadfilmlg vkltdsstga fthhldenkm
301 islnidegii fnkvvedfdf ravvsslsel kgieyegqyi dkqyeefips saplsqdrlw
361 qavesltqsn etivaeqgts ffgastiflk snsrfigqpl wgsigytfpa algsqiadke
421 srhllfigdg slqltvqelg lsireklnpi cfiinndgyt vereihgptq syndipmwny
481 sklpetfgat edrvvskivr tenefvsvmk eaqadvnrmy wielvleked apkllkkmgk
541 lfaeqnk

SEQ ID NO:101 (Chain A, branched-chain ketoacid decarboxylase (Kdca) (Lactococcus Lactis))

1 mgsshhhhhh ssglvprgsh masmytvgdy lldrlhelgi eeifgvpgdy nlqfldqiis
61 redmkwigna nelnasymad gyartkkaaa flttfgvgel sainglagsy aenlpvveiv
121 gsptskvqnd gkfvhhtlad gdfkhfmkmh epvtaartll taenatyeid rvlsqllker

181 kpvyinlpvd vaaakaekpa lslekesstt ntteqvilsk ieeslknaqk pvviaghevi
241 sfglektvtq fvsetklpit tlnfgksavd eslpsflgiy ngklseislk nfvesadfil
301 mlgvkltdss tgafthhlde nkmislnide giifnkvved fdfravvssl selkgieyeg
361 qyidkqyeef ipssaplsqd rlwqaveslt qsnetivaeq gtsffgasti flksnsrfig
421 qplwgsigyt fpaalgsqia dkesrhllfi gdgslqltvq elglsirekl npicfiinnd
481 gytvereihg ptqsyndipm wnysklpetf gatedrvvsk ivrtenefvs vmkeaqadvn
541 rmywielvle kedapkllkk mgklfaeqnk

SEQ ID NO:102 (Indole-3-pyruvate decarboxylase (Lactococcus lactis subsp. lactis Il1403))
1 mytvgdylld rlhelgieei fgvpgdynlq fldqiisrkd mkwvgnanel nasymadgya
61 rtkkaaaflt tfgvgelsav nglagsyaen lpvveivgsp tskvqnegkf vhhtladgdf
121 khfmkmhepv taartlltae natveidrvl sallkerkpv yinlpvdvaa akaekpslpl
181 kkenptsnts dqeilnkiqe slknakkpiv itgheiisfg lektvtqfis ktklpittln
241 fgkssvdetl psflgiyngk lsepnlkefv esadfilmlg vkltdsstga fthhlnenkm
301 islninegki fneriqnfdf eslisslldl sgieykgkyi dkkqedfvps nallsqdrlw
361 qavenltqsn etivaeqgts ffgassiflk pkshfigqpl wgsigytfpa algsqiadke
421 srhllfigdg slqltvqerk lqvqvsqpss shmnsys

Alcohol dehydrogenase yqhD

SEQ ID NO:103 (YP_001459806.1; GI:157162488; Alcohol dehydrogenase yqhD (*Escherichia coli* HS))
1 mnnfnlhtpt rilfgkgaia glreqiphda rvlitygggs vkktgvldqv ldalkgmdvl
61 efggiepnpa yetlmnavkl vreqkvtfll avgggsvldg tkfiaaaany penidpwhil
121 qtggkeiksa ipmgcvltlp atgsesnaga visrkttgdk qafhsahvqp vfavldpvyt
181 ytlpprqvan gvvdafvhtv eqyvtkpvda kiqdrfaegi lltliedgpk alkepenydv
241 ranvmwaatq alngligagv pqdwathmlg heltamhgld haqtlaivlp alwnekrdtk
301 rakllqyaer vwnitegsdd eridaaiaat rnffeqlgvp thlsdygldg ssipallkkl
361 eehgmtqlge nhditldvsr riyeaar

SEQ ID NO:104 (Alcohol dehydrogenase, iron-dependent (Escherichia coli 97.0259))
1 mnnfnlhtpt rilfgkgaia glreqiphda rvlitygggs vkktgvldqv ldalkgmdvl

61 efggiepnpa yetlmnavkl vreqkvtfll avgggsvldg tkfiaaaany penidpwhil
121 qtggkeiksa ipmgcvltlp atgsesnaga visrkttgdk qafhsahvqp vfavldpvyt
181 ytlpprqvan gvvdafvhtv eqyvtkpvda kiqdrfaegi lltliedgpk alkepenydv
241 ranvmwaatq alngligagv pqdwathmlg heltamhgld haqtlaivlp alwnekrdtk
301 rakllqyaer iwnitegsdd eridaaiaat rnffeqlgvp thlsdygldg ssipallkkl
361 eehgmtqlge nhditldvsr riyeaar

SEQ ID NO:105 (Alcohol dehydrogenase (*Escherichia coli* MS 200-1))

1 mnnfnlhtpt rilfgkgaia glreqiphda rvlitygggs vkktgvldqv lnalkgmdvl
61 efggiepnpa yetlmnavkl vreqkvtfll avgggsvldg tkfiaaaany penidpwhil
121 qtggkeiksa ipmgcvltlp atgsesnaga visrkttgdk qafhsahvqp vfavldpvyt
181 ytlpprqvan gvvdafvhtv eqyvtkpvda kiqdrfaegi lltliedgpk alkepenydv
241 ranvmwaatq alngligagv pqdwathmlg heltamhgld haqtlaivlp alwnekrdtk
301 rakllqyaer vwnitegsdd eridaaiaat rnffeqlgvp thlsdygldg ssipallkkl
361 eehgmtqlge nhditldvsr riyeaar

SEQ ID NO:106 (Alcohol dehydrogenase yqhD (*Escherichia coli* B7A))

1 mnnfnlhtpt rilfgkgaia glreqiphda rvlitygggs vkktgvldqv ldalkgmdvl
61 efggiepnpa yetlmnavkl vreqkvtfll avgggsvldg tkfiaaaany penidpwhil
121 qtggkeiksa ipmgcvltlp atgsesnaga visrkttgdk qafhsahvqp vfavldpvyt
181 ytlpprqvan gvvdafvhtv eqyvtkpvda kiqdrfaegi lltliedgpk alkepenydv
241 ranvmwaatq alngligagv pqdwathmlg heltamhgld haqtlaivlp alwnekretk
301 rakllqyaer vwnitegsdd eridaaiaat rnffeqlgvp thlsdygldg ssipallkkl
361 eehgmtqlge nhditldvsr riyeaar

SEQ ID NO:107 (Alcohol dehydrogenase (*Escherichia coli* MS 196-1))

1 mnnfnlhtpt rilfgkgaia glreqiphda rvlitygggs vkktgvldqv ldalkgmdvl
61 efggiepnpa yetlmnavkl vreqkvtfll avgggsvldg tkfiaaaany penidpwhil
121 qtggkeiksa ipmgcvltlp atgsesnaga visrkttgdk qafhsahvqp vfavldpvyt
181 ytlpprqvan gvvdafvhtv eqyvtkpvda kiqdrfaegi lltliedgpk alkepenydv
241 ranvmwaatq alngligagv pqdwathmlg hkltamhgld haqtlaivlp alwnekrdtk

301 rakllqyaer vwnitegsdd eridaaiaat rnffeqlgvp thlsdygldg
ssipallkkl
361 eehgmtqlge nhditldvsr riyeaar

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What is claimed is:

1. A recombinant microbial cell modified to exhibit increased biosynthesis of a TCA derivative compared to a wild-type control.

2. The recombinant cell of claim 1 wherein the TCA derivative comprises 1,4-butanediol.

3. The recombinant microbial cell any preceding claim wherein the microbial cell is a fungal cell.

4. The recombinant cell of claim 3 wherein the fungal cell is a member of the Saccharomycetaceae family.

5. The recombinant cell of claim 3 wherein the fungal cell is *Saccharomyces cerevisiae*, *Candida rugosa*, or *Candida albicans*.

6. The recombinant cell of claim 1 or claim 2 wherein the microbial cell is a bacterial cell.

7. The recombinant cell of claim 6 wherein the bacterial cell is a member of the phylum Protobacteria.

8. The recombinant cell of claim 7 wherein the bacterial cell is a member of the Enterobacteriaceae family.

9. The recombinant cell of claim 8 wherein the bacterial cell is *Escherichia coli*.

10. The recombinant cell of claim 7 wherein the bacterial cell is a member of the Pseudomonaceae family.

11. The recombinant cell of claim 10 wherein the bacterial cell is *Pseudomonas putida*.

12. The recombinant cell of claim 6 wherein the bacterial cell is a member of the phylum Firmicutes.

13. The recombinant cell of claim 12 wherein the bacterial cell is a member of the Bacillaceae family.

14. The recombinant cell of claim 13 wherein the bacterial cell is *Bacillus subtilis*.

15. The recombinant cell of claim 12 wherein the bacterial cell is a member of the Streptococcaceae family.

16. The recombinant cell of claim 15 wherein the bacterial cell is *Lactococcus lactis*.

17. The recombinant cell of claim 12 wherein the bacterial cell is a member of the Clostridiaceae family.

18. The recombinant cell of claim 17 wherein the bacterial cell is *Clostridium cellulolyticum*.

19. The recombinant cell of claim 6 wherein the bacterial cell is a member of the phylum Cyanobacteria.

20. The recombinant cell of any preceding claim wherein the microbial cell is photosynthetic.

21. The recombinant cell of any preceding claim wherein the microbial cell is cellulolytic.

22. The recombinant cell of any preceding claim wherein the increased biosynthesis of the TCA derivative comprises an increase in xylose dehydrogenase activity, xylonolactonase activity, xylonate dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity.

23. The recombinant cell of claim 22 wherein the increased biosynthesis of the TCA derivative further comprises an increase in benzoylformate decarboxylase activity and an increase in alcohol dehydrogenase activity.

24. The recombinant cell of claim 23 wherein the benzoylformate decarboxylase comprises BFD of *Pseudomonas putida*.

25. The recombinant cell of claim 23 wherein the alcohol dehydrogenase comprises yqhD of *E. coli*.

26. The recombinant cell of claim 22 wherein the increased biosynthesis of the TCA derivative further comprises an increase in decarboxylase activity and an increase in alcohol dehydrogenase activity.

27. The recombinant cell of claim 26 wherein the decarboxylase comprises KIVD of *Lactococcus lactis*.

28. The recombinant cell of claim 26 wherein the alcohol dehydrogenase comprises yqhD of *E. coli*.

29. The recombinant cell of preceding claim wherein the increased biosynthesis of the TCA derivative comprises a decrease in α-ketoglutaric semialdehyde dehydrogenase activity.

30. The recombinant cell of preceding claim wherein the increased biosynthesis of the TCA derivative comprises a decrease in aldolase activity.

31. The recombinant cell of preceding claim wherein the increased biosynthesis of the TCA derivative comprises a decrease in 2-keto-3-deoxy gluconate aldolase activity.

32. The recombinant cell of any preceding claim comprising an engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol.

33. The recombinant cell of claim 32 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 2,5-dioxopentonoic acid into succinaldehyde.

34. The recombinant cell of claim 33 wherein the enzyme that converts 2,5-dioxopentonoic acid into succinaldehyde comprises a 2-ketoacid decareboxylase or a 2-oxoglutarate decarboxylase.

35. The recombinant cell of claim 33 or 34 wherein the enzyme that converts 2,5-dioxopentonoic acid into succinaldehyde comprises KIVD, BFD, or IPDC.

36. The recombinant cell of any one of claims 32-35 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts succinaldehyde to 1,4-butanediol.

37. The recombinant cell of claim 36 wherein the enzyme that converts succinaldehyde to 1,4-butanediol comprises an alcohol dehydrogenase.

38. The recombinant cell of claim 36 or claim 37 wherein the enzyme that converts succinaldehyde to 1,4-butanediol comprises YqhD, ADH6, YjgB, or YahK.

39. The recombinant cell of claim 32 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 2,5-dioxopentonoic acid into 2-keto-5-hydroxy-pentanoic acid.

40. The recombinant cell of claim 39 wherein the enzyme that converts 2,5-dioxopentonoic acid into 2-keto-5-hydroxy-pentanoic acid comprises an alcohol dehydrogenase.

41. The recombinant cell of claim 39 or claim 40 wherein the enzyme that converts 2,5-dioxopentonoic acid into 2-keto-5-hydroxy-pentanoic acid comprises YqhD, ADH6, YjgB, or YahK.

42. The recombinant cell of any one of claims 39-41 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 2-keto-5-hydroxy-pentanoic acid to 4-hydroxy-1-butyraldehyde.

43. The recombinant cell of claim 42 wherein the enzyme that converts 2-keto-5-hydroxy-pentanoic acid to 4-hydroxy-1-butyraldehyde comprises a 2-ketoacid decareboxylase or a 2-oxoglutarate decarboxylase.

44. The recombainant cell of claim 42 or claim 43 wherein the enzyme that converts 2-keto-5-hydroxy-pentanoic acid to 4-hydroxy-1-butyraldehyde comprises Kivd, BFD, or IPDC.

45. The recombinant cell of any one of claims 42-44 wherein the engineered metabolic pathway for converting 2,5-dioxopentanoic acid to 1,4-butanediol comprises an enzyme that converts 4-hydroxy-1-butyraldehyde to 1,4-butanediol.

46. The recombinant cell of claim 45 wherein the enzyme that converts 4-hydroxy-1-butyraldehyde to 1,4-butanediol comprises an alcohol dehydrogenase.

47. The recombinant cell of claim 45 or claim 46 wherein the enzyme that converts 4-hydroxy-1-butyraldehyde to 1,4-butanediol comprises YqhD, ADH6, YjgB, or YahK.

48. The recombinant cell of any preceding claim comprising an engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid.

49. The recombinant cell of claim 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-arabinose into D-arabinolactone.

50. The recombinant cell of claim 49 wherein the enzyme that can convert D-arabinose into D-arabinolactone comprises a pentose dehydrogenase.

51. The recombinant cell of claim 49 or claim 50 wherein the enzyme that can convert D-arabinose into D-arabinonolactone comprises AraDH.

52. The recombinant cell of any one of claims 49-51 wherein the recombinant cell exhibits conversion of D-arabinose into D-arabinonolactone at a level at least 110% of a wild-type control cell.

53. The recombinant cell of any one of claims 49-52 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid.

54. The recombanant cell of claim 53 wherein the enzyme that converts D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid comprises an aldonic acid dehydratase.

55. The recombanant cell of claim 53 or claim 54 wherein the enzyme that converts D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid comprises AraD.

56. The recombinant cell of any one of claims 53-55 wherein the recombinant cell exhibits conversion of D-arabononic acid to 2-oxo-4(s),5-dihydroxy-pentanoic acid at a level at least 110% of a wild-type control cell.

57. The recombinant cell of any one of claims 49-56 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid.

58. The recombinant cell of claim 57 wherein the enzyme that converts 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

59. The recombinant cell of claim 57 or claim 58 wherein the enzyme that converts 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises KdaD.

60. The recombinant cell of any one of claims 57-59 wherein the recombinant cell exhibits conversion of 2-oxo-4(s),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid at a level at least 110% of a wild-type control cell.

61. The recombinant cell of claim 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-xylose to D-xylonolactone.

62. The recombinant cell of claim 61 wherein the enzyme that converts D-xylose to D-xylonolactone comprises a pentose dehydrogenase.

63. The recombinant cell of claim 61 or claim 62 wherein enzyme that converts D-xylose to D-xylonolactone comprises XylB or rrnAC3034.

64. The recombinant cell of any one of claims 61-63 wherein the recombinant cell exhibits conversion of D-xylose to D-xylonolactone at a level at least 110% of a wild-type control.

65. The recombinant cell of any one of claims 61-64 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-xylonolactone to D-xylonic acid.

66. The recombinant cell of claim 65 wherein the enzyme that converts D-xylonolactone to D-xylonic acid comprises a pentonolactonase.

67. The recombinant cell of claim 65 or claim 66 wherein the enzyme that converts D-xylonolactone to D-xylonic acid comprises XylC or rrnAC3033.

68. The recombinant cell of any one of claims 65-67 wherein the recombinant cell exhibits conversion of D-xylonolactone to D-xylonic acid at a level at least 110% of a wild-type control.

69. The recombinant cell of any one of claims 61-68 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid.

70. The recombinant cell of claim 69 wherein the enzyme that converts D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid comprises an aldonic acid dehydratase.

71. The recombinant cell of claim 69 or claim 70 wherein the enzyme that converts D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid comprises XylD or rrnAC3032.

72. The recombinant cell of any one of claims 69-71 wherein the recombinant cell exhibits conversion of D-xylonic acid to 2-oxo-4(S),5-dihydroxy-pentanoic acid at a level at least 110% of a wild-type control.

73. The recombinant cell of any one of claims 61-72 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid.

74. The recombinant cell of claim 73 wherein the enzyme that converts 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

75. The recombinant cell of claim 73 or claim 74 wherein the enzyme that converts 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid comprises XylX or rrnAC3039.

76. The recombinant cell of any one of claims 73-75 wherein the recombinant cell exhibits conversion of 2-oxo-4(S),5-dihydroxy-pentanoic acid to 2,5-dioxopenatnoic acid at a level at least 110% of a wild-type control.

77. The recombinant cell of claim 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts L-arabinose to L-arabinolactone.

78. The recombinant cell of claim 77 wherein the enzyme that converts L-arabinose to L-arabinolactone comprises a pentose dehydrogenase.

79. The recombinant cell of claim 77 or claim 78 wherein the enzyme that converts L-arabinose to L-arabinolactone comprises AraE.

80. The recombinant cell of any one of claims 77-79 wherein the recombinant cell exhibits conversion of L-arabinose to L-arabinolactone at a level at least 110% of a wild-type control.

81. The recombinant cell of any one of claims 77-80 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts L-arabinolactone to L-arabinonic acid.

82. The recombinant cell of claim 81 wherein the enzyme that converts L-arabinolactone to L-arabinonic acid comprises a pentonolactonase.

83. The recombinant cell of claim 81 or claim 82 wherein the enzyme that converts L-arabinolactone to L-arabinonic acid comprises AraI.

84. The recombinant cell of any one of claims 81-83 wherein the recombinant cell exhibits conversion of L-arabinolactone to L-arabinonic acid at a level at least 110% of a wild-type control.

85. The recombinant cell of any one of claims 77-84 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid.

86. The recombinant cell of claim 85 wherein the enzyme that converts L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid comprises an aldonic acid dehydratase.

87. The recombinant cell of claim 85 or claim 86 wherein the enzyme that converts L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid comprises AraB.

88. The recombinant cell of any one of claims 81-87 wherein the recombinant cell exhibits conversion of L-arabinonic acid to 2-oxo-4(R),5-dihydroxy-pentanoic acid at a level at least 110% of a wild-type control.

89. The recombinant cell of any one of claims 77-88 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid.

90. The recombinant cell of claims 89 wherein the enzyme that converts 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

91. The recombinant cell of claims 89 wherein the enzyme that converts 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid comprises AraD.

92. The recombinant cell of any one of claims 89-90 wherein the recombinant cell exhibits conversion of 2-oxo-4(R),5-dihydroxy-pentanoic acid to 2,5-dioxopentanoic acid at a level at least 110% of a wild-type control.

93. The recombinant cell of claim 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid.

94. The recombinant cell of claim 93 wherein the enzyme that converts D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid comprises an aldonic acid dehydratase.

95. The recombinant cell of claim 93 or claim 94 wherein the enzyme that converts D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid comprises YcbF.

96. The recombinant cell of any one of claims 93-95 wherein the recombinant cell exhibits conversion of D-glucaric acid to 4-deoxy-5-keto-D-glucaric acid at a level at least 110% of a wild-type control.

97. The recombinant cell of any one of claims 93-96 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid.

98. The recombinant cell of claim 97 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

99. The recombinant cell of claim 97 or claim 98 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises YcbC.

100. The recombinant cell of claim 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid.

101. The recombinant cell of claim 100 wherein the enzyme that converts D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid comprises an aldonic acid dehydratase.

102. The recombinant cell of claim 100 or claim 101 wherein the enzyme that converts D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid comprises YcbH.

103. The recombinant cell of any one of claims 100-102 wherein the recombinant cell exhibits conversion of D-galactaric acid to 4-deoxy-5-keto-D-glucaric acid at a level at least 110% of a wild-type control.

104. The recombinant cell of any one of claims 100-103 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid.

105. The recombinant cell of claim 104 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

106. The recombinant cell of claim 104 or claim 105 wherein the enzyme that converts 4-deoxy-5-keto-D-glucaric acid to 2,5-dioxopentanoic acid comprises YcbC.

107. The recombinant cell of claim 48 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline.

108. The recombinant cell of claim 107 wherein the enzyme that converts 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline comprises an amino acid transporter.

109. The recombinant cell of claim 107 or claim 108 wherein the enzyme that converts 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline comprises LysE or HypE.

110. The recombinant cell of any one of claims 107-109 wherein the recombinant cell exhibits conversion of 4(R)-hydroxy-L-proline to 4(R)-hydroxy-D-proline at a level at least 110% of a wild-type control.

111. The recombinant cell of any one of claims 107-110 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline.

112. The recombinant cell of claim 111 wherein the enzyme that converts 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline comprises HypOX.

113. The recombinant cell of claim 111 or claim 112 wherein the recombinant cell exhibits conversion of 4(R)-hydroxy-D-proline to 2-carboxy-4(R)-hydroxy-δ-pyrroline at a level at least 110% of a wild-type control.

114. The recombinant cell of any one of claims 107-113 wherein the engineered metabolic pathway for converting a carbon source to 2,5-dioxopentanoic acid comprises an enzyme that converts 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid.

115. The recombinant cell of claim 114 wherein the enzyme that converts 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid comprises a 2-keto-3-deoxyaldonic acid dehydratase.

116. The recombinant cell of claim 114 or claim 115 wherein the enzyme that converts 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid comprises PP1247.

117. The recombinant cell of any one of claims 114-116 wherein the recombinant cell exhibits conversion of 2-oxo-4(R),5-hydroxy-5-aminopentanoic acid to 2,5-dioxopentanoic acid at a level at least 110% of a wild-type control.

118. The recombinant cell of any one of claims 48-117 modified to exhibit increased α-ketoglutaric semialdehyde dehydrogenase activity compared to a wild-type control.

119. The recombinant cell of claim 118 exhibiting increased conversion of 2,5-dioxopentanoic acid to a TCA derivative compared to a wild-typ control.

120. The recombinant cell of claim 119 wherein the TCA derivative comprises succinate, fumarate, malate, glutamate, lysine, threonine, or 4-hydroxybutyrate.

121. The recombinant cell of any preceding claim genetically modified to increase consumption of xylose, arabinose, glucaric acid, galactaric acid, or hydroxyproline compared to a wild-type control.

122. The recombinant cell of any preceding claim genetically modified to in crease consumption of a uronic acid compared to a wild-type control.

123. The recombinant cell of claim 122 wherein the urnic acid comprises galacturonic acid or glucuronic acid.

124. The recombinant cell of claim 122 or claim 123 genetically modified to increase conversion of the uronic acid to an aldonic acid compared to a wild-type control.

125. The recombinant cell of any one of claims 122-124 wherein the recombinant cell comprises an exogenous urinate dehydrogenase.

126. A method comprising:

incubating a recombinant cell of any preceding claim in medium that comprises a carbon source under conditions effective for the recombinant cell to produce a TCA derivative.

127. The method of claim 126 wherein the TCA derivative comprises 1,4-butanediol.

128. The method of claim 126 wherein the carbon source comprises xylose, arabinose, glucaric acid, galactaric acid, or hydroxyproline.

129. The method of any one of claims 126-128 wherein the increased biosynthesis of the TCA derivative comprises an increase in pentose dehydrogenase activity, pentonolactonase activity, aldonic acid dehydratase activity, or 2-keto-3-deoxyaldonic acid dehydratase activity.

130. The method of any one of claims 126-129 wherein the increased biosynthesis of the TCA derivative comprises an increase in hexic acid dehydratase activity or 5-dehydro-4-deoxyglucarate dehydratase activity.

131. A method comprising:

introducing into a host cell a heterologous polynucleotide encoding at least one polypeptide that catalyzes conversion of a carbon source to a TCA derivative, wherein the at least one polypeptide is operably linked to a promoter so that the modified host cell catalyzes conversion of the carbon source to TCA derivative.

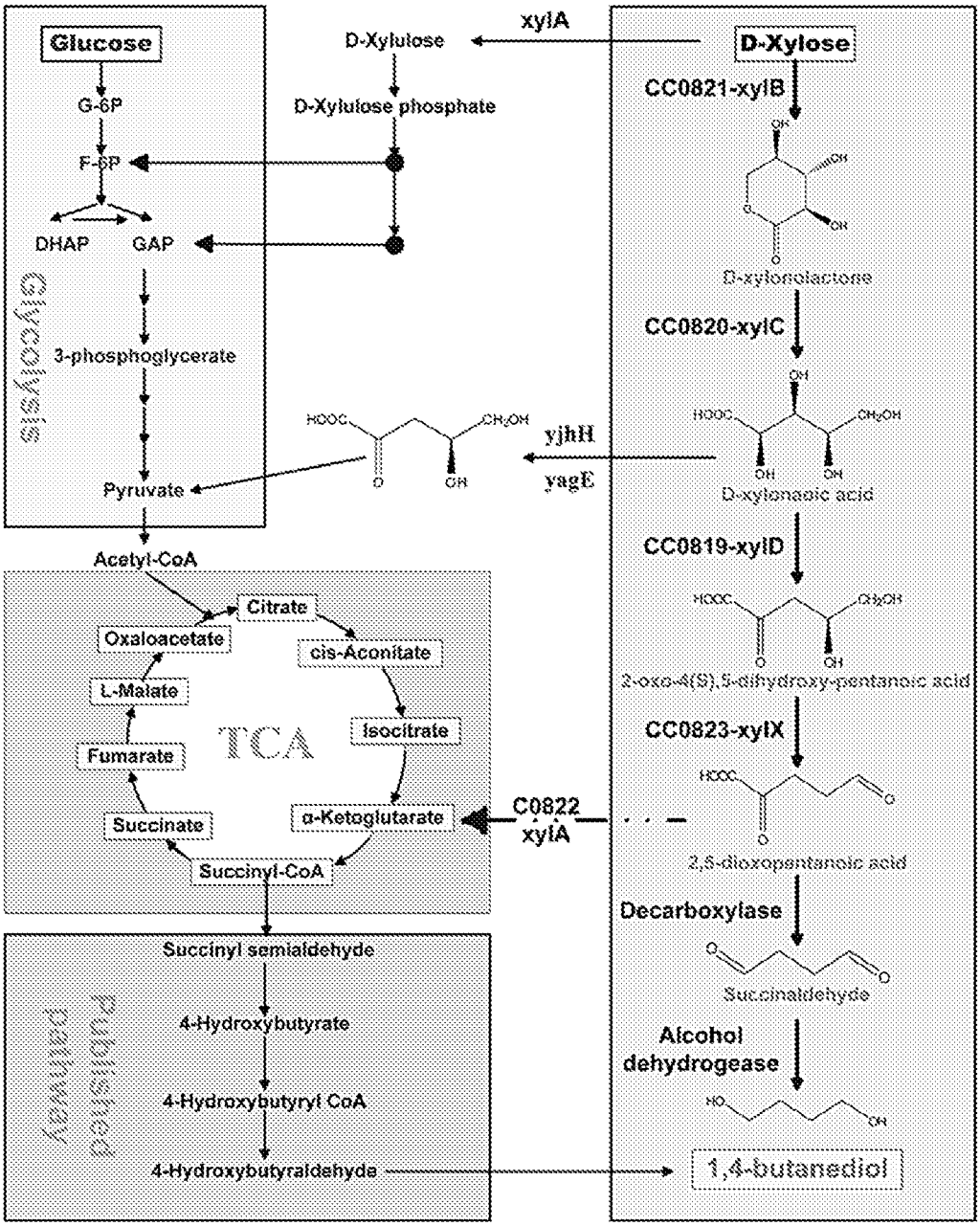
132. The method of claim 131 wherein the TCA derivative comprises 1,4-butanediol.

133. The method of claim 131 wherein the carbon source comprises xylose.

134. The method of claim 131 wherein the TCA derivative comprises succinate, fumarate, malate, glutamate, lysine, threonine, 4-hydroxybutyrate.

Figure 1

A.


Glucose
G-6P
F-6P
DHAP
GAP
3-phosphoglycerate
Pyruvate
Glycolysis
Acetyl-CoA
Citrate
Oxaloacetate
cis-Aconitate
L-Malate
Isocitrate
Fumarate
TCA
Succinate
α-Ketoglutarate
Succinyl-CoA
Succinyl semialdehyde
4-Hydroxybutyrate
4-Hydroxybutyryl CoA
4-Hydroxybutyraldehyde
Anaerobic pathway
D-Xylulose
D-Xylulose phosphate
xylA
yjhH
yagE
C0822
xylA
D-Xylose
CC0821-xylB
D-xylonolactone
CC0820-xylC
D-xylonaaic acid
CC0819-xylD
2-oxo-4(S),5-dihydroxy-pentanoic acid
CC0823-xylX
2,5-dioxopentanoic acid
Decarboxylase
Succinaldehyde
Alcohol dehydrogease
1,4-butanediol

B.

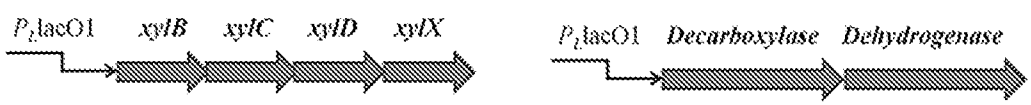

$P_L$lacO1 xylB xylC xylD xylX
$P_L$lacO1 Decarboxylase Dehydrogenase

Figure 2

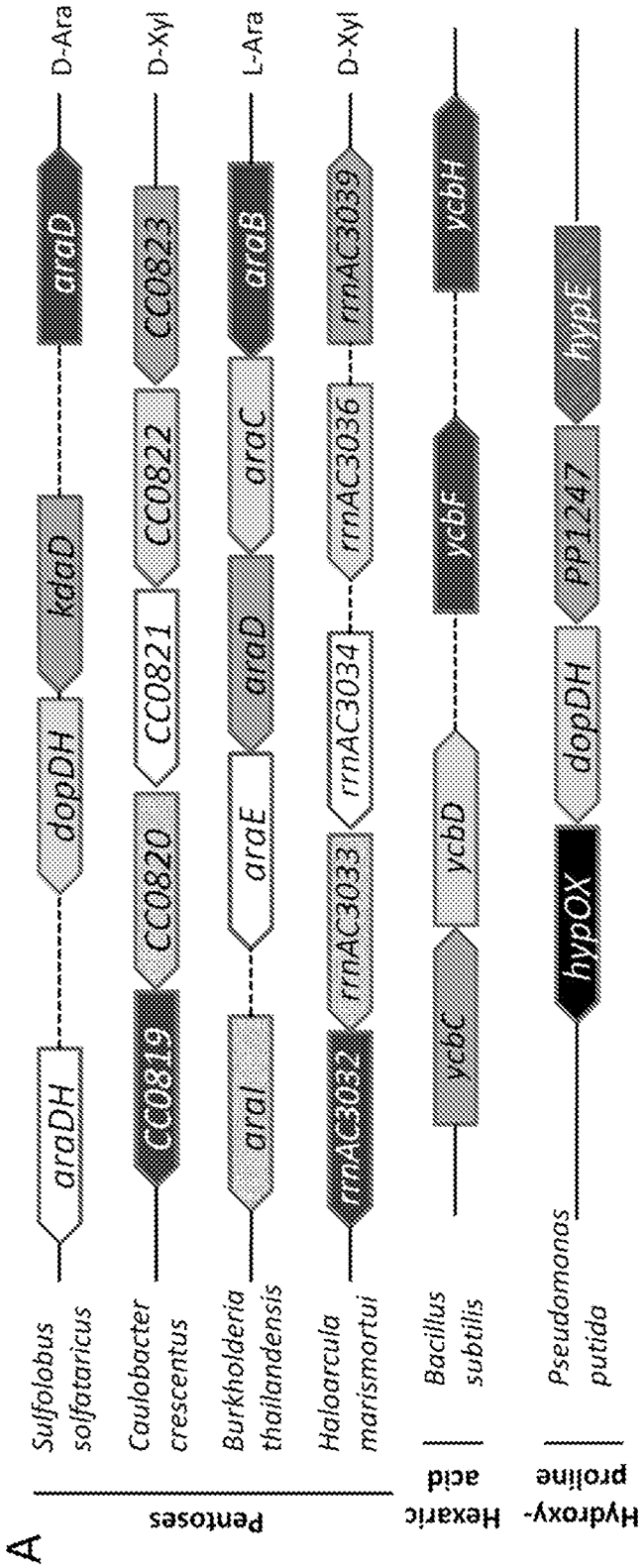

A
Pentoses
Hexaric acid
Hydroxy-proline
Sulfolobus solfataricus
araDH
dopDH
kdaD
aldB
D-Ara
Caulobacter crescentus
CC0819
CC0820
CC0821
CC0822
CC0823
D-Xyl
Burkholderia thailandensis
araI
araE
araD
araC
aldB
L-Ara
Haloarcula marismortui
rrnAC3032
rrnAC3033
rrnAC3034
rrnAC3036
rrnAC3039
D-Xyl
Bacillus subtilis
ycbC
ycbD
ycbJ
ycbH
Pseudomonas putida
hypOX
dopDH
PP1247
hmgE

Figure 2 (continued)

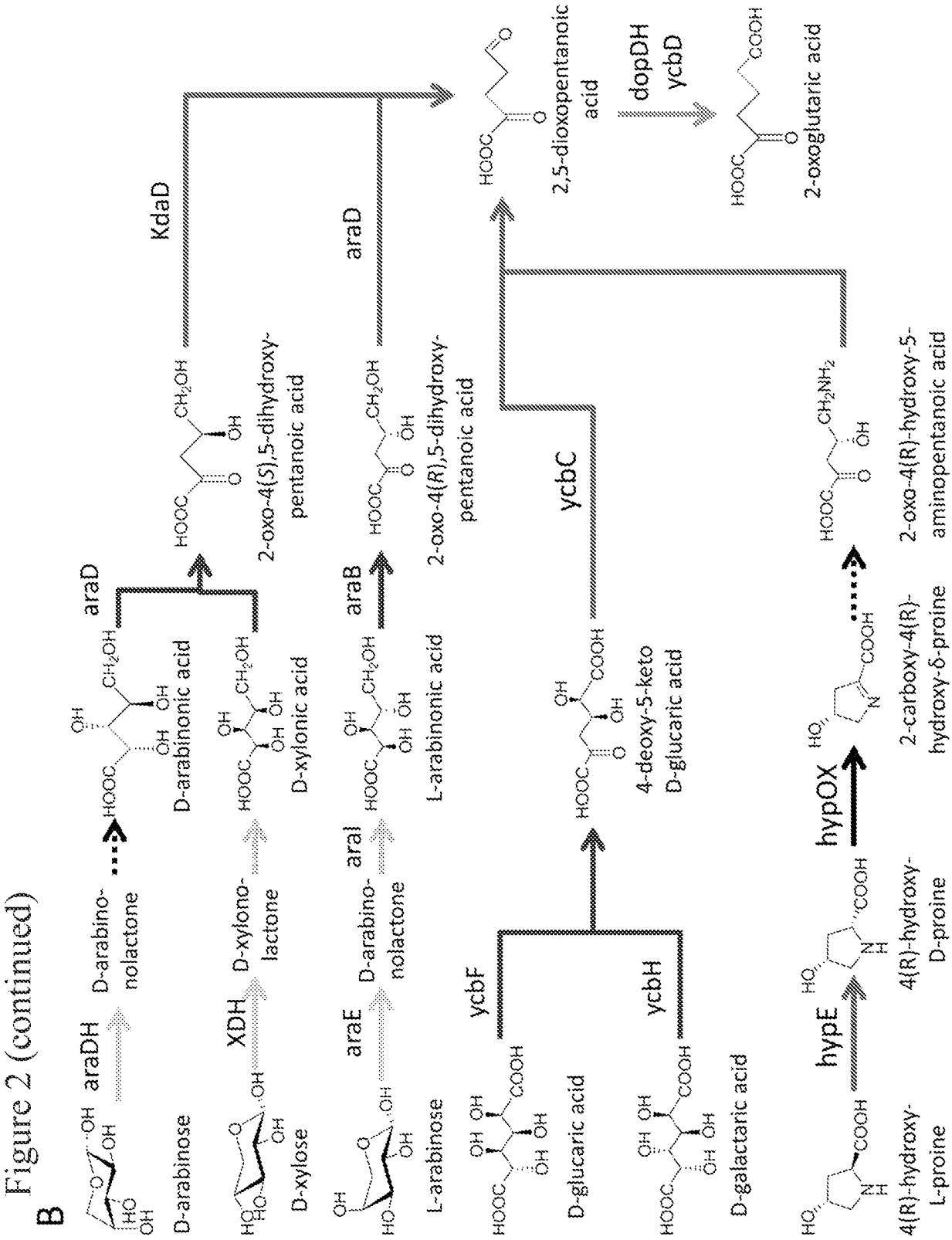


Figure 3

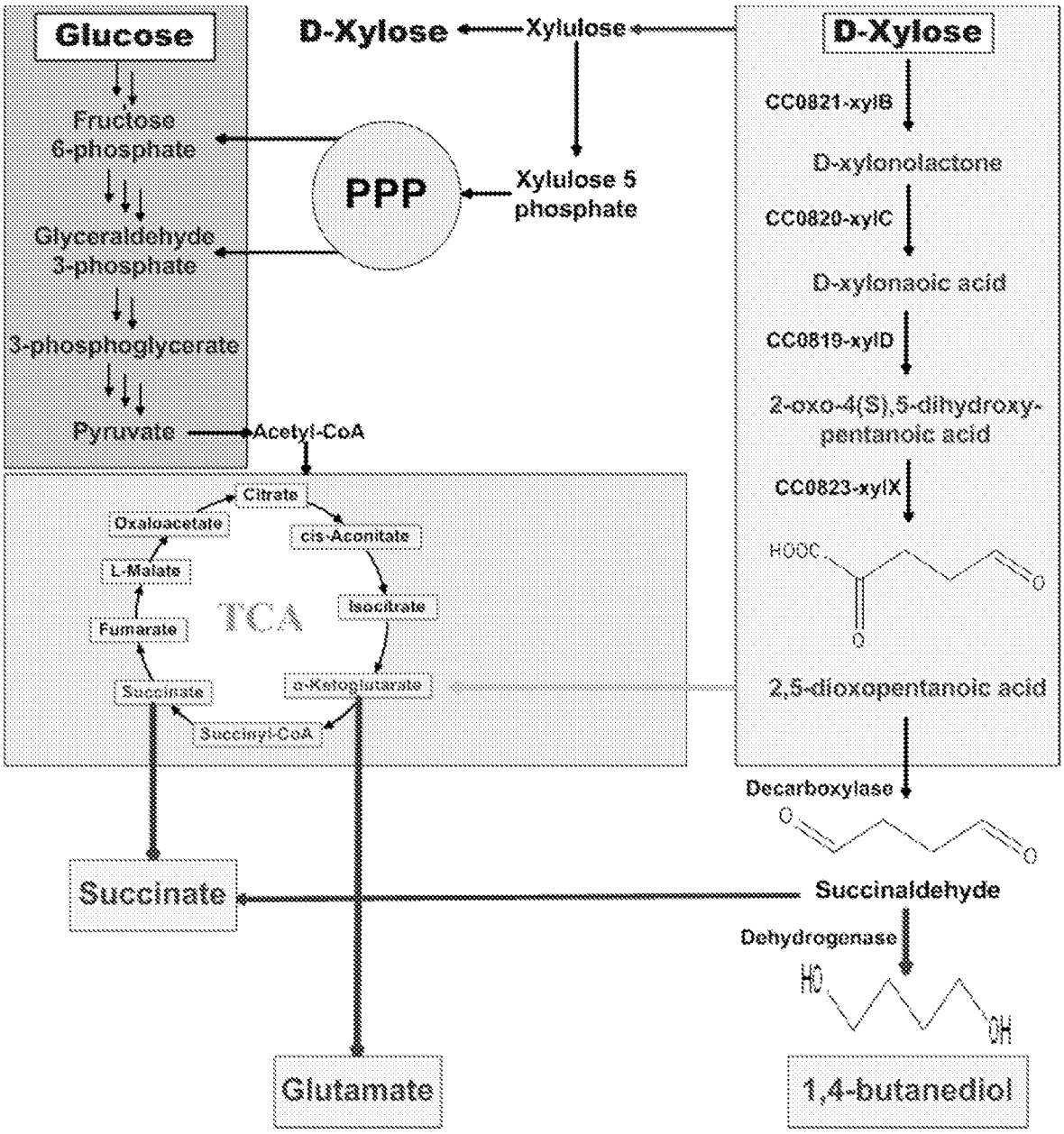
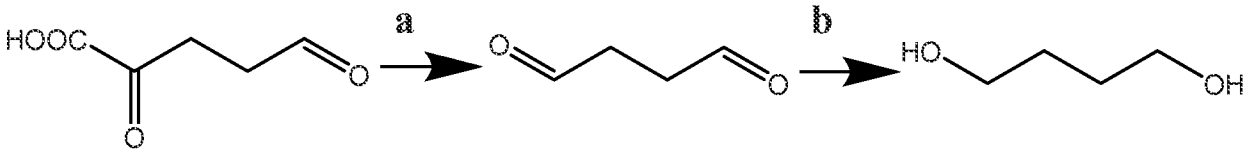
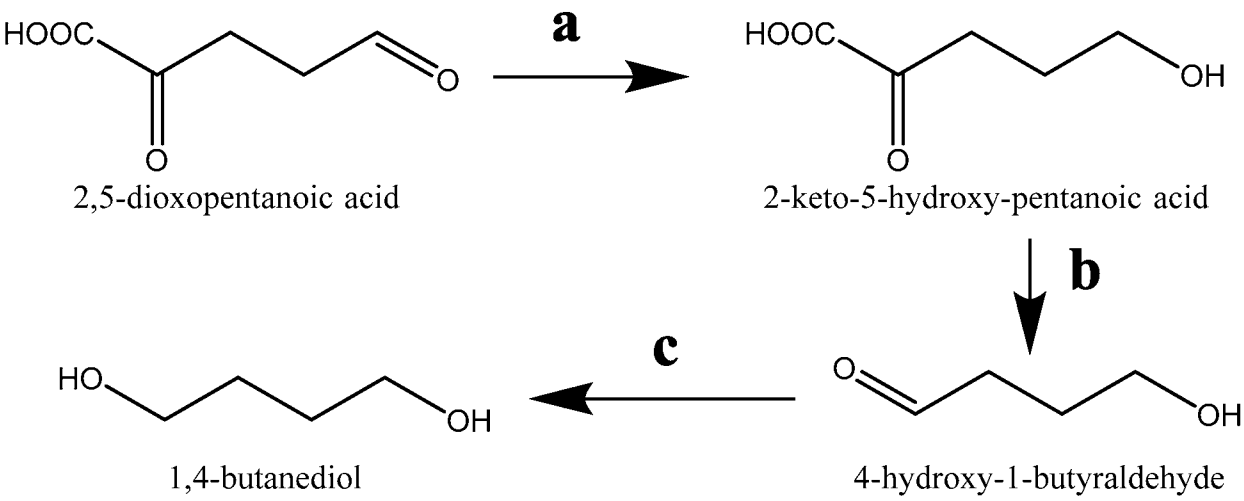

Glucose
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
3-phosphoglycerate
Pyruvate
Acetyl-CoA
D-Xylose
Xylulose
PPP
Xylulose 5 phosphate
Citrate
cis-Aconitate
Isocitrate
α-Ketoglutarate
Succinyl-CoA
Succinate
Fumarate
L-Malate
Oxaloacetate
TCA
D-Xylose
CC0821-xylB
D-xylonolactone
CC0820-xylC
D-xylonaoic acid
CC0819-xylD
2-oxo-4(S),5-dihydroxy-pentanoic acid
CC0823-xylX
2,5-dioxopentanoic acid
Decarboxylase
Succinaldehyde
Dehydrogenase
1,4-butanediol
Succinate
Glutamate

Figure 4



2,5-dioxopentanoic acid succinaldehyde 1,4-butanediol

Figure 5



2,5-dioxopentanoic acid

2-keto-5-hydroxy-pentanoic acid

1,4-butanediol

4-hydroxy-1-butyraldehyde

Figure 6

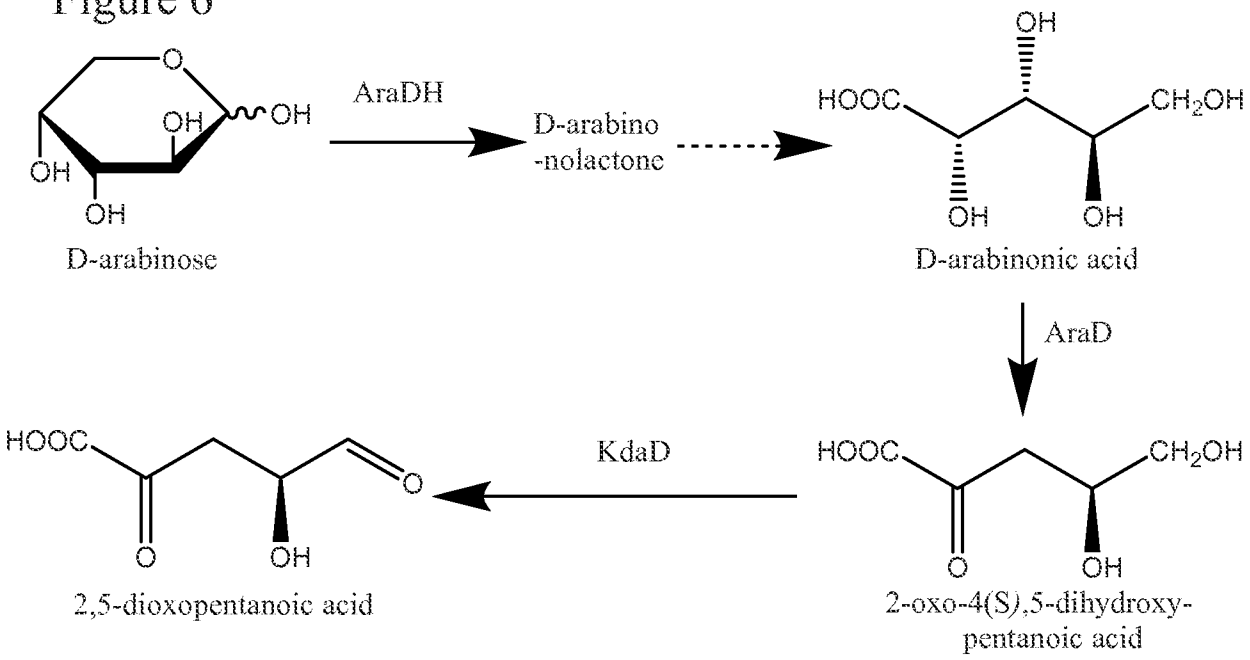


Figure 7

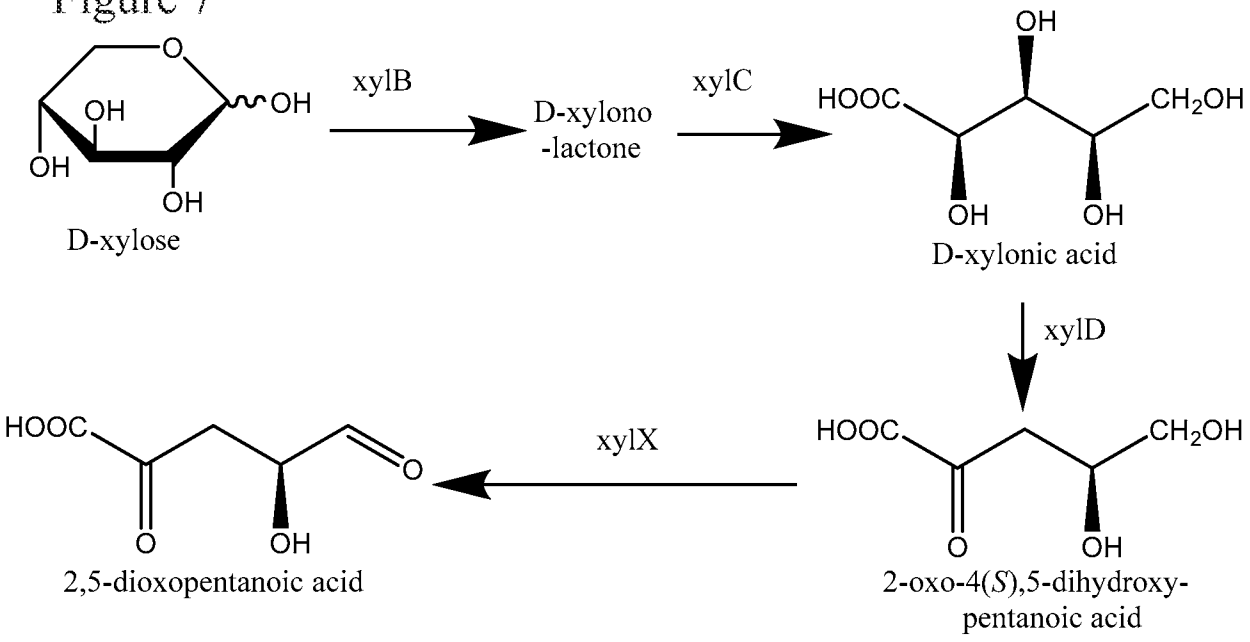


Figure 8

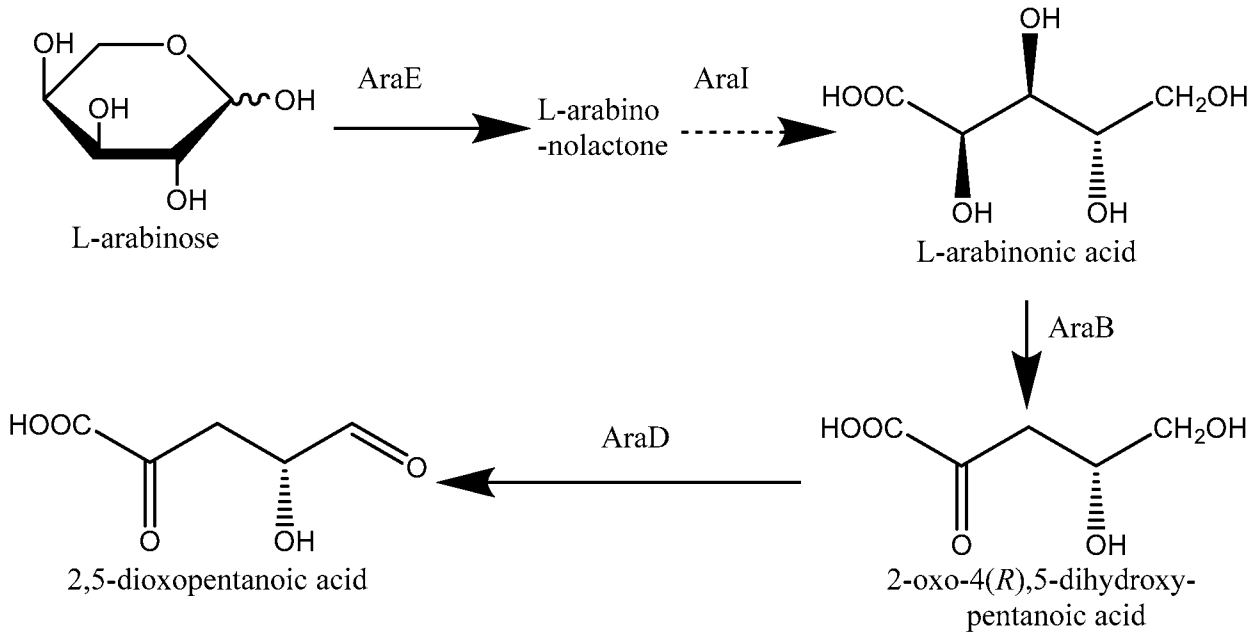


Figure 9

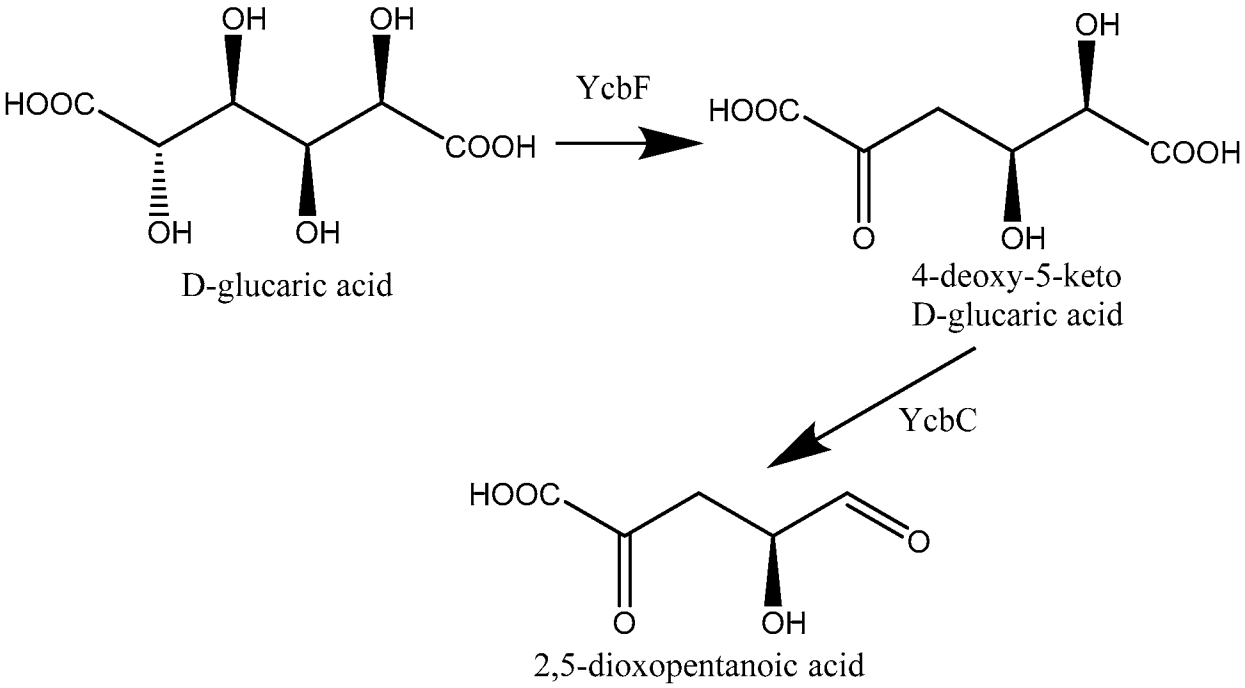


Figure 10

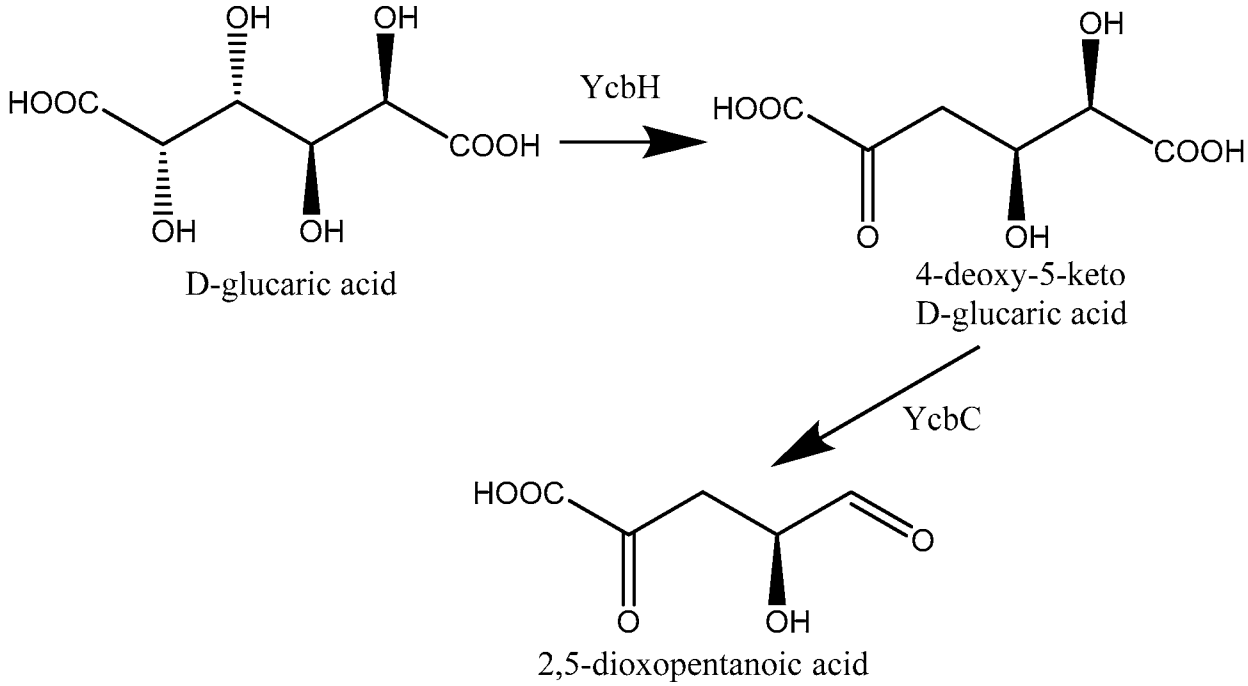


Figure 11

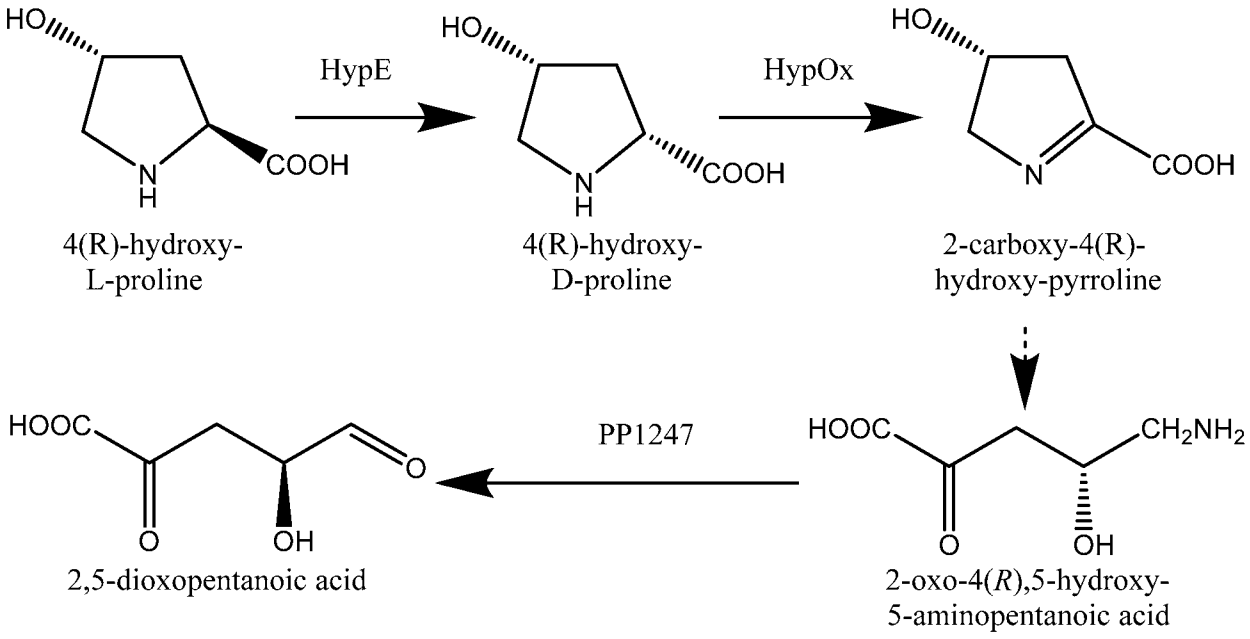


Figure 12

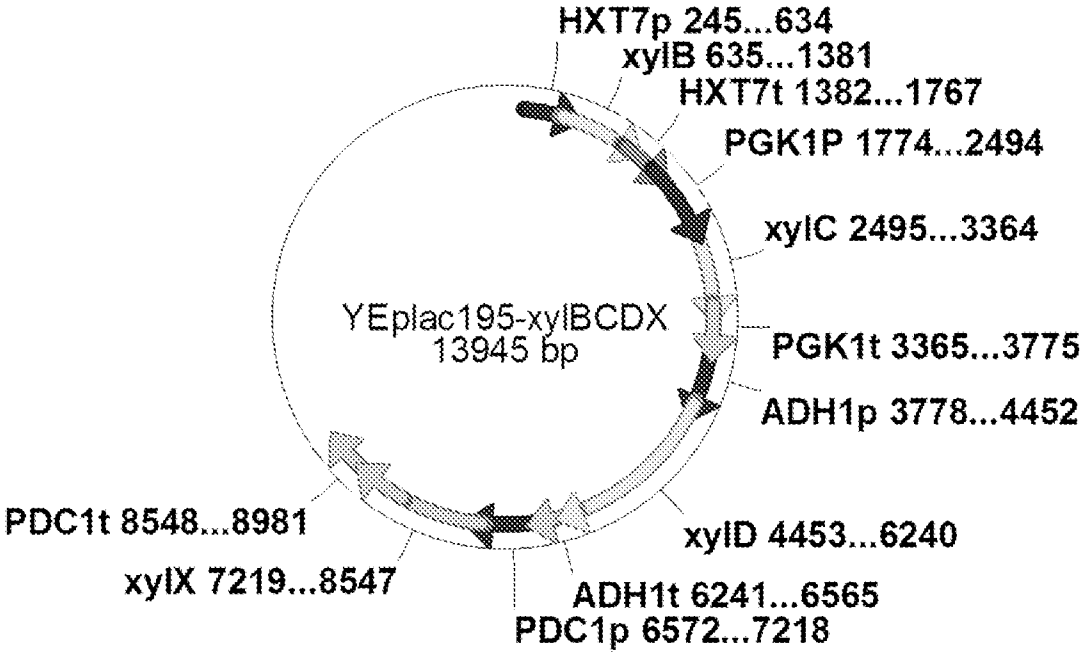


Figure 13

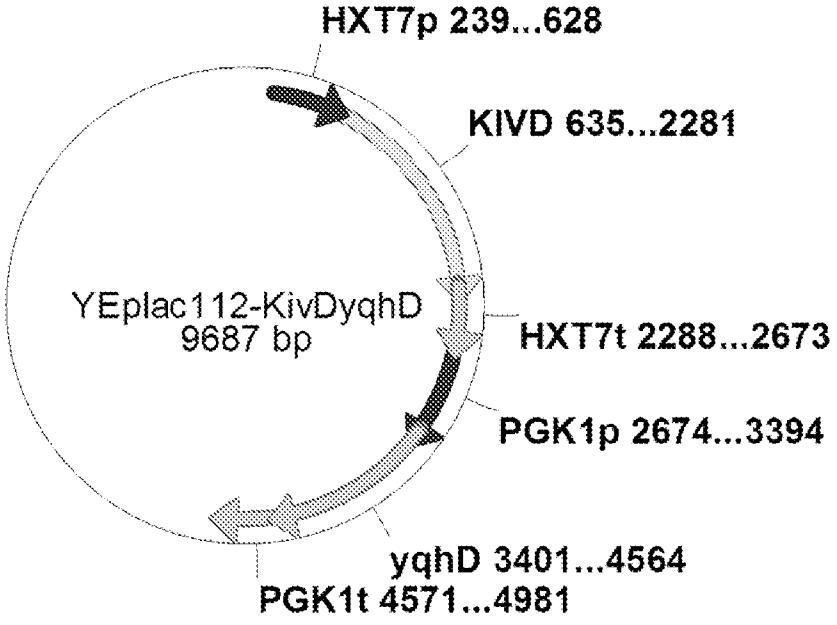
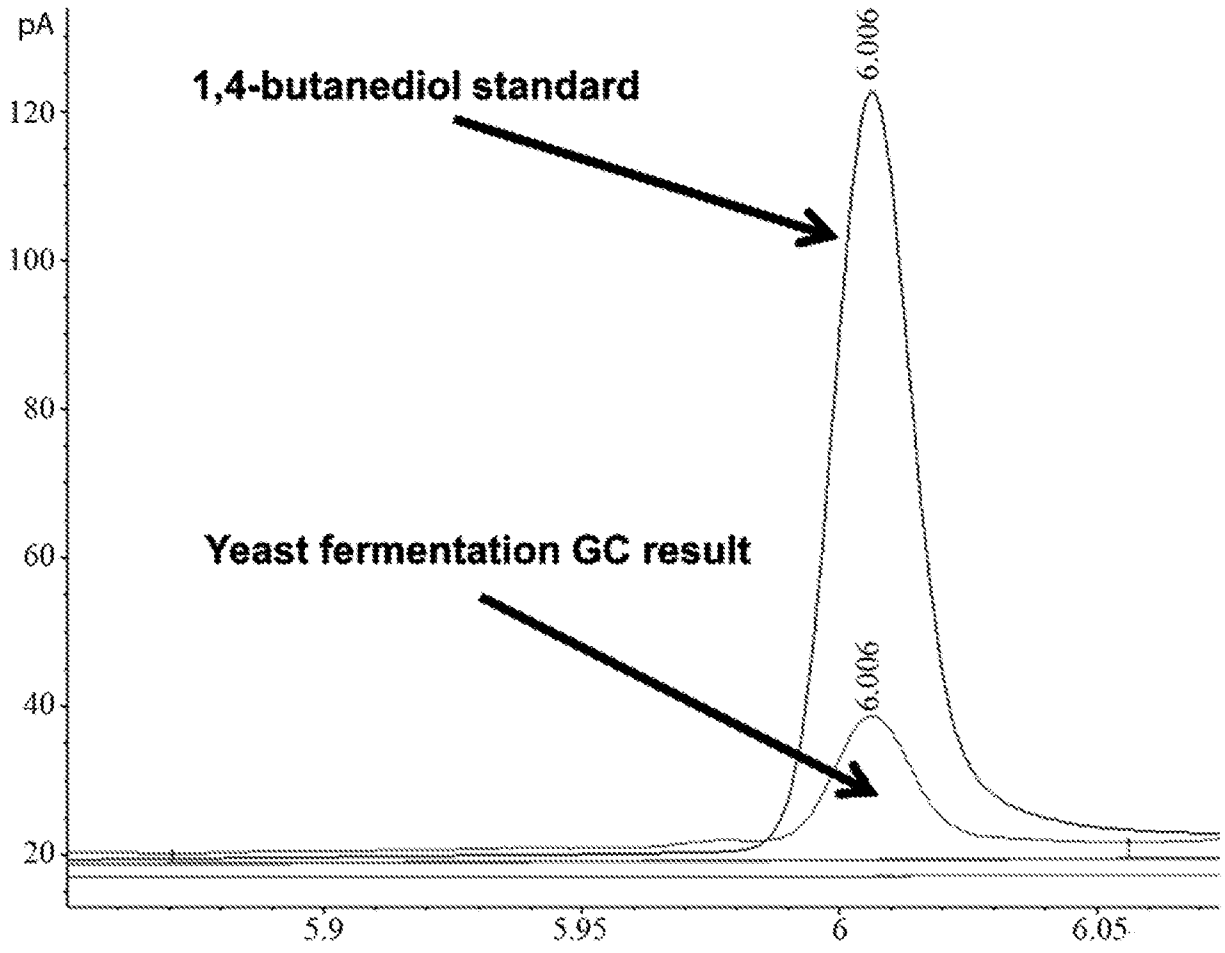


Figure 14


pA
120
100
80
60
40
20
1,4-butanediol standard
Yeast fermentation GC result
6.006
6.006
5.9
5.95
6
6.05

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/076118

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P7/16
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARRY YIM ET AL: "Metabolic engineering of Escherichia coli for direct production of 1,4-butanediol", NATURE CHEMICAL BIOLOGY, NATURE PUB. GROUP , vol. 7, no. 7 1 July 2011 (2011-07-01), pages 445-452, XP002706396, ISSN: 1552-4450, DOI: 10.1038/NCHEMBIO.580 Retrieved from the Internet: URL:http://www.nature.com/nchembio/journal/v7/n7/full/nchembio.580.html [retrieved on 2011-05-22] cited in the application abstract page 451, left-hand column, paragraph 2 ----- -/--	1-134

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2014	31/03/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Herrmann, Klaus

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/076118

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/076118

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/137192 A1 (UNIV CALIFORNIA [US]; LIAO JAMES C [US]; YAN YAJUN [US]) 3 November 2011 (2011-11-03) the whole document -----	1-134

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/076118

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011137192 A1	03-11-2011	US 2013203141 A1	08-08-2013
		WO 2011137192 A1	03-11-2011