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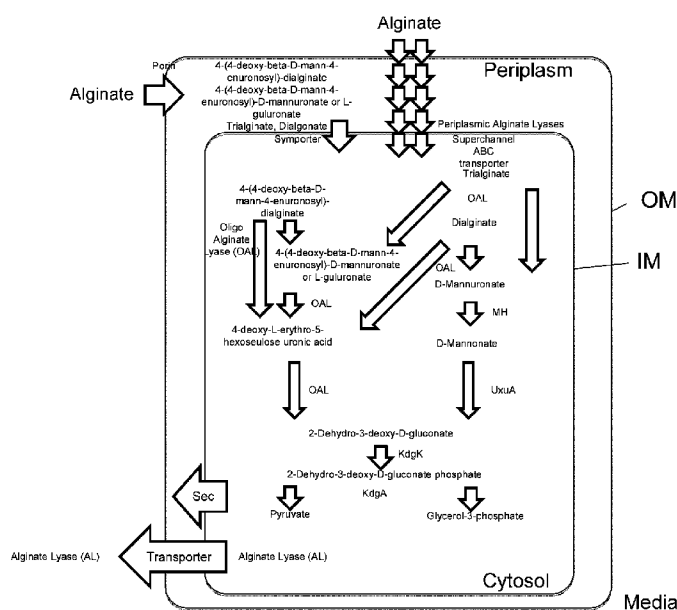


FIG. 2

(57) **Abstract:** Methods, enzymes, recombinant microorganism, and microbial systems are provided for converting polysaccharides, such as those derived from biomass, into suitable monosaccharides or oligosaccharides, as well as for converting suitable monosaccharides or oligosaccharides into commodity chemicals, such as biofuels. Commodity chemicals produced by the methods described herein are also provided. Commodity chemical enriched, refinery-produced petroleum products are also provided, as well as methods for producing the same.

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BIOFUEL PRODUCTION

Priority Information

This application claims priority to U.S. Provisional Application No. 60/977,628, filed on October 4, 2007, which is hereby incorporated by reference in its entirety.

Statement Regarding Sequence Listing

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 150097_40101_SEQUENCE_LISTING.txt. The text file is 519 KB, was created on October 3, 2008, and is being submitted electronically via EFS-Web.

Technical Field

The present application relates generally to the use of microbial and chemical systems to convert biomass to commodity chemicals, such as biofuels/biopetrols.

Background

Petroleum is facing declining global reserves and contributes to more than 30% of greenhouse gas emissions driving global warming. Annually 800 billion barrels of transportation fuel are consumed globally. Diesel and jet fuels account for greater than 50% of global transportation fuels.

Significant legislation has been passed, requiring fuel producers to cap or reduce the carbon emissions from the production and use of transportation fuels.

Fuel producers are seeking substantially similar, low carbon fuels that can be blended and distributed through existing infrastructure (*e.g.*, refineries, pipelines, tankers).

Due to increasing petroleum costs and reliance on petrochemical feedstocks, the chemicals industry is also looking for ways to improve margin and price stability, while reducing its environmental footprint. The chemicals industry is striving to develop greener products that are more energy, water, and CO₂ efficient than current products. Fuels produced from biological sources, such as biomass, represent one aspect of process.

Presents method for converting biomass into biofuels focus on the use of lignocellulosic biomass, and there are many problems associated with using this process. Large-scale cultivation of lignocellulosic biomass requires substantial amount of cultivated land, which can be only achieved by replacing food crop production with energy crop production, deforestation, and by recultivating currently uncultivated land. Other problems include a decrease in water availability and quality and an increase in the use of pesticides and fertilizers.

The degradation of lignocellulosic biomass using biological systems is a very difficult challenge due to its substantial mechanistic strength and the complex chemical components. Approximately thirty different enzymes are required to fully convert lignocellulose to monosaccharides. The only available alternate to this complex approach requires a substantial amount of heat, pressure, and strong acids. The art therefore needs an economic and technically simple process for converting biomass into hydrocarbons for use as biofuels or biopetrols.

Brief Description of the Drawings

Figure 1 shows the *Vibrio splendidus* genomic region of the fosmid clone described in Example 1. Genes are indicated with orange arrows. Labels show the numerical gene indices and the predicted function of the proteins.

Figure 2 illustrates the pathways involved in certain embodiment in which *E. coli* may be engineered to grow on alginate as a sole source of carbon.

Figure 3 illustrates the pathways involved in certain embodiment in which *E. coli* may be engineered to grow on pectin as a sole source of carbon.

Figure 4 shows the results of engineered or recombinant *E. coli* growing on alginate as a sole source of carbon (*see* solid circles). *Agrobacterium tumefaciens* cells provide a positive control (*see* hatched circles). The well to the immediate left of the of the *A. tumefaciens* positive control contains DH10B *E. coli* cells, which provide a negative control.

Figure 5 shows the growth of recombinant strain of *E. coli* on galacturonates and pectin. Figure 5A shows the growth of *E. coli* on various lengths of galacturonate after 24hr. The recombinant strain in Figure 5A is the *E. coli* BL21(DE3) strain harboring pTrlogl-kdgR + pBBRGal3P, and the control strain is the BL21(DE3) strain harboring pTrc99A + pBBR1MCS-2, as described in Example 2. Figure 5B shows the growth of recombinant *E. coli* on pectin after 3-4 days. The recombinant strain in Figure 5B is *E. coli* DH5a strain containing pPEL74 (Ctrl) and pPEL74 and pROU2, as described in Example 2.

Figure 6 shows the degradation of alginate to form pyruvate. Figure 6A illustrates a simplified metabolic pathway for alginate degradation and metabolism. Figure 6B shows the results of *in vitro* degradation of alginate to form pyruvate by an enzymatic degradation route. Figure 6C shows the results of *in vitro* degradation of alginate to form pyruvate by a chemical degradation route.

Figure 7 shows the biological activity of various alcohol dehydrogenases isolated from *Agrobacterium tumefaciens* C58. Figure 7A shows DEHU hydrogenase activity as monitored by NADPH consumption, and Figure 7B shows mannuronate hydrogenase activity as monitored by NADPH consumption.

Figure 8 shows the GC-MS chromatogram results for the control sample (Figure 8A) and for isobutyraldehyde, 3-methylpentanol, and 2-methylpentanal production from pBADalsS-ilvCD-leuABCD2 and pTrcBALK (Figure 8B).

Figure 9 shows the GC-MS chromatogram results for the control sample (Figure 9A) and for 4-hydroxyphenylethanol and indole-3-ethanol production from pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK (Figure 9B).

Figure 10 shows the mass spectrometry results for isobutanol (Figure 10A), 3-methylpentanol (Figure 10B), and 2-methylpentanol (Figure 10C).

Figure 11 shows the mass spectrometry results for phenylethanol (Figure 11A), 4-hydroxyphenylethanol (Figure 11B), and indole-3-ethanol (Figure 11C).

Figure 12 shows the biological activity of diol dehydratases. Figure 12A shows the reduction of butyrolin by *ddh1*, *ddh2*, and *ddh3* as monitored by NADH consumption. Figure 12B shows the oxidation activity of *ddh3* towards 1,2-cyclopentanediol and 1,2-cyclohexanediol as measured by NADH production.

Figure 13 summarizes the results of kinetic studies for various substrates in the oxidation reactions catalyzed by the DDH polypeptides. These reactions were NAD⁺ dependent.

Figure 14 shows the nucleotide sequence (Figure 14A) (SEQ ID NO:97) and polypeptide sequence (Figure 14B) (SEQ ID NO:98) of diol dehydrogenase DDH1 isolated from *Lactobacillus brevis* ATCC 367.

Figure 15 shows the nucleotide sequence (Figure 15A) (SEQ ID NO:99) and polypeptide sequence (Figure 15B) (SEQ ID NO:100) of diol dehydrogenase DDH2 isolated from *Pseudomonas putida* KT2440.

Figure 16 shows the nucleotide sequence (Figure 16A) (SEQ ID NO:101) and polypeptide sequence (Figure 16B) (SEQ ID NO:102) of diol dehydrogenase DDH3 isolated from *Klebsiella pneumoniae* MGH78578.

Figure 17 shows the sequential *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This reaction illustrates the sequential conversion of butanal into 5-hydroxy-4-octanone and then 4,5-octanediol. Figure 17A shows the detection of butyrolin (5-hydroxy-4-octanone) at 5.36 minutes, and Figure 17B shows the detection of 4,5-octanediol at 6.49 and 6.65 minutes.

Figure 18 shows the sequential *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of *n*-pentanal into 6-hydroxy-5-decanone and then 5,6-decanediol. Figure 18A shows the detection of valeroiin (6-hydroxy-5-decanone) at 8.22 minutes, and Figure 18B shows the detection of 5,6 decanediol at 9.22 and 9.35 minutes.

Figure 19 shows the sequential *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of 3-methylbutanal into 2,7-dimethyl-5-hydroxy-4-octanone and then 2,7-dimethyl-4,5-octanediol. Figure 19A shows the detection of isoveraloin (2,7-dimethyl-5-hydroxy-4-octanone) at 6.79 minutes, and Figure 19B shows the detection of 2,7-dimethyl-4,5-octanediol at 7.95 and 8.15 minutes.

Figure 20 shows the sequential *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of *n*-hexanal into 7-hydroxy-6-dodecanone and then 6,7-dodecanediol. Figure 20A shows the detection of hexanoin (7-hydroxy-6-decanone) at

10.42 minutes, and Figure 20B shows the detection of 6,7 dodecanediol at 10.89 and 10.95 minutes.

Figure 21 shows the sequential *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of 4-methylpentanal into 2,9-dimethyl-6-hydroxy-5-decanone and then 2,9-dimethyl-5,6-decanediol. Figure 21A shows the detection of isohexanoin (2,9-Dimethyl-6-hydroxy-5-decanone) at 9.45 minutes, and Figure 21B shows the detection of 2,9-dimethyl-5,6-decanediol at 10.38 and 10.44 minutes.

Figure 22 shows the *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the conversion of n-octanal into 9-hydroxy-8-hexadecanone by showing the detection of octanoin (9-hydroxy-8-hexadecanone) at 12.35 minutes.

Figure 23 shows the *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the conversion of acetaldehyde into 3-hydroxy-2-butanone by showing the detection of acetoin (3-hydroxy-2-butanone) at $rt=0.91$ minutes.

Figure 24 shows the sequential *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of *n*-propanal into 4-hydroxy-3-hexanone and then 3,4-hexanediol. Figure 24A shows the detection of propioid (4-hydroxy-3-hexanone) at

rt=2.62 minutes, and Figure 24B shows the detection of 3,4-hexanediol at rt=3.79 minutes.

Figure 25 the *in vivo* biological activity of a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a *ddh* gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (DDH3). This Figure illustrates the conversion of phenylacetoaldehyde into 1,4-diphenyl-3-hydroxy-2-butanone by showing the detection of 1,4-diphenyl-3-hydroxy-2-butanone at rt=13.66 minutes.

Figure 26 shows the sequential biological activity of a diol dehydrogenase *ddh* from *Klebsiella pneumoniae* MGH 78578 (DDH3) and a diol dehydratase *pduCDE* from *Klebsiella pneumoniae* MGH 78578. Figure 26A shows GC-MS data which confirms the presence of 4,5-octanediol in the sample extraction, which is the expected product resulting from the reduction of butyrolin by *ddh3*. Figure 26B shows GC-MS data confirming the presence of 4-octanone in the sample extraction, which is the expected product resulting from the sequential dehydrogenation of butyrolin and dehydration of 4,5-octanediol by *ddh3* and *pduCDE*, respectively.

Figure 27 shows the sequential biological activity of a diol dehydrogenase *ddh* from *Klebsiella pneumoniae* MGH 78578 (DDH3) and a diol dehydratase *pduCDE* from *Klebsiella pneumoniae* MGH 78578. Figures 27A and 27B show comparisons between the sample extraction gas chromatograph/mass spectrum and the 4-octanone standard gas chromatograph/mass spectrum, confirming that 4-octanone was produced from butyrolin using the enzymes diol dehydrogenase (*ddh3*) and a diol dehydratase (*pduCDE*).

Figure 28 shows the nucleotide sequence (Figure 28A) (SEQ ID NO:103) and polypeptide sequence (Figure 28B) (SEQ ID NO:104) of a diol dehydratase large subunit (*pduC*) isolated from *Klebsiella pneumoniae* MGH78578.

Figure 29 shows the nucleotide sequence (Figure 29A) (SEQ ID NO:105) and polypeptide sequence (Figure 29B) (SEQ ID NO:106) of a diol dehydratase medium subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduD*), in addition to the nucleotide sequence (Figure 29C) (SEQ ID NO:107) and polypeptide sequence (Figure 29D) (SEQ ID NO:108) of a diol dehydratase small subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduE*).

Figure 30 shows the oxidation of 4-octanol by secondary alcohol dehydrogenases as monitored by NADH production (Figure 30A) and NADPH production (Figure 30B).

Figure 31 shows the oxidation of 4-octanol by secondary alcohol dehydrogenases as monitored by NADH production (Figure 31A) and NADPH production (Figure 31B).

Figure 32 shows the oxidation of 2,7-dimethyl octanol by secondary alcohol dehydrogenases as monitored by NADH production (Figure 32A) and NADPH production (Figure 32B).

Figure 33 shows the oxidation and reduction activity of 2ADH11 and 2ADH16. Figure 33A shows the reduction of 2,7-dimethyl-4-octanone as measured by NADPH consumption. Figure 33B shows the reduction of 2,7-dimethyl-4-octanone, 4-octanone, and cyclopentanone.

Figure 34 shows the oxidation and reduction of cyclopentanol by secondary alcohol dehydrogenases. Figure 34A shows the oxidation of cyclopentanol as monitored by NADH or NADPH formation. Figure 34B shows the reduction of cyclopentanol as monitored by NADPH consumption.

Figure 35 shows the calculated rate constants for the illustrated reduction reactions for each substrate catalyzed by secondary alcohol dehydrogenase ADH-16 (SEQ ID NO:138).

Figure 36 shows the calculated rate constants for the illustrated oxidation reactions for each substrate catalyzed by secondary alcohol dehydrogenase ADH-16 (SEQ ID NO:138).

Figure 37 shows a list of alginate lyases genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

Figure 38 shows a list of pectate lyase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

Figure 39A shows a list of rhamnogalacturonan lyase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein. Figure 39B shows a list of rhamnogalacturonate hydrolase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

Figure 40 shows a list of pectin methyl esterase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

Figure 41 shows a list of pectin acetyl esterase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

Figure 42 shows the production of 2-phenyl ethanol (Figure 42A), 2-(4-hydroxyphenyl) ethanol (Figure 42B), and 2-(indole-3-)ethanol (Figure 42C) at 24 hours from the recombinant microorganisms described in Example 4, which comprise functional 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-)ethanol biosynthesis pathways.

Figure 43 shows the GC-MS chromatogram results that confirm the production of 2-phenyl ethanol (Figure 43B) at one week from the recombinant microorganisms described in Example 4 (pBADpheA-aroLAC-aroG-tktA-aroBDE and pTrcBALK). Figure 43A shows the negative control cells (pBAD33 and pTrc99A).

Figure 44 shows the GC-MS chromatogram results that confirm the production of 2-(4-hydroxyphenyl) ethanol (9.36 min) and 2-(indole-3) ethanol (10.32 min) at one week from the recombinant microorganisms described in Example 4 (pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK).

Figure 45 confirms both the formation of 1-propanal from 1,2-propanediol (Figure 45A), and the formation of 2-butanone from meso-2,3-butanediol (Figure 45B), both of which were catalyzed *in vitro* by an isolated B12 independent diol dehydratase, as described in Example 9.

Figure 46A shows the *in vivo* production of 1-propanol from 1,2-propanediol. Figure 46B shows the *in vivo* production of 2-butanol from meso-2,3-butanediol. Figure 46C shows the *in vivo* production of cyclopentanone from trans-1,2-cyclopentanediol. These experiments were performed as described in Example 9.

Figure 47 shows the results of the TBA assay, as performed in Example 10. The left tube in Figure 47 represents media taken from an overnight culture of cells expressing Vs24254, showing secretion of an alginate lyase, while the right hand tube shows the TBA reaction using media from cells expressing Vs24259 (negative control). The lack of pink coloration in the negative control indicates that little or no cleavage of the alginate polymer has occurred.

Figure 48 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized benzaldehyde lyase (BAL) catalyzed the *in vivo* production of 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone from a ligation reaction between acetaldehyde and propionaldehyde (Figure 48A), and catalyzed the *in vivo* production of 4-hydroxy-3-heptanone and 3-hydroxy-4-heptanone from a ligation reaction between propionaldehyde and butyraldehyde (Figure 48B).

Figure 49 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram

results show that codon-optimized BAL catalyzed the *in vivo* production of 3-hydroxy-2-heptanone from a ligation reaction between acetaldehyde and pentanal (Figure 49A), and catalyzed the *in vivo* production of 4-hydroxy-3-octanone and 3-hydroxy-4-octanone from a ligation reaction between pentanal and propionaldehyde (Figure 49B).

Figure 50 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the *in vivo* production of 5-hydroxy-4-nonanone from ligation reaction between butyraldehyde and pentanal (Figure 50A), and catalyzed the *in vivo* production of 2-methyl-5-hydroxy-4-decanone and 2-methyl-4-hydroxy-5-decanone from ligation reaction between hexanal and 3-methylbutyraldehyde (Figure 50B).

Figure 51 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the *in vivo* production of 6-methyl-3-hydroxy-2-heptanone from ligation reaction between acetaldehyde and 4-methylhexanal (Figure 51A), and catalyzed the *in vivo* production of 7-methyl-4-hydroxy-3-octanone from a ligation reaction between 4-methylhexanal and propionaldehyde (Figure 51B).

Figure 52 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the *in vivo* production of 8-methyl-5-hydroxy-4-nonanone from ligation reaction between 4-methylhexanal and butyraldehyde (Figure 52A), and catalyzed the *in vivo* production of 3-hydroxy-2-decanone from a ligation reaction between acetaldehyde and octanal (Figure 52B).

Figure 53 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the *in vivo* production of 4-hydroxy-3-undecanone from ligation reaction between octanal and propionaldehyde (Figure

53A), and catalyzed the *in vivo* production of 5-hydroxy-4-dodecanone from a ligation reaction between octanal and butyraldehyde (Figure 53B).

Figure 54 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the *in vivo* production of 6-hydroxy-5-tridecanone (Figure 54A) from ligation reaction between octanal and pentanal, and catalyzed the *in vivo* production of 2-methyl-5-hydroxy-4-dodecanone and 2-methyl-4-hydroxy-5-decanone from a ligation reaction between octanal and 3-methylbutyraldehyde (Figure 54B).

Figure 55 shows the *in vivo* biological activity of a C-C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the *in vivo* production of 2-methyl-6-hydroxy-5-tridecanone from a ligation reaction between octanal and 4-methylpentanal.

Figure 56 shows the growth of recombinant *E. coli* on alginate as a sole source of carbon (Figure 56A), as described in Example 10. Growth on glucose (Figure 56B) provides a positive control. The cells were transformed with either no plasmid (BL21 – negative control), one plasmid (e.g., Da or 3a), or two plasmids (e.g., Dk3a and Da3k). The plasmids are indicated by the lower case letter: “a” refers to the pET-DEST42 plasmid backbone and “k” refers to the pENTR/D/TOPO backbone. “D” indicates that the plasmid contains the genomic region Vs24214-24249, while “3” indicates that the plasmid contains the genomic region Vs24189-24209. Thus, Da would be pET-DEST42-Vs24214-24249, Da3k would be pET-DEST42-Vs24214-24249 and pENTR/D/TOPO-Vs24189-24209 and so on. These results show that the combined genomic regions Vs24214-24249 and Vs24189-24209 are sufficient to confer on *E. coli* the ability to grow on alginate as a sole source of carbon.

Figure 57 shows the production of ethanol by *E. coli* growing on alginate, as performed in Example 11. *E. coli* was transformed with either pBBRPdc-

AdhA/B or pBBRPdc-AdhA/B + 1.5 FOS and allowed to grow in m9 media containing alginate.

Brief Summary

Embodiments of the present invention include methods for converting a polysaccharide to a commodity chemical, comprising (a) contacting the polysaccharide, wherein the polysaccharide is optionally derived from biomass, with a polysaccharide degrading or depolymerizing metabolic system, wherein the metabolic system is selected from; (i) enzymatic or chemical catalysis, and (ii) a microbial system, wherein the microbial system comprises a recombinant microorganism, wherein the recombinant microorganism comprises one or exogenous genes that allow it to grow on the polysaccharide as a sole source of carbon, thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide; and (b) contacting the suitable monosaccharide or oligosaccharide with commodity chemical biosynthesis pathway, wherein the commodity chemical biosynthesis pathway comprises an aldehyde or ketone biosynthesis pathway, thereby converting the polysaccharide to the commodity chemical.

In certain aspects, the biomass is selected from marine biomass and vegetable/fruit/plant biomass. In certain aspects, the marine biomass is selected from kelp, giant kelp, sargasso, seaweed, algae, marine microflora, microalgae, and sea grass. In certain aspects, the vegetable/fruit/plant biomass comprises plant peel or pomace. In certain aspects, the vegetable/fruit/plant biomass is selected from citrus, potato, tomato, grape, gooseberry, carrot, mango, sugar-beet, apple, switchgrass, wood, and stover.

In certain aspects, the polysaccharide is selected from alginate, agar, carrageenan, fucoidan, pectin, polygalacturonate, cellulose, hemicellulose, xylan, arabinan, and mannan. In certain aspects, the suitable monosaccharide or oligosaccharide is selected from 2-keto-3-deoxy D-gluconate (KDG), D-mannitol, guluronate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose.

In certain aspects, the commodity chemical is selected from methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butanediol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl) ethanol, , 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanediol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanedione, indolyethane, indolyethene, 2-(indole-3-yl)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pentene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione , 4-methyl-1-phenyl-3-

hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 4-methyl-1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl-1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-

hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2,3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3,4-hexanedione, 2,5-dimethyl-3-hydroxy-4-hexanone, 5-methyl-1-phenylhexane, 4-methyl-1-phenylhexane, 5-methyl-1-phenyl-1-hexene, 5-methyl-1-phenyl-2-hexene, 5-methyl-1-phenyl-3-hexene, 4-methyl-1-phenyl-1-hexene, 4-methyl-1-phenyl-2-hexene, 4-methyl-1-phenyl-3-hexene, 5-methyl-1-phenyl-2-hexanol, 5-methyl-1-phenyl-3-hexanol, 4-methyl-1-phenyl-2-hexanol, 4-methyl-1-phenyl-3-hexanol, 5-methyl-1-phenyl-2-hexanone, 5-methyl-1-phenyl-3-hexanone, 4-methyl-1-phenyl-2-hexanone, 4-methyl-1-phenyl-3-hexanone, 5-methyl-1-phenyl-2,3-hexanediol, 4-methyl-1-phenyl-2,3-hexanediol, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-3-hydroxy-2-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 5-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)hexane, 5-methyl-1-(4-hydroxyphenyl)-1-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexene, 5-methyl-1-(4-hydroxyphenyl)-3-hexene, 4-methyl-1-(4-hydroxyphenyl)-1-hexene, 4-methyl-1-(4-hydroxyphenyl)-2-hexene, 4-methyl-1-(4-hydroxyphenyl)-3-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexanol, 5-methyl-1-(4-hydroxyphenyl)-3-hexanol, 4-methyl-1-(4-hydroxyphenyl)-2-hexanol, 4-methyl-1-(4-hydroxyphenyl)-3-hexanol, 5-methyl-1-(4-hydroxyphenyl)-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(indole-3)hexane, 5-methyl-1-(indole-3)-1-hexene, 5-methyl-1-(indole-3)-2-hexene, 5-methyl-1-(indole-3)-3-hexene, 4-

methyl-1-(indole-3)-1-hexene, 4-methyl-1-(indole-3)-2-hexene, 4-methyl-1-(indole-3)-3-hexene, 5-methyl-1-(indole-3)-2-hexanol, 5-methyl-1-(indole-3)-3-hexanol, 4-methyl-1-(indole-3)-2-hexanol, 4-methyl-1-(indole-3)-3-hexanol, 5-methyl-1-(indole-3)-2-hexanone, 5-methyl-1-(indole-3)-3-hexanone, 4-methyl-1-(indole-3)-2-hexanone, 4-methyl-1-(indole-3)-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanediol, 4-methyl-1-(indole-3)-2,3-hexanediol, 5-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 5-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 4-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 4-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanedione, 4-methyl-1-(indole-3)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2,5-dimethyl-2-heptene, 2,5-dimethyl-3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2,6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone,

2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol, nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-

hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedione, undecane, 1-undecene, 1-undecanol, undecanal, undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decylidene, 1-dodecanol, dodecanal, dodecanoate, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1, 3-propanediol, 4-hydroxybutanal, 1, 4-butanediol, 3-hydroxy-2-butanone, 2, 3-butanediol, 1,5-pentane diol, homocitrate, homoisocitrate, β -hydroxy adipate, glutarate, glutaraldehyde, glutaraldehyde, 2-hydroxy-1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2,3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino pentaldehyde, 1,10-diaminodecane, 1,10-diamino-5-decene, 1,10-diamino-5-hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-phenylbutane, 1-(4-hydroxyphenyl)-4-phenyl-1-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butanol, 1-(4-hydroxyphenyl)-4-phenyl-2-butanone, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydroxyphenyl)-4-phenyl-3-hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-

butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3-)butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl)-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetaldehyde, 1,4-di(indole-3-)butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, and phosphate.

Certain embodiments of the present invention include methods for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising: (a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a microbial system for a time sufficient to convert the polysaccharide to a suitable monosaccharide or oligosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase and/or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide.

Certain embodiments of the present invention include methods for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising: (a) contacting the polysaccharide, wherein the polysaccharide is optionally

obtained from biomass, with a chemical or enzymatic catalysis pathway for a time sufficient to convert the polysaccharide to a first monosaccharide or oligosaccharide; and (b) contacting the first monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert the first monosaccharide or oligosaccharide to the suitable monosaccharide or oligosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to the suitable monosaccharide or oligosaccharide.

In certain aspects, the lyase is selected from an alginate lyase, a pectate lyase, a polymannuronate lyase, a polygluronate lyase, a polygalacturonate lyase and a rhamnogalacturonate lyase. In certain aspects, the hydrolase is selected from an alginate hydrolase, a rhamnogalacturonate hydrolase, a polymannuronate hydrolase, a pectin hydrolase, and a polygalacturonate hydrolase. In certain aspects, the transporter is selected from an ABC transporter, a symporter, and an outer membrane porin. In certain aspects, the ABC transporter is selected from Atu3021, Atu3022, Atu3023, Atu3024, algM1, algM2, AlgQ1, AlgQ2, AlgS, OG2516_05558, OG2516_05563, OG2516_05568, OG2516_05573, TogM, TogN, TogA, TogB, and functional variants thereof. In certain aspects, the symporter is selected from V12B01_24239 (SEQ ID NO:26), V12B01_24194 (SEQ ID NO:8), and TogT, and functional variants thereof. In certain aspects, the outermembrane porin comprises a porin selected from V12B01_24269, KdgM, and KdgN, and functional variants thereof.

Certain embodiments include a recombinant microorganism that is capable of growing on a polysaccharide as a sole source of carbon, wherein the polysaccharide is selected from alginate, pectin, tri-galacturonate, di-galacturonate, cellulose, and hemi-cellulose. In certain aspects, the polysaccharide is alginate. In

certain aspects, the polysaccharide is pectin. In certain aspects, the polysaccharide is tri-galacturonate.

Certain embodiments include a recombinant microorganism, comprising (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase. In certain aspects, the microorganism is capable of growing on a polysaccharide as a sole source of carbon. In certain aspects, the polysaccharide is selected from alginate, pectin, and tri-galacturonate.

Certain embodiments include methods for converting a suitable monosaccharide or oligosaccharide to a first commodity chemical comprising, (a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises a recombinant microorganism, wherein the microorganism comprises a commodity chemical biosynthesis pathway, thereby converting the suitable monosaccharide or oligosaccharide to the first commodity chemical. In certain aspects, the commodity chemical pathway comprises one or more genes encoding an aldehyde or ketone biosynthesis pathway.

In certain aspects, the aldehyde or ketone biosynthesis pathway is selected from one or more of an acetaldehyde, a propionaldehyde, a butyraldehyde, an isobutyraldehyde, a 2-methyl-butyraldehyde, a 3-methyl-butyraldehyde, a 2-phenyl acetaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, a 2-Indole-3-acetaldehyde, a glutaraldehyde, a 5-amino-pentaldehyde, a succinate semialdehyde, and a succinate 4-hydroxyphenyl acetaldehyde biosynthesis pathway. In certain aspects, the aldehyde or

ketone biosynthesis pathway comprises an acetoaldehyde biosynthesis pathway and a biosynthesis pathway selected from a propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a propionaldehyde biosynthesis pathway and a biosynthesis pathway selected from a butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, and phenylacetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from an isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises an isobutyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 2-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 3-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 2-phenyl acetoaldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-(4-hydroxyphenyl) acetaldehyde and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 2-(4-hydroxyphenyl) acetaldehyde biosynthesis pathway and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the first commodity chemical is further enzymatically and/or chemically reduced and dehydrated to a second commodity chemical.

Certain embodiments include methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical comprising, (a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises; (i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and (ii) one or more genes encoding and expressing an aldehyde reductase, thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical. In certain aspects, the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC). In certain aspects, the IPDC comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 312. In certain aspects, the aldehyde reductase enzyme is a phenylacetaldehyde reductase (PAR). In certain aspects, the PAR comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 313. In certain aspects, the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol.

Certain embodiments include a recombinant microorganism, comprising (i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and (ii) one or more genes encoding and expressing an aldehyde reductase. In certain aspects, the aldehyde biosynthesis pathway further comprises one or more genes encoding and expressing an enzyme

selected from a CoA-linked aldehyde dehydrogenase, an aldehyde dehydrogenase, and an alcohol dehydrogenase. In certain aspects, the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC). In certain aspects, the aldehyde reductase enzyme is a phenylacetaldehyde reductase (PAR). In certain aspects, the microorganism is capable of converting a suitable monosaccharide or oligosaccharide to a commodity chemical. In certain aspects, the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol.

Certain embodiments include a recombinant microorganism, wherein the microorganism comprises reduced ethanol production capability compared to a wild-type microorganism. In certain aspects, the microorganism comprises a reduction or inhibition in the conversion of acetyl-coA to ethanol. In certain aspects, the recombinant microorganism comprises a reduction of an ethanol dehydrogenase, thereby providing a reduced ethanol production capability. In certain aspects, the ethanol dehydrogenase is an adhE, homolog or variant thereof. In certain aspects, the microorganism comprises a deletion or knockout of an adhE, homolog or variant thereof. In certain aspects, the recombinant microorganism comprises one or more deletions or knockouts in a gene encoding an enzyme selected from an enzyme that catalyzes the conversion of acetyl-coA to ethanol, an enzyme that catalyzes the conversion of pyruvate to lactate, an enzyme that catalyzes the conversion of fumarate to succinate, an enzyme that catalyzes the conversion of acetyl-coA and phosphate to coA and acetyl phosphate, an enzyme that catalyzes the conversion of acetyl-coA and formate to coA and pyruvate, and an enzyme that catalyzes the conversion of alpha-keto acid to branched chain amino acids.

Certain embodiments include wherein the microbial systems or recombinant microorganisms described herein comprise a microorganism selected from *Acetobacter aceti*, *Achromobacter*, *Acidiphilium*, *Acinetobacter*, *Actinomadura*, *Actinoplanes*, *Aeropyrum pernix*, *Agrobacterium*, *Alcaligenes*, *Ananas comosus (M)*, *Arthrobacter*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus melleus*, *Aspergillus pulverulentus*, *Aspergillus saitoi*, *Aspergillus sojae*, *Aspergillus usamii*, *Bacillus*

alcalophilus, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bifidobacterium*, *Brevibacillus brevis*, *Burkholderia cepacia*, *Candida cylindracea*, *Candida rugosa*, *Carica papaya* (L), *Cellulosimicrobium*, *Cephalosporium*, *Chaetomium erraticum*, *Chaetomium gracile*, *Clostridium*, *Clostridium butyricum*, *Clostridium acetobutylicum*, *Clostridium thermocellum*, *Corynebacterium (glutamicum)*, *Corynebacterium efficiens*, *Escherichia coli*, *Enterococcus*, *Erwina chrysanthemi*, *Gliconobacter*, *Gluconacetobacter*, *Haloarcula*, *Humicola insolens*, *Humicola nsolens*, *Kitasatospora setae*, *Klebsiella*, *Klebsiella oxytoca*, *Kluyveromyces*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Kocuria*, *Lactlactis*, *Lactobacillus*, *Lactobacillus fermentum*, *Lactobacillus sake*, *Lactococcus*, *Lactococcus lactis*, *Leuconostoc*, *Methylocystis*, *Methanolobus siciliae*, *Methanogenium organophilum*, *Methanobacterium bryantii*, *Microbacterium imperiale*, *Micrococcus lysodeikticus*, *Micrococcus luteus*, *Mucor javanicus*, *Mycobacterium*, *Myrothecium*, *Nitrobacter*, *Nitrosomonas*, *Nocardia*, *Papaya carica*, *Pediococcus*, *Pediococcus halophilus*, *Penicillium*, *Penicillium camemberti*, *Penicillium citrinum*, *Penicillium emersonii*, *Penicillium roqueforti*, *Penicillium lilactinum*, *Penicillium multicolor*, *Paracoccus pantotrophus*, *Propionibacterium*, *Pseudomonas*, *Pseudomonas fluorescens*, *Pseudomonas denitrificans*, *Pyrococcus*, *Pyrococcus furiosus*, *Pyrococcus horikoshii*, *Rhizobium*, *Rhizomucor miehei*, *Rhizomucor pusillus* Lindt, *Rhizopus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Rhizopus niveus*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Rhodococcus*, *Sccharomyces cerevisiae*, *Sclerotinia libertina*, *Sphingobacterium multivorum*, *Sphingobium*, *Sphingomonas*, *Streptococcus*, *Streptococcus thermophilus* Y-1, *Streptomyces*, *Streptomyces griseus*, *Streptomyces lividans*, *Streptomyces murinus*, *Streptomyces rubiginosus*, *Streptomyces violaceoruber*, *Streptoverticillium mobaraense*, *Tetragenococcus*, *Thermus*, *Thiosphaera pantotrophica*, *Trametes*, *Trichoderma*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma viride*, *Trichosporon penicillatum*, *Vibrio alginolyticus*, *Xanthomonas*, yeast, *Zygosaccharomyces rouxii*, *Zymomonas*, and *Zymomonas mobilis*.

Certain embodiments include a commodity chemical produced by the methods described herein. Certain aspects include a blended commodity chemical comprising a commodity chemical produced by the methods provided herein and a refinery-produced petroleum product. In certain aspects, the commodity chemical is selected from a C10-C12 hydrocarbon, 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol. In certain aspects, the C10-C12 hydrocarbon is selected from 2,7-dimethyloctane and 2,9-dimethyldecane. In certain aspects, the refinery-produced petroleum product is selected from jet fuel and diesel fuel.

Certain embodiments include methods of producing a commodity chemical enriched refinery-produced petroleum product, comprising (a) blending the refinery-produced petroleum product with the commodity chemical produced by the methods described herein, thereby producing the commodity chemical enriched refinery-produced petroleum product.

Detailed Description

Embodiments of the present invention relate to the unexpected discovery that microorganisms which are otherwise incapable of growing on certain polysaccharides derived from biomass as a sole source of carbon, can be engineered to grow on these polysaccharides as a sole source of carbon. Such microorganisms can include both prokaryotic and eukaryotic microorganisms, such as bacteria and yeast. In some aspects, certain laboratory and/or wild-type strains of *E. coli* can be engineered to grow on biomass derived from either alginate or pectin as a sole source of carbon to produce suitable monosaccharides or other molecules. Among other uses apparent to a person skilled in the art, the monosaccharides and other molecules produced by the growth of these engineered or recombinant microorganisms on alginate or pectin may be utilized as feedstock in the production of various commodity chemicals, such as biofuels.

Alginate and pectin provide advantages over other biomass sources in the production of biofuel feedstocks. For example, large-scale aquatic-farming can

generate a significant amount of biomass without replacing food crop production with energy crop production, deforestation, and recultivating currently uncultivated land, as most of hydrosphere including oceans, rivers, and lakes remains untapped. As one particular example, the Pacific coast of North America is abundant in minerals necessary for large-scale aqua-farming. Giant kelp, which lives in the area, grows as fast as 1m/day, the fastest among plants on earth, and grows up to 50 m. Additionally, aqua-farming has other benefits including the prevention of a red tide outbreak and the creation of a fish-friendly environment.

As an additional advantage, and in contrast to lignocellulolic biomass, biomass derived from aquatic, fruit, plant and/or vegetable sources is easy to degrade. Such biomass typically lacks lignin and is significantly more fragile than lignocellulolic biomass and can thus be easily degraded using either enzymes or chemical catalysts (*e.g.*, formate). As one example, aquatic biomass such as seaweed may be easily converted to monosaccharides using either enzymes or chemical catalysis, as seaweed has significantly simpler major sugar components (Alginate: 30%, Mannitol: 15%) as compared to lignocellulose (Glucose: 24.1-39%, Mannose: 0.2-4.6%, Galactose: 0.5-2.4%, Xylose: 0.4-22.1%, Arabinose 1.5-2.8%, and Uronic acids: 1.2-20.7%, and total sugar contents are corresponding to 36.5-70% of dried weight).

As an additional example, biomass from plants such as fruit and/or vegetable contains pectin, a heteropolysaccharide derived from the plant cell wall. The characteristic structure of pectin is a linear chain of α -(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan. Pectin can be easily converted to oligosaccharides or suitable monosaccharides using either enzymes, chemical catalysis, and/or microbial systems designed to utilize pectin as a source of carbon, as described herein. Saccharification and fermentation using aquatic, fruit, and/or vegetable biomass is much easier than using lignocellulose.

In this regard, embodiments of the present invention also relate to the surprising discovery that certain microorganisms can be engineered to produce various commodity chemicals, such as biofuels. In certain aspects, these biofuels may include

alkanes, such as medium to long chain alkanes, which provide advantages over ethanol based biofuels. In certain aspects, the monosaccharides (*e.g.*, 2-keto-3-deoxy D-gluconate; KDG) and other molecules produced by the growth of various engineered or recombinant microorganisms (*e.g.*, recombinant microorganisms growing on pectin or alginate as a source of carbon) may be useful in the production of commodity chemicals, such as biofuels. As one example, suitable monosaccharides such as KDG may be utilized by recombinant microorganisms to produce alkanes, such as medium to long chain alkanes, among other chemicals. In certain aspects, such recombinant microorganisms may be utilized to produce such commodity chemical as 2,7 dimethyl octane and 2,9 dimethyl decane, among others provided herein and known in the art.

Such processes produce biofuels with significant advantages over other biofuels. In particular, medium to long chain alkanes provide a number of important advantages over the existing common biofuels such as ethanol and butanol, and are attractive long-term replacements of petroleum-based fuels such as gasoline, diesels, kerosene, and heavy oils in the future. As one example, medium to long chain alkanes and alcohols are major components in all petroleum products and jet fuel in particular, and hence alkanes we produce can be utilized directly by existing engines. By way of further example, medium to long chain alcohols are far better fuels than ethanol, and have a nearly comparable energy density to gasoline.

As another example, *n*-alkanes are major components of all oil products including gasoline, diesels, kerosene, and heavy oils. Microbial systems or recombinant microorganisms may be used to produce *n*-alkanes with different carbon lengths ranging, for example, from C7 to over C20: C7 for gasoline (*e.g.*, motor vehicles), C10-C15 for diesels (*e.g.*, motor vehicles, trains, and ships), and C8-C16 for kerosene (*e.g.*, aviations and ships), and for all heavy oils.

As one aspect of the invention, the commodity chemicals produced by the methods and recombinant microorganisms described herein may be utilized by existing petroleum refineries for the purposes of blending with petroleum products produced by traditional refinery methods. To this end, as noted above, fuel producers

are seeking substantially similar, low carbon fuels that can be blended and distributed through existing infrastructure (refineries, pipelines, tankers). As hydrocarbons, the commodity chemicals produced according to the methods herein are substantially similar to petroleum derived fuels, reduce green house gas emissions by more than 80% from petroleum derived fuels, and are compatible with existing infrastructure in the oil and gas industry. For instance, certain of the commodity chemicals produced herein, including, for example, various C10-C12 hydrocarbons such as 2,7 dimethyloctane, 2,7 dimethyldecanone, among others, are blendable directly into refinery-produced petroleum products, such as jet and diesel fuels. By using such biologically produced commodity chemicals as a blendstock for jet and diesel fuels, refineries may reduce Green House Gas emissions by more than 80%.

Accordingly, certain embodiments of the present invention relate generally to methods for converting biomass to a commodity chemical, comprising obtaining a polysaccharide from biomass; contacting the polysaccharide with a polysaccharide degrading or depolymerizing pathway, thereby converting the polysaccharide to a suitable monosaccharide. The suitable monosaccharide obtained from such as process may be used for any desired purpose. For instance, in certain aspects, the suitable monosaccharide may then be converted to a commodity chemical (e.g., biofuel) by contacting the suitable monosaccharide with a biofuel biosynthesis pathway, whether as part of a recombinant microorganism, an *in vitro* enzymatic or chemical pathway, or a combination thereof, thereby converting the monosaccharide to a commodity chemical.

In other aspects, in producing a commodity chemical such as a biofuel, a suitable monosaccharide may be obtained directly from any available source and converted to a commodity chemical by contacting the suitable monosaccharide with a biofuel biosynthesis pathway, as described herein. Among other uses apparent to a person skilled in the art, such biofuels may then be blended directly with refinery produced petroleum products, such as jet and diesel fuels, to produce commodity chemical enriched, refinery-produced petroleum products.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below. All references referred to herein are incorporated by reference in their entirety.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.* to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

By “about” is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 25, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 % to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

The term “biologically active fragment”, as applied to fragments of a reference polynucleotide or polypeptide sequence, refers to a fragment that has at least about 0.1, 0.5, 1, 2, 5, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 100, 110, 120, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000% or more of the activity of a reference sequence.

The term “reference sequence” refers generally to a nucleic acid coding sequence, or amino acid sequence, of any enzyme having a biological activity described herein (*e.g.*, saccharide dehydrogenase, alcohol dehydrogenase, dehydratase, lyase, transporter, decarboxylase, hydrolase, etc.), such as a “wild-type” sequence, including those reference sequences exemplified by SEQ ID NOS:1-144, and 308-313. A reference sequence may also include naturally-occurring, functional variants (*i.e.*, orthologs or homologs) of the sequences described herein.

Included within the scope of the present invention are biologically active fragments of at least about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 500, 600 or more contiguous nucleotides or amino acid residues in length, including all integers in between, which comprise or encode a polypeptide having an enzymatic activity of a reference polynucleotide or polypeptide. Representative biologically active fragments generally participate in an interaction, *e.g.*, an intra-molecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction. Examples of enzymatic interactions or activities include saccharide dehydrogenase activities, alcohol dehydrogenase activities, dehydratases activities, lyase activities, transporter activities, isomerase activities, kinase activities, among others described herein. Biologically active fragments typically comprise one or more active sites or enzymatic/binding motifs, as described herein and known in the art.

By “coding sequence” is meant any nucleic acid sequence that contributes to the code for the polypeptide product of a gene. By contrast, the term “non-coding sequence” refers to any nucleic acid sequence that does not contribute to the code for the polypeptide product of a gene.

Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present.

By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the

phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

The terms "complementary" and "complementarity" refer to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

By "corresponds to" or "corresponding to" is meant (a) a polynucleotide having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein; or (b) a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

By "derivative" is meant a polypeptide that has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties (*e.g.*, pegylation) or by post-translational modification techniques as would be understood in the art. The term "derivative" also includes within its scope alterations that have been made to a parent sequence including additions or deletions that provide for functionally equivalent molecules.

By "enzyme reactive conditions" it is meant that any necessary conditions are available in an environment (*i.e.*, such factors as temperature, pH, lack of inhibiting substances) which will permit the enzyme to function. Enzyme reactive conditions can be either *in vitro*, such as in a test tube, or *in vivo*, such as within a cell.

As used herein, the terms “function” and “functional” and the like refer to a biological or enzymatic function.

By “gene” is meant a unit of inheritance that occupies a specific locus on a chromosome and consists of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (*i.e.*, introns, 5' and 3' untranslated sequences).

“Homology” refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux *et al.*, 1984, *Nucleic Acids Research* 12, 387-395) which is incorporated herein by reference. In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

The term “host cell” includes an individual cell or cell culture which can be or has been a recipient of any recombinant vector(s) or isolated polynucleotide of the invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transfected, transformed, or infected *in vivo* or *in vitro* with a recombinant vector or a polynucleotide of the invention. A host cell which comprises a recombinant vector of the invention is a recombinant host cell, recombinant cell, or recombinant microorganism.

By “isolated” is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an “isolated polynucleotide”, as used herein, refers to a polynucleotide, which has been purified from the sequences which flank it in a naturally-occurring state, *e.g.*, a DNA fragment which has been removed from the sequences that are normally adjacent to the fragment. Alternatively, an “isolated peptide” or an “isolated polypeptide” and the like,

as used herein, refer to *in vitro* isolation and/or purification of a peptide or polypeptide molecule from its natural cellular environment, and from association with other components of the cell, *i.e.*, it is not associated with *in vivo* substances.

By “increased” or “increasing” is meant the ability of one or more recombinant microorganisms to produce a greater amount of a given product or molecule (*e.g.*, commodity chemical, biofuel, or intermediate product thereof) as compared to a control microorganism, such as an unmodified microorganism or a differently modified microorganism. An “increased” amount is typically a “statistically significant” amount, and may include an increase that is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (including all integers and decimal points in between, *e.g.*, 1.5, 1.6, 1.7, 1.8, etc.) the amount produced by an unmodified microorganism or a differently modified microorganism.

By “obtained from” is meant that a sample such as, for example, a polynucleotide extract or polypeptide extract is isolated from, or derived from, a particular source, such as a desired organism, typically a microorganism. “Obtained from” can also refer to the situation in which a polynucleotide or polypeptide sequence is isolated from, or derived from, a particular organism or microorganism. For example, a polynucleotide sequence encoding a benzaldehyde lyase enzyme may be isolated from a variety of prokaryotic or eukaryotic microorganisms, such as *Pseudomonas*.

The term “operably linked” as used herein means placing a gene under the regulatory control of a promoter, which then controls the transcription and optionally the translation of the gene. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the genetic sequence or promoter at a distance from the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the gene it controls in its natural setting; *i.e.* the gene from which the genetic sequence or promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a

regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting; *i.e.*, the genes from which it is derived. "Constitutive promoters" are typically active, *i.e.*, promote transcription, under most conditions. "Inducible promoters" are typically active only under certain conditions, such as in the presence of a given molecule factor (*e.g.*, IPTG) or a given environmental condition (*e.g.*, CO₂ concentration, nutrient levels, light, heat). In the absence of that condition, inducible promoters typically do not allow significant or measurable levels of transcriptional activity.

The recitation "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, rRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

As will be understood by those skilled in the art, the polynucleotide sequences of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence) or may comprise a variant, or a biological functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the enzymatic activity of the encoded polypeptide is not substantially diminished relative to

the unmodified polypeptide, and preferably such that the enzymatic activity of the encoded polypeptide is improved (*e.g.*, optimized) relative to the unmodified polypeptide. The effect on the enzymatic activity of the encoded polypeptide may generally be assessed as described herein.

The polynucleotides of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a polynucleotide fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

The terms “polynucleotide variant” and “variant” and the like refer to polynucleotides that display substantial sequence identity with any of the reference polynucleotide sequences or genes described herein, and to polynucleotides that hybridize with any polynucleotide reference sequence described herein, or any polynucleotide coding sequence of any gene or protein referred to herein, under low stringency, medium stringency, high stringency, or very high stringency conditions that are defined hereinafter and known in the art. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion or substitution of at least one nucleotide. Accordingly, the terms “polynucleotide variant” and “variant” include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide, or has increased activity in relation to the reference polynucleotide (*i.e.*, optimized). Polynucleotide variants include, for example, polynucleotides having at least 50% (and at least 51% to at least 99% and all integer

percentages in between) sequence identity with a reference polynucleotide described herein.

The terms "polynucleotide variant" and "variant" also include naturally-occurring allelic variants that encode these enzymes. Examples of naturally-occurring variants include allelic variants (same locus), homologs (different locus), and orthologs (different organism). Naturally occurring variants such as these can be identified and isolated using well-known molecular biology techniques including, for example, various polymerase chain reaction (PCR) and hybridization-based techniques as known in the art. Naturally occurring variants can be isolated from any organism that encodes one or more genes having a suitable enzymatic activity described herein (*e.g.*, C-C ligase, diol dehydrogenase, pectate lyase, alginate lyase, diol dehydratase, transporter, etc.).

Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. In certain aspects, non-naturally occurring variants may have been optimized for use in a given microorganism (*e.g.*, *E. coli*), such as by engineering and screening the enzymes for increased activity, stability, or any other desirable feature. The variations can produce both conservative and non-conservative amino acid substitutions (as compared to the originally encoded product). For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of a reference polypeptide. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a biologically active polypeptide. Generally, variants of a particular reference nucleotide sequence will have at least about 30%, 40% 50%, 55%, 60%, 65%, 70%, generally at least about 75%, 80%, 85%, 90% to 95% or more, and even about 97% or 98% or more sequence identity to that

particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

As used herein, the term “hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions” describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Ausubel *et al.*, “Current Protocols in Molecular Biology”, John Wiley & Sons Inc, 1994-1998, Sections 6.3.1-6.3.6. Aqueous and non-aqueous methods are described in that reference and either can be used.

Reference herein to “low stringency” conditions include and encompass from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization at 42°C, and at least about 1 M to at least about 2 M salt for washing at 42°C. Low stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 5% SDS for washing at room temperature. One embodiment of low stringency conditions includes hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2 × SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions).

“Medium stringency” conditions include and encompass from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization at 42°C, and at least about 0.1 M to at least about 0.2 M salt for washing at 55°C. Medium stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7% SDS for hybridization at 65° C, and (i) 2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 5% SDS for washing at 60-65°C. One embodiment of medium stringency conditions includes hybridizing in 6 × SSC at about 45°C, followed by one or more washes in 0.2 × SSC, 0.1% SDS at 60°C.

“High stringency” conditions include and encompass from at least about 31% v/v to at least about 50% v/v formamide and from about 0.01 M to about 0.15 M salt for hybridization at 42°C, and about 0.01 M to about 0.02 M salt for washing at 55°C. High stringency conditions also may include 1% BSA, 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 0.2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 1% SDS for washing at a temperature in excess of 65°C. One embodiment of high stringency conditions includes hybridizing in 6× SSC at about 45°C, followed by one or more washes in 0.2 × SSC, 0.1% SDS at 65°C.

One embodiment of “very high stringency” conditions includes hybridizing in 0.5 M sodium phosphate, 7% SDS at 65°C, followed by one or more washes in 0.2 × SSC, 1% SDS at 65°C.

Other stringency conditions are well known in the art and a skilled addressee will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization. For detailed examples, see Ausubel *et al.*, *supra* at pages 2.10.1 to 2.10.16 and Sambrook *et al.*, Current Protocols in Molecular Biology (1989), at sections 1.101 to 1.104.

While stringent washes are typically carried out at temperatures from about 42°C to 68°C, one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridization rate typically occurs at about 20° C to 25° C below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating T_m are well known in the art (see Ausubel *et al.*, *supra* at page 2.10.8).

In general, the T_m of a perfectly matched duplex of DNA may be predicted as an approximation by the formula: $T_m = 81.5 + 16.6 (\log_{10} M) + 0.41 (\%G+C) - 0.63 (\% \text{ formamide}) - (600/\text{length})$ wherein: M is the concentration of Na⁺,

preferably in the range of 0.01 molar to 0.4 molar; %G+C is the sum of guano sine and cytosine bases as a percentage of the total number of bases, within the range between 30% and 75% G+C; % formamide is the percent formamide concentration by volume; length is the number of base pairs in the DNA duplex. The T_m of a duplex DNA decreases by approximately 1°C with every increase of 1% in the number of randomly mismatched base pairs. Washing is generally carried out at $T_m - 15^\circ\text{C}$ for high stringency, or $T_m - 30^\circ\text{C}$ for moderate stringency.

In one example of a hybridization procedure, a membrane (*e.g.*, a nitrocellulose membrane or a nylon membrane) containing immobilized DNA is hybridized overnight at 42° C in a hybridization buffer (50% deionizer formamide, 5 × SSC, 5 × Reinhardt's solution (0.1% fecal, 0.1% polyvinylpyrrolidone and 0.1% bovine serum albumin), 0.1% SDS and 200 mg/mL denatured salmon sperm DNA) containing a labeled probe. The membrane is then subjected to two sequential medium stringency washes (*i.e.*, 2 × SSC, 0.1% SDS for 15 min at 45°C, followed by 2 × SSC, 0.1% SDS for 15 min at 50°C), followed by two sequential higher stringency washes (*i.e.*, 0.2 × SSC, 0.1% SDS for 12 min at 55°C followed by 0.2 × SSC and 0.1% SDS solution for 12 min at 65-68°C).

Polynucleotides and fusions thereof may be prepared, manipulated and/or expressed using any of a variety of well established techniques known and available in the art. For example, polynucleotide sequences which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a selected enzyme in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular

prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence. Such nucleotides are typically referred to as "codon-optimized." Any of the nucleotide sequences described herein may be utilized in such a "codon-optimized" form. For example, the nucleotide coding sequence of the benzaldehyde lyase from *Pseudomonas fluorescens* may be codon-optimized for expression in *E. coli*.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, expression and/or activity of the gene product.

In order to express a desired polypeptide, a nucleotide sequence encoding the polypeptide, or a functional equivalent, may be inserted into appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (1989), and Ausubel *et al.*, Current Protocols in Molecular Biology (1989).

"Polypeptide," "polypeptide fragment," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. In certain aspects, polypeptides may include enzymatic polypeptides, or "enzymes," which typically catalyze (*i.e.*, increase the rate of) various chemical reactions.

The recitation polypeptide “variant” refers to polypeptides that are distinguished from a reference polypeptide sequence by the addition, deletion or substitution of at least one amino acid residue. In certain embodiments, a polypeptide variant is distinguished from a reference polypeptide by one or more substitutions, which may be conservative or non-conservative. In certain embodiments, the polypeptide variant comprises conservative substitutions and, in this regard, it is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Polypeptide variants also encompass polypeptides in which one or more amino acids have been added or deleted, or replaced with different amino acid residues.

The present invention contemplates the use in the methods described herein of variants of full-length polypeptides having any of the enzymatic activities described herein, truncated fragments of these full-length polypeptides, variants of truncated fragments, as well as their related biologically active fragments. Typically, biologically active fragments of a polypeptide may participate in an interaction, for example, an intra-molecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction (*e.g.*, the interaction can be transient and a covalent bond is formed or broken). Biologically active fragments of a polypeptide/enzyme an enzymatic activity described herein include peptides comprising amino acid sequences sufficiently similar to, or derived from, the amino acid sequences of a (putative) full-length reference polypeptide sequence. Typically, biologically active fragments comprise a domain or motif with at least one enzymatic activity, and may include one or more (and in some cases all) of the various active domains. A biologically active fragment of a an enzyme can be a polypeptide fragment which is, for example, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 450, 500, 600 or more contiguous amino acids, including all integers in between, of a reference polypeptide sequence. In certain embodiments, a biologically active fragment comprises a conserved enzymatic sequence, domain, or motif, as described elsewhere

herein and known in the art. Suitably, the biologically-active fragment has no less than about 1%, 10%, 25%, 50% of an activity of the wild-type polypeptide from which it is derived.

The term “exogenous” refers generally to a polynucleotide sequence or polypeptide that does not naturally occur in a wild-type cell or organism, but is typically introduced into the cell by molecular biological techniques, *i.e.*, engineering to produce a recombinant microorganism. Examples of “exogenous” polynucleotides include vectors, plasmids, and/or man-made nucleic acid constructs encoding a desired protein or enzyme. The term “endogenous” refers generally to naturally occurring polynucleotide sequences or polypeptides that may be found in a given wild-type cell or organism. For example, certain naturally-occurring bacterial or yeast species do not typically contain a benzaldehyde lyase gene, and, therefore, do not comprise an “endogenous” polynucleotide sequence that encodes a benzaldehyde lyase. In this regard, it is also noted that even though an organism may comprise an endogenous copy of a given polynucleotide sequence or gene, the introduction of a plasmid or vector encoding that sequence, such as to over-express or otherwise regulate the expression of the encoded protein, represents an “exogenous” copy of that gene or polynucleotide sequence. Any of the of pathways, genes, or enzymes described herein may utilize or rely on an “endogenous” sequence, or may be provided as one or more “exogenous” polynucleotide sequences, and/or may be utilized according to the endogenous sequences already contained within a given microorganism.

A “recombinant” microorganism typically comprises one or more exogenous nucleotide sequences, such as in a plasmid or vector.

The recitations “sequence identity” or, for example, comprising a “sequence 50% identical to,” as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a “percentage of sequence identity” may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T,

C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997, *Nucl. Acids Res.*

25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

"Transformation" refers generally to the permanent, heritable alteration in a cell resulting from the uptake and incorporation of foreign DNA into the host-cell genome; also, the transfer of an exogenous gene from one organism into the genome of another organism.

By "vector" is meant a polynucleotide molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, yeast or virus, into which a polynucleotide can be inserted or cloned. A vector preferably contains one or more unique restriction sites and can be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating vector, *i.e.*, a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a linear or closed circular plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial chromosome. The vector can contain any means for assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Such a vector may comprise specific sequences that allow recombination into a particular, desired site of the host chromosome. A vector system can comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. In the present case, the vector is preferably one which is operably functional in a bacterial cell, such as a cyanobacterial cell. The vector can include a reporter gene, such as a green fluorescent protein (GFP), which can be either fused in frame to one or more of the encoded polypeptides, or expressed separately.

The vector can also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants.

The terms "wild-type" and "naturally occurring" are used interchangeably to refer to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild type gene or gene product (*e.g.*, a polypeptide) is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

Examples of "biomass" include aquatic or marine biomass, fruit-based biomass such as fruit waste, and vegetable-based biomass such as vegetable waste, among others. Examples of aquatic or marine biomass include, but are not limited to, kelp, giant kelp, seaweed, algae, and marine microflora, microalgae, sea grass, and the like. In certain aspects, biomass does not include fossilized sources of carbon, such as hydrocarbons that are typically found within the top layer of the Earth's crust (*e.g.*, natural gas, nonvolatile materials composed of almost pure carbon, like anthracite coal, etc).

Examples of fruit and/or vegetable biomass include, but are not limited to, any source of pectin such as plant peel and pomace including citrus, orange, grapefruit, potato, tomato, grape, mango, gooseberry, carrot, sugar-beet, and apple, among others.

Examples of polysaccharides, oligosaccharides, monosaccharides or other sugar components of biomass include, but are not limited to, alginate, agar, carrageenan, fucoidan, pectin, gluronate, mannuronate, mannitol, lyxose, cellulose, hemicellulose, glycerol, xylitol, glucose, mannose, galactose, xylose, xylan, mannan, arabinan, arabinose, glucuronate, galacturonate (including di- and tri-galacturonates), rhamnose, and the like.

Certain examples of alginate-derived polysaccharides include saturated polysaccharides, such as β -D-mannuronate, α -L-gluronate, dialginate, trialginate, pentalginate, hexalginate, heptalginate, octalginate, nonalginate, decalginate, undecalginate, dodecalginate and polyalginate, as well as unsaturated polysaccharides such as 4-deoxy-L-erythro-5-hexoseulose uronic acid, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-D-mannuronate or L-guluronate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-dialginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-trialginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-tetralginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-pentalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-hexalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-heptalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-octalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-nonalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-undecalginate, and 4-(4-deoxy-beta-D-mann-4-enuronosyl)-dodecalginate.

Certain examples of pectin-derived polysaccharides include saturated polysaccharides, such as galacturonate, digalacturonate, trigalacturonate, tetragalacturonate, pentagalacturonate, hexagalacturonate, heptagalacturonate, octagalacturonate, nonagalacturonate, decagalacturonate, dodecagalacturonate, polygalacturonate, and rhamnopolygalacturonate, as well as saturated polysaccharides such as 4-deoxy-L-threo-5-hexosulose uronate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-galacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-digalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-trigalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-tetragalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-pentagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-hexagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-heptagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-octagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-nonagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-decagalacturonate, and 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-dodecagalacturonate.

These polysaccharide or oligosaccharide components may be converted into "suitable monosaccharides" or other "suitable saccharides," such as "suitable

oligosaccharides,” by the microorganisms described herein which are capable of growing on such polysaccharides or other sugar components as a source of carbon (*e.g.*, a sole source of carbon).

A “suitable monosaccharide” or “suitable saccharide” refers generally to any saccharide that may be produced by a recombinant microorganism growing on pectin, alginate, or other saccharide (*e.g.*, galacturonate, cellulose, hemi-cellulose etc.) as a source or sole source of carbon, and also refers generally to any saccharide that may be utilized in a biofuel biosynthesis pathway of the present invention to produce hydrocarbons such as biofuels or biopetrols. Examples of suitable monosaccharides or oligosaccharides include, but are not limited to, 2-keto-3-deoxy D-gluconate (KDG), D-mannitol, gluronate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose, and the like. As noted herein, a “suitable monosaccharide” or “suitable saccharide” as used herein may be produced by an engineered or recombinant microorganism of the present invention, or may be obtained from commercially available sources.

The recitation “commodity chemical” as used herein includes any saleable or marketable chemical that can be produced either directly or as a by-product of the methods provided herein, including biofuels and/or biopetrols. General examples of “commodity chemicals” include, but are not limited to, biofuels, minerals, polymer precursors, fatty alcohols, surfactants, plasticizers, and solvents. The recitation “biofuels” as used herein includes solid, liquid, or gas fuels derived, at least in part, from a biological source, such as a recombinant microorganism.

Examples of commodity chemicals include, but are not limited to, methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-

butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butandiol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl) ethanol, , 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butandiol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanedione, indolyethane, indolyethene, 2-(indole-3-)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pentene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione , 4-methyl-1-phenyl-3-hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-

methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 4-methyl-1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl-1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2,3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3,4-hexanedione, 2,5-dimethyl-3-hydroxy-4-

hexanone, 5-methyl-1-phenylhexane, 4-methyl-1-phenylhexane, 5-methyl-1-phenyl-1-hexene, 5-methyl-1-phenyl-2-hexene, 5-methyl-1-phenyl-3-hexene, 4-methyl-1-phenyl-1-hexene, 4-methyl-1-phenyl-2-hexene, 4-methyl-1-phenyl-3-hexene, 5-methyl-1-phenyl-2-hexanol, 5-methyl-1-phenyl-3-hexanol, 4-methyl-1-phenyl-2-hexanol, 4-methyl-1-phenyl-3-hexanol, 5-methyl-1-phenyl-2-hexanone, 5-methyl-1-phenyl-3-hexanone, 4-methyl-1-phenyl-2-hexanone, 4-methyl-1-phenyl-3-hexanone, 5-methyl-1-phenyl-2,3-hexanediol, 4-methyl-1-phenyl-2,3-hexanediol, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-3-hydroxy-2-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 5-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)hexane, 5-methyl-1-(4-hydroxyphenyl)-1-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexene, 5-methyl-1-(4-hydroxyphenyl)-3-hexene, 4-methyl-1-(4-hydroxyphenyl)-1-hexene, 4-methyl-1-(4-hydroxyphenyl)-2-hexene, 4-methyl-1-(4-hydroxyphenyl)-3-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexanol, 5-methyl-1-(4-hydroxyphenyl)-3-hexanol, 4-methyl-1-(4-hydroxyphenyl)-2-hexanol, 4-methyl-1-(4-hydroxyphenyl)-3-hexanol, 5-methyl-1-(4-hydroxyphenyl)-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(indole-3-)hexane, 5-methyl-1-(indole-3)-1-hexene, 5-methyl-1-(indole-3)-2-hexene, 5-methyl-1-(indole-3)-3-hexene, 4-methyl-1-(indole-3)-1-hexene, 4-methyl-1-(indole-3)-2-hexene, 4-methyl-1-(indole-3)-3-hexene, 5-methyl-1-(indole-3)-2-hexanol, 5-methyl-1-(indole-3)-3-hexanol, 4-methyl-1-(indole-3)-2-hexanol, 4-methyl-1-(indole-3)-3-hexanol, 5-methyl-1-(indole-3)-2-hexanone, 5-methyl-1-(indole-3)-3-hexanone, 4-methyl-1-(indole-3)-2-hexanone, 4-methyl-1-(indole-3)-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanediol, 4-methyl-1-(indole-3)-2,3-hexanediol, 5-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 5-methyl-1-

(indole-3)-2-hydroxy-3-hexanone, 4-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 4-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanedione, 4-methyl-1-(indole-3)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2,5-dimethyl-2-heptene, 2,5-dimethyl-3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2,6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone, 2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-

methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol, nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedione, 1-undecene, 1-undecanol, undecanal, undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decdecene, 1-dodecanol, ddodecanal, dodecanoate, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-

hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1, 3-propanediol, 4-hydroxybutanal, 1, 4-butanediol, 3-hydroxy-2-butanone, 2, 3-butanediol, 1,5-pentane diol, homocitrate, homoisocitrate, β -hydroxy adipate, glutarate, glutaraldehyde, glutaraldehyde, 2-hydroxy-1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2,3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino pentaldehyde, 1,10-diaminodecane, 1,10-diamino-5-decene, 1,10-diamino-5-hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-phenylbutane, 1-(4-hydroxyphenyl)-4-phenyl-1-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butanol, 1-(4-hydroxyphenyl)-4-phenyl-2-butanone, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydroxyphenyl)-4-phenyl-3-hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl)-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetaldehyde, 1,4-di(indole-3)-butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-

butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, phosphate, and the like.

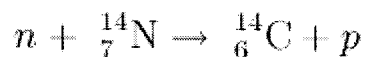
The recitation “optimized” as used herein refers to a pathway, gene, polypeptide, enzyme, or other molecule having an altered biological activity, such as by the genetic alteration of a polypeptide’s amino acid sequence or by the alteration/modification of the polypeptide’s surrounding cellular environment, to improve its functional characteristics in relation to the original molecule or original cellular environment (*e.g.*, a wild-type sequence of a given polypeptide or a wild-type microorganism). Any of the polypeptides or enzymes described herein may be optionally “optimized,” and any of the genes or nucleotide sequences described herein may optionally encode an optimized polypeptide or enzyme. Any of the pathways described herein may optionally contain one or more “optimized” enzymes, or one or more nucleotide sequences encoding for an optimized enzyme or polypeptide.

Typically, the improved functional characteristics of the polypeptide, enzyme, or other molecule relate to the suitability of the polypeptide or other molecule for use in a biological pathway (*e.g.*, a biosynthesis pathway, a C-C ligation pathway) to convert a monosaccharide or oligosaccharide into a biofuel. Certain embodiments, therefore, contemplate the use of “optimized” biological pathways. An exemplary “optimized” polypeptide may contain one or more alterations or mutations in its amino acid coding sequence (*e.g.*, point mutations, deletions, addition of heterologous sequences) that facilitate improved expression and/or stability in a given microbial system or microorganism, allow regulation of polypeptide activity in relation to a desired substrate (*e.g.*, inducible or repressible activity), modulate the localization of the polypeptide within a cell (*e.g.*, intracellular localization, extracellular secretion), and/or effect the polypeptide’s overall level of activity in relation to a desired substrate

(*e.g.*, reduce or increase enzymatic activity). A polypeptide or other molecule may also be “optimized” for use with a given microbial system or microorganism by altering one or more pathways within that system or organism, such as by altering a pathway that regulates the expression (*e.g.*, up-regulation), localization, and/or activity of the “optimized” polypeptide or other molecule, or by altering a pathway that minimizes the production of undesirable by-products, among other alterations. In this manner, a polypeptide or other molecule may be “optimized” with or without altering its wild-type amino acid sequence or original chemical structure. Optimized polypeptides or biological pathways may be obtained, for example, by direct mutagenesis or by natural selection for a desired phenotype, according to techniques known in the art.

In certain aspects, “optimized” genes or polypeptides may comprise a nucleotide coding sequence or amino acid sequence that is 50% to 99% identical (including all integers in between) to the nucleotide or amino acid sequence of a reference (*e.g.*, wild-type) gene or polypeptide. In certain aspects, an “optimized” polypeptide or enzyme may have about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 (including all integers and decimal points in between *e.g.*, 1.2, 1.3, 1.4, 1.5, 5.5, 5.6, 5.7, 60, 70, etc.), or more times the biological activity of a reference polypeptide.

Certain aspects of the invention also include a commodity chemical, such as a biofuel, that is produced according to the methods and recombinant microorganisms described herein. Such a biofuel (*e.g.*, medium to long chain alkane) may be distinguished from other fuels, such as those fuels produced by traditional refinery from crude carbon sources, by radio-carbon dating techniques. For instance, carbon has two stable, nonradioactive isotopes: carbon-12 (^{12}C), and carbon-13 (^{13}C). In addition, there are trace amounts of the unstable isotope carbon-14 (^{14}C) on Earth. Carbon-14 has a half-life of 5730 years, and would have long ago vanished from Earth were it not for the unremitting impact of cosmic rays on nitrogen in the Earth's atmosphere, which create more of this isotope. The neutrons resulting from the cosmic ray interactions participate in the following nuclear reaction on the atoms of nitrogen molecules (N_2) in the atmospheric air:



Plants and other photosynthetic organisms take up atmospheric carbon dioxide by photosynthesis. Since many plants are ingested by animals, every living organism on Earth is constantly exchanging carbon-14 with its environment for the duration of its existence. Once an organism dies, however, this exchange stops, and the amount of carbon-14 gradually decreases over time through radioactive beta decay.

Most hydrocarbon-based fuels, such as crude oil and natural gas derived from mining operations, are the result of compression and heating of ancient organic materials (*i.e.*, kerogen) over geological time. Formation of petroleum typically occurs from hydrocarbon pyrolysis, in a variety of mostly endothermic reactions at high temperature and/or pressure. Today's oil formed from the preserved remains of prehistoric zooplankton and algae, which had settled to a sea or lake bottom in large quantities under anoxic conditions (the remains of prehistoric terrestrial plants, on the other hand, tended to form coal). Over geological time the organic matter mixed with mud, and was buried under heavy layers of sediment resulting in high levels of heat and pressure (known as diagenesis). This process caused the organic matter to chemically change, first into a waxy material known as kerogen which is found in various oil shales around the world, and then with more heat into liquid and gaseous hydrocarbons in a process known as catagenesis. Most hydrocarbon based fuels derived from crude oil have been undergoing a process of carbon-14 decay over geological time, and, thus, will have little to no detectable carbon-14. In contrast, certain biofuels produced by the living microorganisms of the present invention will comprise carbon-14 at a level comparable to all other presently living things (*i.e.*, an equilibrium level). In this manner, by measuring the carbon-12 to carbon-14 ratio of a hydrocarbon-based biofuel of the present invention, and comparing that ratio to a hydrocarbon based fuel derived from crude oil, the biofuels produced by the methods provided herein can be structurally distinguished from typical sources of hydrocarbon based fuels.

Embodiments of the present invention include methods for converting a polysaccharide to a suitable monosaccharide comprising, (a) obtaining the

polysaccharide; and (b) contacting the polysaccharide with a recombinant microorganism or microbial system comprising such a microorganism for a time sufficient to convert the polysaccharide to a suitable monosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase and/or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, and a polysaccharide transporter; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide.

Alternatively, certain aspects may include methods for converting a polysaccharide to a suitable monosaccharide comprising, (a) obtaining the polysaccharide; and (b) contacting the polysaccharide with a microbial system for a time sufficient to convert the polysaccharide to a suitable monosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase; (ii) at least one gene encoding and expressing a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide.

In certain embodiments, a microbial system or isolated microorganism is capable of growing using a polysaccharide (*e.g.*, alginate, pectin, etc.) as a sole source of carbon and/or energy. A "sole source of carbon" refers generally to the ability to grow on a given carbon source as the only carbon source in a given growth medium.

With regard to alginate, approximately 50 percent of seaweed dry-weight comprises various sugar components, among which alginate and mannitol are major components corresponding to 30 and 15 percent of seaweed dry-weight, respectively. With regard to pectin, Although microorganisms such as *E. coli* are generally

considered as a host organisms in synthetic biology, and although such microorganism are able to metabolize mannitol, they completely lack the ability to degrade and metabolize alginate. In this regard, many laboratory or wild-type microorganisms, such as *E. coli*, are unable to grow on alginate as a sole source of carbon. Similarly, many organisms such as *E. coli* are unable to degrade and metabolize pectin, a polysaccharide found in many food waste products, and, thus are unable to grown on pectin as a sole source of carbon. Accordingly, embodiments of the present application include engineered microorganisms, such as *E. coli*, or microbial systems containing such engineered microorganisms, that are capable of using polysaccharides, such as alginate and pectin, as a sole source of carbon and/or energy.

Alginate is a block co-polymer of β -D-mannuronate (M) and α -D-gluronate (G) (M and G are epimeric about the C5-carboxyl group). Each alginate polymer comprises regions of all M (polyM), all G (polyG), and/or the mixture of M and G (polyMG). To utilize alginate to produce one or more suitable monosaccharides, certain aspects of the present invention provide an engineered or recombinant microorganism or microbial system that is able to degrade or de-polymerize alginate and to use it as a source of carbon and/or energy. As one means of accomplishing this purpose, such recombinant microorganisms may incorporate a set of polysaccharide degrading or depolymerizing enzymes such as alginate lyases (ALs) to the microbial system.

ALs are mainly classified into two distinctive subfamilies depending on their acts of catalysis: endo- (EC 4.2.2.3) and exo-acting (EC 4.2.2.-) ALs. Endo-acting ALs are further classified based on their catalytic specificity; M specific and G specific ALs. The endo-acting ALs randomly cleave alginate via a β -elimination mechanism and mainly depolymerize alginate to di-, tri- and tetrasaccharides. The uronate at the non-reducing terminus of each oligosaccharide are converted to unsaturated sugar uronate, 4-deoxy- α -L-erythro-hex-4-ene pyranosyl uronates. The exo-acting ALs catalyze further depolymerization of these oligosaccharides and release unsaturated monosaccharides, which may be non-enzymatically converted to monosaccharides,

including α -keto acid, 4-deoxy- α -L-erythro-hexoselulose uronate (DEHU). Certain embodiments of an engineered microbial system or isolated, engineered microorganism may include endoM-, endoG- and exo-acting ALs to degrade or depolymerize aquatic or marine-biomass polysaccharides such as alginate to a monosaccharide such as DEHU.

Embodiments of the present invention may also include lyases such as alginate lyases isolated from various sources, including, but not limited to, marine algae, mollusks, and wide varieties of microbes such as genus *Pseudomonas*, *Vibrio*, and *Sphingomonas*. Many alginate lyases are endo-acting M specific, several are G specific, and few are exo-acting. For example, ALs isolated from *Sphingomonas* sp. strain A1 include five endo-acting ALs, A1-I, A1-II, A1-II', A1-III, and A1-IV' and an exo-acting AL, A1-IV.

Typically, A1-I, A1-II, and A1-III have molecular weights of 66 kDa, 25 kDa, and 40 kDa, respectively. A1-II and A1-III are self-splicing products of A1-I. A1-II may be more specific to G and A1-III may be specific to M. A1-I may have high activity for both M and G. A1-IV has molecular weight of about 85 kDa and catalyzes exo-lytic depolymerization of oligoalginate. Although both A1-II' and A1-IV' are functional homologues of A1-II and A1-IV. A1-II' has endo-lytic activity and may have no preference to M or G. A1-IV has primarily endo-lytic activity. In addition to these ALs, exo-lytic AL Atu3025 derived from *Agrobacterium tumefaciens* has high activity for depolymerization of oligoalginate, and may be used in certain embodiments of the present invention. Certain embodiments may incorporate into the microbial system or isolated microorganism the genes encoding A1-I, A1-II', A1-IV, and Atu3025, and may include optimal codon usage for the suitable host organisms, such as *E. coli*.

Certain examples of alginate lyases or oligoalginate lyases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to SEQ ID NOS:67-68, which show the nucleotide (SEQ ID NO:67) and polypeptide (SEQ ID

NO:68) sequences of oligoalginate lyase Atu3025 isolated from *Agrobacterium tumefaciens*. Certain examples of alginate lyases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the alginate lyase enzymes described in Figure 37, as well as the secreted alginate lyase encoded by Vs24254 from *Vibrio splendidus*.

In certain embodiments, a microbial system or recombinant microorganism may be engineered to secrete or display the lyases or alginate lyases (ALs) to the culture media, such as by incorporating a signal peptide or autotransporter domain into the lyase. In this regard, it is typically understood that bacteria have at least four different types of protein secretion machinery (type I, II, III and IV). For example, in *E. coli*, the type II secretion machinery is used for the secretion of recombinant proteins. The type II secretion machinery may comprise a two-step process: the translocation of premature proteins tagged with signal peptides to the periplasm fraction and processing to the mature proteins followed by secretion to media.

The first process may proceed by any of three different pathways: secB-dependent pathway, signal recognition particle (SRP) pathway, or twin-arginine translocation (TAT) pathway. Recombinant proteins may be secreted into periplasm fraction. The fates of the mature proteins vary dependent on the type of proteins. For example, some proteins are secreted spontaneously by diffusion or passively by a secretion apparatus named secretion that consists of 12-16 proteins, and others stay in periplasm fraction and are eventually degraded.

Some proteins may also be secreted by an autotransporter apparatus, such as by utilizing an autotransporter domain. The proteins secreted by autotransporter domains typically comprise an N-terminal signal peptide that plays a role in translocation to the periplasm, which may be mediated by secB or SRP pathways, passenger domain, and/or C-terminal translocation unit (UT) having a characteristic β -barrel structure. The β -barrel portion of the UT builds an aqueous pore channel across

the outer membrane and helps the transportation of passenger domain to media. Autodisplayed passenger proteins are often cleaved by the autotransporter and set free to media.

The type I secretion machinery may also be used for the secretion of recombinant proteins in *E. coli*. The type I secretion machinery may be used for the secretion of high-molecular-weight toxins and exoenzymes. The type I secretion machinery consist of two inner membrane proteins (HlyB and HlyD) that are the member of the ATP binding cassette (ABC) transporter family, and an endogenous outer membrane protein (TolC). The secretion of recombinant proteins based on type I secretion machinery may utilize the C-terminal region of α -haemolysin (HlyA) as a signal sequence. The recombinant proteins may readily pass through the inner membrane, periplasm, and outer membrane through the type I secretion machinery.

Depending on the types of linker and signal peptides utilized by various embodiments of the present application, both autotransporter and type I secretion machinery can be altered to the cell surface display machinery. Alternatively, a system specific to cell surface display may be used. For example, in this system, target proteins may be fused to PgsA protein (a poly- γ -glutamate synthetase complex) that is natively displayed on the surface of *Bacillus subtilis*.

Certain embodiments may include lyases such as alginate lyases fused with various signal peptides and/or autotransporter domains found in proteins secreted by both type I and type II secretion machinery. Other embodiments may include lyases such as alginate lyases fused with any combination of signal peptides and or autotransporter domains found in proteins secreted transport machinery as described herein or known to a person skilled in the art. Embodiments may also include signal peptides or autotransporter domains that are experimentally redesigned to maximize the secretion of lyases such as alginate lyases to the culture media, and may also include the use of many different linker sequences that fuse signal peptides, lyases, and autotransporters that improve the efficiency of secretion or the cell surface presentation of lyases.

Certain embodiments may include a microbial system or isolated microorganism that comprise saccharide transporters, which are able to transport monosaccharides (*e.g.*, DEHU) and oligosaccharides from the media to the cytosol to efficiently utilize these monosaccharides as a source of carbon and/or energy. For instance, genes encoding monosaccharide permeases (*i.e.*, monosaccharide transporters) such as DEHU permeases may be isolated from bacteria that grow on polysaccharides such as alginate as a source of carbon and/or energy, and may be incorporated into embodiments of the present microbial system or isolated microorganism. As an additional example, embodiments may also include redesigned native permeases or transporters with altered specificity for monosaccharide (*e.g.*, DEHU) transportation.

In this regard, *E.coli* contains several permeases able to transport monosaccharides, which include, but are not limited to, KdgT for 2-keto-3-deoxy-D-gluconate (KDG) transporter, ExuT for aldohexuronates such as D-galacturonate and D-glucuronate transporter, GntT, GntU, GntP, and GntT for gluconate transporter, and KgtP for proton-driven α -ketoglutarate transporter. Microbial systems or recombinant microorganisms described herein may comprise any of these permeases, in addition to those permeases known to a person of skill in the art and not mentioned herein, and may also include permease enzymes redesigned to transport other monosaccharides, such as DEHU.

A microbial system or recombinant microorganism according to the present invention may also comprise permeases/transporters/superchannels/porins that catalyze the transport of monosaccharides (*e.g.*, D-mannuronate and D-lyxose) from media to the periplasm or cytosol of a microorganism. For example, genes encoding the permeases of D-mannuronate in soil *Aeromonas* may be incorporated into a microbial system as described herein.

As one alternative example, a microbial system or microorganism may comprise native permeases/transporters that are redesigned to alter their specificity for efficient monosaccharide transportation, such as for D-mannuronate and D-lyxose transportation. For instance, *E.coli* contains several permeases that are able to transport

monosaccharides or sugars such as D-mannonate and D-lyxose, including KdgT for 2-keto-3-deoxy-D-gluconate (KDG) transporter, ExuT for aldohexuronates such as D-galacturonate and D-glucuronate transporter, GntPTU for gluconate/fructuronate transporter, uidB for glucuronide transporter, fucP for L-fucose transporter, galP for galactose transporter, yghK for glycolate transporter, dgoT for D-galactonate transporter, uhpT for hexose phosphate transporter, dctA for orotate/citrate transporter, gntUT for gluconate transporter, malEGF for maltose transporter: alsABC for D-allose transporter, idnT for L-idonate/D-gluconate transporter, KgtP for proton-driven α -ketoglutarate transporter, lacY for lactose/galactose transporter, xylEFGH for D-xylose transporter, araEFGH for L-arabinose transporter, and rbsABC for D-ribose transporter. In certain embodiments, a microbial system or recombinant microorganism may comprise permeases or transporters as described above, including those that are redesigned or optimized for improved transport of certain monosaccharides, such as D-mannuronate, DEHU, and D-lyxose.

Certain aspects may employ a recombinant microorganism that comprises a "superchannel," by which aquatic or marine-biomass polysaccharides such as alginate polymers, or fruit or vegetable biomass such as pectin polymers, may be directly incorporated into the cytosol and degraded inside the microbial system. For instance, a group of bacteria characterized as *Sphingomonads* have a wide range in capability of degrading environmentally hazardous compounds such as polychlorinated polycyclic aromatics (dioxin). These bacteria contain characteristic large pleat-like molecules on their cell surfaces. In this regard, certain *Sphingomonads* have structures characterized as "superchannels" that enable the bacteria to directly take up macromolecules.

As one particular example of a microorganism comprising a superchannel, *Sphingomonas* sp. strain A1 directly incorporates polysaccharides such as alginate through a superchannel. Such superchannels may consist of a pit on the outer membrane (e.g., AlgR), alginate-binding proteins in the periplasm (e.g., AlgQ1 and AlgQ2), and an ATP-binding cassette (ABC) transporter (e.g., AlgM1, AlgM2, and AlgS).

Incorporated polysaccharides such as alginate may be readily depolymerized by lyases such as alginate lyases produced in the cytosol. Thus, certain embodiments may incorporate genes encoding a superchannel (*e.g.*, *ccpA*, *algS*, *algM1*, *algM2*, *algQ1*, *algQ2*) to introduce this ability to the microbial system or recombinant microorganism. Other embodiments may include microorganisms such as *Sphingomonas subarctica* IFO 16058^T, which harbor the plasmid containing genes that encode a superchannel, and which have significantly improved ability to utilize marine or aquatic biomass polysaccharides such as alginate as a source of carbon and/or energy. Certain recombinant microorganisms may employ these superchannel encoding plasmid sequences contained within *Sphingomonas subarctica* IFO 16058^T.

Certain examples of alginate ABC transporters that may be utilized herein, include ABC transporters Atu3021, Atu3022, Atu3023, Atu3024, *algM1*, *algM2*, *AlgQ1*, *AlgQ2*, *AlgS*, OG2516_05558, OG2516_05563, OG2516_05568, and OG2516_05573, including functional variants thereof. Certain examples of alginate symporters that may be utilized herein include symporters V12B01_24239 and V12B01_24194, among others, including functional variants thereof. One additional example of an alginate porin includes V12B01_24269, and variants thereof.

As noted above, certain embodiments may include recombinant microorganisms that comprise one or more monosaccharide dehydrogenases, isomerases, dehydratases, kinases, and aldolases. With regard to monosaccharide dehydrogenases, certain microbial systems or recombinant microorganism may incorporate enzymes that reduce various monosaccharides (*e.g.*, DEHU, mannuronate) to a monosaccharide that is suitable for biofuel biosynthesis, such as 2-keto-3-deoxy-D-gluconate (KDG) or D-mannitol. Such exemplary enzymes, include, for example, DEHU hydrogenases and mannuronate hydrogenases, in addition to various alcohol dehydrogenases having DEHU hydrogenase and/or mannuronate dehydrogenase activity, such as the novel ADH1 through ADH12 enzymes isolated from *Agrobacterium tumefaciens* C58 (*see, e.g.*, SEQ ID NOS:69-92).

For more detail on the ADH1 through ADH12 enzymes, SEQ ID NO:69 shows the nucleotide and SEQ ID NO:70 shows the polypeptide sequence of ADH1 Atu1557 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:71 shows the nucleotide and SEQ ID NO:72 shows the polypeptide sequence of ADH2 Atu2022 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:73 shows the nucleotide and SEQ ID NO:74 shows the polypeptide sequence of ADH3 Atu0626 isolated from *Agrobacterium tumefaciens* C58.

SEQ ID NO:75 shows the nucleotide and SEQ ID NO:76 shows the polypeptide sequence of ADH4 Atu5240 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:77 shows the nucleotide and SEQ ID NO:78 shows the polypeptide sequence of ADH5 Atu3163 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:79 shows the nucleotide and SEQ ID NO:80 shows the polypeptide sequence of ADH6 Atu2151 isolated from *Agrobacterium tumefaciens* C58.

SEQ ID NO:81 shows the nucleotide and SEQ ID NO:82 shows the polypeptide sequence of ADH7 Atu2814 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:83 shows the nucleotide and SEQ ID NO:84 shows the polypeptide sequence of ADH8 Atu5447 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:85 shows the nucleotide and SEQ ID NO:86 shows the polypeptide sequence of ADH9 Atu4087 isolated from *Agrobacterium tumefaciens* C58.

SEQ ID NO:87 shows the nucleotide and SEQ ID NO:88 shows the polypeptide sequence of ADH10 Atu4289 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:89 shows the nucleotide and SEQ ID NO:90 shows the polypeptide sequence of ADH11 Atu3027 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:91 shows the nucleotide and SEQ ID NO:92 shows the polypeptide sequence of ADH12 Atu3026 isolated from *Agrobacterium tumefaciens* C58.

Further examples of enzymes having dehydrogenase activity include Atu3026, Atu3027, OG2516_05543, OG2516_05538 and V12B01_24244. The microorganisms and methods of the present invention may also utilize biologically

active fragments and variants of these hydrogenase enzymes, including optimized variants thereof.

As a further example, *Pseudomonas* grown using alginate as a sole source of carbon and energy comprises a DEHU hydrogenase enzyme that uses NADPH as a co-factor, is more stable when NADP^+ is present in the solution, and is active at ambient pH. Thus, certain embodiments of a microbial system or a recombinant microorganism as described herein may incorporate genes encoding hydrogenases such as DEHU or mannuronate hydrogenase derived or obtained from various microbes, in which these microbes may be capable of growing on polysaccharides such as alginate or pectin as a source of carbon and/or energy.

Certain embodiments may incorporate components of a microbial system or isolated microorganism that is capable of efficiently growing on monosaccharides such as D-mannuronate or D-lyxose as a source of carbon and energy. For instance, both *Aeromonas* and *Aerobacter aerogenes* PRL-R3 comprise genes encoding monosaccharide dehydrogenases such as D-mannuronate hydrogenase and D-lyxose isomerase. Thus, certain microbial systems or recombinant microorganisms may comprise monosaccharide dehydrogenases such as D-mannuronate hydrogenase and D-lyxose isomerase from *Aeromonas*, *Aerobacter aerogenes* PRL-R3, or various other suitable microorganisms, including those microorganisms capable of growing on D-mannuronate or D-lyxose as a source of carbon and energy.

Certain embodiments may include a microbial system or isolated microorganism with enhanced efficiency for converting monosaccharides such as D-mannuronate and D-xylulose into monosaccharides suitable for a biofuel biosynthesis pathway such as KDG. Merely by way of explanation, D-mannuronate and D-xylulose are metabolites in microbes such as *E. coli*. D-mannuronate is converted by a D-mannuronate dehydratase to KDG. D-xylulose enters the pentose phosphate pathway. Thus, to increase conversion of D-mannuronate to KDG, an exogenous or endogenous D-mannuronate dehydratase (e.g., *uxuA*) gene may be over-expressed in a recombinant microorganism of the invention. Similarly, in other embodiments, suitable endogenous

or exogenous genes such as kinases (*e.g.*, *kdgK*), *nad*, as well as KDG aldolases (*e.g.*, *kdgA* and *eda*) may be either incorporated or overexpressed in a given recombinant microorganism (*see* SEQ ID NOS:93-96), including biologically active variants or fragments thereof, such as optimized variants of these genes. SEQ ID NO:93 shows the nucleotide sequence and SEQ ID NO:94 shows the polypeptide sequence of a 2-keto-deoxy gluconate kinase (*KdgK*) from *Escherichia coli* DH10B. SEQ ID NO:95 shows the nucleotide sequence and SEQ ID NO:96 shows the polypeptide sequence of a 2-keto-deoxy gluconate-6-phosphate aldorase (*KdgA*) from *Escherichia coli* DH10B.

In certain aspects, as noted above, a recombinant microorganism that is capable of growing on alginate or pectin as a sole source of carbon may utilize a naturally-occurring or endogenous copy of a dehydratase, kinase, and/or aldolase. For instance, *E. coli* contains endogenous dehydratases, kinases, and aldolases that are capable of catalyzing the appropriate steps in the conversion of polysaccharides to a suitable monosaccharide. In these and other related aspects, the naturally-occurring dehydratase or kinase may also be over-expressed, such as by providing an exogenous copy of the naturally-occurring dehydratase, kinase or aldolase operable linked to a highly constitutive or inducible promoter.

As one exemplary source of enzymes for engineering a recombinant microorganism to grow on alginate as a sole source of carbon, *Vibrio splendidus* is known to be able to metabolize alginate to support growth. For example, SEQ ID NO:1 shows a secretome region carrying certain *Vibrio splendidus* genes (V12B01_02425 to V12B01_02480), which encodes a type II secretion apparatus. SEQ ID NO:2 shows the nucleotide sequence of an entire genomic region between V12B01_24189 to V12B01_24249, which was derived from *Vibrio splendidus*, and which when transformed into *E. coli* as a fosmid clone was sufficient to confer the ability to grow on alginate as a sole source of carbon. SEQ ID NOS:3-64 show the individual putative genes contained within SEQ ID NO:2. Thus, in certain aspects, a recombinant microorganism (*e.g.*, *E. coli*) that is able to grow on alginate as a sole source of carbon and/or energy may comprise one or more nucleotide or polypeptide reference sequences

described in SEQ ID NOS:1-64, including biologically active fragments or variants thereof, such as optimized variants.

In certain aspects, a recombinant microorganism that is able to grow on alginate as a sole source of carbon may contain certain coding nucleotide or polypeptide sequences contained within SEQ ID NO:2, such as the sequences in SEQ ID NOS:3-64, or biologically active fragments or variants thereof, including optimized variants. These sequences are described in further detail below.

SEQ ID NO:3 shows the nucleotide coding sequence of the putative protein V12B01_24184. This putative coding sequence is contained within the polynucleotide sequence of SEQ ID NO:2, and encodes a polypeptide that is similar to an autotransporter adhesion or type I secretion target ggxgxdxxx (SEQ ID NO:145) repeat. SEQ ID NO:4 shows the polypeptide sequence of putative protein V12B01_24184, encoded by the polynucleotide of SEQ ID NO:3. This putative polypeptide is similar to autotransporter adhesion or type I secretion target ggxgxdxxx (SEQ ID NO:145) repeat.

SEQ ID NO:5 shows the nucleotide sequence that encodes the putative protein V12B01_24189. SEQ ID NO:6 shows the polypeptide sequence of the putative protein V12B01_24189, which is similar to cyclohexadienyl dehydratase.

SEQ ID NO:7 shows the nucleotide sequence that encodes the putative protein V12B01_24194. SEQ ID NO:8 shows the polypeptide sequence of the putative protein V12B01_24194, which is similar to a Na/proline transporter.

SEQ ID NO:9 shows the nucleotide sequence that encodes the putative protein V12B01_24199. SEQ ID NO:10 shows the polypeptide sequence of the putative protein V12B01_24199, which is similar to a *keto*-deoxy-phosphogluconate aldolase.

SEQ ID NO:11 shows the nucleotide sequence that encodes the putative protein V12B01_24204. SEQ ID NO:12 shows the polypeptide sequence of the putative protein V12B01_24204, which is similar to 2-dehydro-3-deoxygluconokinase.

SEQ ID NO:13 shows the nucleotide sequence that encodes the putative protein V12B01_241209. SEQ ID NO:14 shows the polypeptide sequence of the putative protein V12B01_241209.

SEQ ID NO:15 shows the nucleotide sequence that encodes the putative protein V12B01_24214. SEQ ID NO:16 shows the polypeptide sequence of the putative protein V12B01_24214, which is similar to a chondroitin AC/alginate lyase.

SEQ ID NO:17 shows the nucleotide sequence that encodes the putative protein V12B01_24219. SEQ ID NO:18 shows the polypeptide sequence of the putative protein V12B01_24219, which is similar to a chondroitin AC/alginate lyase.

SEQ ID NO:19 shows the nucleotide sequence that encodes the putative protein V12B01_24224. SEQ ID NO:20 shows the polypeptide sequence of the putative protein V12B01_24224, which is similar to a 2-*keto*-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase.

SEQ ID NO:21 shows the nucleotide sequence that encodes the putative protein V12B01_24229. SEQ ID NO:22 shows the polypeptide sequence of the putative protein V12B01_24229, which is similar to a GntR-family transcriptional regulator.

SEQ ID NO:23 shows the nucleotide sequence that encodes the putative protein V12B01_24234. SEQ ID NO:24 shows the polypeptide sequence of the putative protein V12B01_24234, which is similar to a Na⁺/proline symporter.

SEQ ID NO:25 shows the nucleotide sequence that encodes the putative protein V12B01_24239. SEQ ID NO:26 shows the polypeptide sequence of the putative protein V12B01_24239, which is similar to an oligoalginate lyase.

SEQ ID NO:27 shows the nucleotide sequence that encodes the putative protein V12B01_24244. SEQ ID NO:28 shows the polypeptide sequence of putative protein V12B01_24244, which is similar to a 3-hydroxyisobutyrate dehydrogenase.

SEQ ID NO:29 shows the nucleotide sequence that encodes the putative protein V12B01_24249. SEQ ID NO:30 shows the polypeptide sequence of the putative protein V12B01_24249, which is similar to a methyl-accepting chemotaxis protein.

SEQ ID NO:31 shows the nucleotide sequence that encodes the putative protein V12B01_24254. SEQ ID NO:32 shows the polypeptide sequence of putative protein V12B01_24254, which is similar to an alginate lyase.

SEQ ID NO:33 shows the nucleotide sequence that encodes the putative protein V12B01_24259. SEQ ID NO:34 shows the polypeptide sequence of putative protein V12B01_24259, which is similar to an alginate lyase.

SEQ ID NO:35 shows the nucleotide sequence that encodes the putative protein V12B01_24264. SEQ ID NO:36 shows the polypeptide sequence of putative protein V12B01_24264.

SEQ ID NO:37 shows the nucleotide sequence that encodes the putative protein V12B01_24269. SEQ ID NO:38 shows the polypeptide sequence of putative protein V12B01_24269, which is similar to a putative oligogalacturonate specific porin.

SEQ ID NO:39 shows the nucleotide sequence that encodes the putative protein V12B01_24274. SEQ ID NO:40 shows the polypeptide sequence of putative protein V12B01_24274, which is similar to an alginate lyase.

Figure 32 shows the nucleotide coding sequence and polypeptide sequence of putative protein V12B01_02425. Figure 32A shows the nucleotide sequence that encodes the putative protein V12B01_02425 (SEQ ID NO:41). Figure

32B shows the polypeptide sequence of putative protein V12B01_02425 (SEQ ID NO:42), which is similar to a type II secretory pathway component EpsC.

SEQ ID NO:43 shows the nucleotide sequence that encodes the putative protein V12B01_02430. SEQ ID NO:44 shows the polypeptide sequence of putative protein V12B01_02430, which is similar to a type II secretory pathway component EpsD.

SEQ ID NO:45 shows the nucleotide sequence that encodes the putative protein V12B01_02435. SEQ ID NO:46 shows the polypeptide sequence of putative protein V12B01_02435, which is similar to a type II secretory pathway component EpsE.

SEQ ID NO:47 shows the nucleotide sequence that encodes the putative protein V12B01_02440. SEQ ID NO:48 shows the polypeptide sequence of putative protein V12B01_02440, which is similar to a type II secretory pathway component EpsF.

SEQ ID NO:49 shows the nucleotide sequence that encodes the putative protein V12B01_02445. SEQ ID NO:50 shows the polypeptide sequence of putative protein V12B01_02445, which is similar to a type II secretory pathway component EpsG.

SEQ ID NO:51 shows the nucleotide sequence that encodes the putative protein V12B01_02450. SEQ ID NO:52 shows the polypeptide sequence of putative protein V12B01_02450, which is similar to a type II secretory pathway component EpsH.

SEQ ID NO:53 shows the nucleotide sequence that encodes the putative protein V12B01_02455. SEQ ID NO:54 shows the polypeptide sequence of putative protein V12B01_02455, which is similar to a type II secretory pathway component EpsI.

SEQ ID NO:55 shows the nucleotide sequence that encodes the putative protein V12B01_02460. SEQ ID NO:56 shows the polypeptide sequence of putative protein V12B01_02460, which is similar to a type II secretory pathway component EpsJ.

SEQ ID NO:57 shows the nucleotide sequence that encodes the putative protein V12B01_02465. SEQ ID NO:58 shows the polypeptide sequence of putative protein V12B01_02465, which is similar to a type II secretory pathway component EpsK.

SEQ ID NO:59 shows the nucleotide sequence that encodes the putative protein V12B01_02470. SEQ ID NO:60 shows the polypeptide sequence of putative protein V12B01_02470, which is similar to a type II secretory pathway component EpsL.

SEQ ID NO:61 shows the nucleotide sequence that encodes the putative protein V12B01_02475. SEQ ID NO:62 shows the polypeptide sequence of putative protein V12B01_02475, which is similar to a type II secretory pathway component EpsM.

SEQ ID NO:63 shows the nucleotide sequence that encodes the putative protein V12B01_02480. SEQ ID NO:64 shows the nucleotide sequence that encodes the putative protein V12B01_02480, which is similar to a type II secretory pathway component EpsC.

As a further exemplary source of enzymes for engineering a microorganism to grow on alginate, *Agrobacterium tumefaciens* C58 is able to metabolize relatively small sizes of alginate molecules (~1000 mers) as a sole source of carbon and energy. Since *A. tumefaciens* C58 has long been used for plant biotechnology, the genetics of this organism has been relatively well studied, and many genetic tools are available and compatible with other gram-negative bacteria such as *E. coli*. Thus, certain aspects may employ this microbe, or the genes therein, for the

production of suitable monosaccharides. For instance, as noted above, the present disclosure provides a series of novel ADH genes having both DEHU and mannuronate hydrogenase activity that were obtained from *Agrobacterium tumefaciens* C58 (see SEQ ID NOS: 67-92).

As noted above, certain aspects may include a recombinant microorganism or microbial system that is capable of growing on pectin as a sole source of carbon and/or energy. Pectin is a linear chain of α -(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan. Into this backbone, there are regions where galacturonic acid is replaced by (1-2)-linked L-rhamnose. From rhamnose, side chains of various neutral sugars typically branch off. This type of pectin is called rhamnogalacturonan I. Over all, about up to every 25th galacturonic acid in the main chain is exchanged with rhamnose. Some stretches consisting of alternating galacturonic acid and rhamnose – “hairy regions”, others with lower density of rhamnose – “smooth regions.” The neutral sugars mainly comprise D-galactose, L-arabinose and D-xylose; the types and proportions of neutral sugars vary with the origin of pectin. In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. Some plants, like sugar-beet, potatoes and pears, contain pectins with acetylated galacturonic acid in addition to methyl esters. Acetylation prevents gel-formation but increases the stabilising and emulsifying effects of pectin. Certain pectin degradation and metabolic pathways are exemplified in Figure 3.

In addition to the genes, enzymes, and biological pathways described above, certain recombinant microorganisms may incorporate features that are useful for growth on pectin as a sole source of carbon. For instance, to degrade and metabolize pectin as a sole source of carbon, pectin methyl and acetyl esterases first catalyze the hydrolysis of methyl and acetyl esters on pectin. Examples of pectin methyl esterases include, but are not limited to, pema and pmeB. Examples of pectin acetyl esterases include, but are not limited to, PaeX and PaeY. Further examples of pectin methyl esterases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in

between) to the pectate methyl esterases in Figure 40. Further examples of pectate acetyl esterases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the pectate acetyl esterases described in Figure 41.

Further to this end, pectate lyases and hydrolases may catalyze the endolytic cleavage of pectate via β -elimination and hydrolysis, respectively, to produce oligopectates. Other enzymes that may be utilized to metabolize pectin include Examples of pectate lyases include, but are not limited to, PelA, PelB, PelC, PelD, PelE, PelF, PelI, PelL, and PelZ. Examples of pectate hydrolases include, but are not limited to, PehA, PehN, PehV, PehW, and PehX. Further examples of pectate lyases include polypeptides or enzymes sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the pectate lyases described in Figure 38.

Polygalacturonases, rhamnogalacturonan lyases, and rhamnogalacturonan hydrolases may also be utilized herein to degrade and metabolize pectin. Examples of rhamnogalacturonan lyases include polypeptides or enzymes sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the rhamnogalacturonan lyases (*i.e.*, rhamnogalacturonases) described in Figure 39A. Examples of rhamnogalacturonate hydrolases include polypeptides or enzymes sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the rhamnogalacturonate hydrolases described in Figure 39B.

Thus, to degrade and metabolize pectin, certain of the the recombinant microorganisms and methods of the present invention may incorporate one or more of the above noted methy and acetyl esterases, lyases, and/or hydrolases, among others known in the art. These may enzymes may be encoded and expressed by endogenous or exogenous genes, and may also include biologically active fragments or variants thereof, such as homologs, orthologs, and/or optimized variants of these enzymes.

To further metabolize the degradation products of pectin, oligopectates may be transported into the periplasm fraction of gram-negative bacteria by outer membrane porins, where they are further degraded into such components as di- and tri-galactonurates. Examples of outer membrane porins include that can transport oligopectates into the periplasm include, but are not limited to, kdgN and kdgM. Certain recombinant microorganism may incorporate these or similar genes.

Di- and tri-galactonurates may then be transported into the cytosol for further degradation. Bacteria contain at least two different transporter systems responsible for di- and tri-galacturonate transportation, including symporter and ABC transporter (*e.g.*, TogT and TogMNAB, respectively). Thus, certain of the recombinant microorganisms provided herein may comprise one or more a di- or tri-galacturonate transoporter systems, such as TogT and/or TogMNAB.

Once di- and trigalacturonate are incorporated into the cytosol, short pectate or galacturonate lyases, break them down to D-galacturonate and (4S)-4,6-dihydroxy-2,5-dioxohexuronate. Examples of short pectate or galacturonate lyases include, but are not limited to, PelW and Ogl, which genes may be either endogenously or exogenously incorporated into certain recombinant microorganisms provided herein. D-galacturonate and (4S)-4,6-dihydroxy-2,5-dioxohexuronate are then converted to 5-dehydro-4-deoxy-D-glucuronate and further to KDG, which steps may be catalyzed by KduI and KduD, respectively. The KduI enzyme has an isomerase activity, and the KduD enzyme has a dehydrogenase activity, such as a 2-deoxy-D-gluconate 3-dehydrogenase activity. Accordingly, certain recombinant microorgansms provided herein may comprise one or more short pectate or galacturonate lyases, such as PelW and/or Ogl, and may optionally comprise one or more isomerases, such as KduI, as well as one or more dehydrogenases, such as KduD, to convert di- and trigalacturonates into a suitable monosaccharide, such as KDG.

In certain aspects, a recombinant microorganism, such as *E. coli*, that is able to grown on pectin or tri-galacturonate as a sole source of carbon and/or energy may comprise one or more of the gene sequences contained within SEQ ID NOS:65 and

66, including biologically active fragments or variants thereof, such as optimized variants. SEQ ID NO:65 shows the nucleotide sequence of the kdgF-PaeX region from *Erwinia carotovora subsp. Atroseptica* SCRI1043. SEQ ID NO:66 shows the nucleotide sequence of ogl-kdgR from *Erwinia carotovora subsp. Atroseptica* SCRI1043.

In certain aspects, a recombinant microorganism, such as *E. coli*, that is able to grown on pectin or tri-galacturonate as a sole source of carbon and/or energy may comprise one or more genomic regions of *Erwinia chrysanthemi*, comprising several genes (*kdgF*, *kduI*, *kduD*, *pelW*, *togM*, *togN*, *togA*, *togB*, *kdgM*, *paeX*, *ogl*, and *kdgR*) encoding enzymes (*kduI*, *kduD*, *ogl*, *pelW*, and *paeX*), transporters (*togM*, *togN*, *togA*, *togB*, and *kdgM*), and regulatory proteins (*kdgR*) responsible for degradation of di- and trigalacturonate, as well as several genes (*pelA*, *pelE*, *paeY*, and *pem*) encoding pectate lyases (*pelA* and *pelE*), pectin acetylsterases (*paeY*), and pectin methylesterase (*pem*) (see Example 2).

Additional examples of isomerases that may be utilized herein include glucuronate isomerases, such as those in the family *uxaC*, as well as 4-deoxy-L-threo-5-hexulose uronate isomerases, such as those in the family *KduI*. Additional examples of reductases that may be utilized herein include tagaturonate reductases, such as those in the family *uxaB*. Additional examples of dehydratases that may be utilized herein include altronate dehydratases, such as those in the family *uxaA*. Additional examples of dehydrogenases that may be utilized herein include 2-deoxy-D-gluconate 3-dehydrogenases, such as those in the family *kduD*.

Certain aspects may also utilize recombinant microorganisms engineered to enhance the efficiency of the KDG degradation pathway. For instance, in bacteria, KDG is a common metabolic intermediate in the degradation of hexuronates such as D-glucuronate and D-galacturonate and enters into Entner Doudoroff pathway where it is converted to pyruvate and glyceraldehyde-3-phosphate (G3P). In this pathway, KDG is first phosphorylated by KDG kinase (KdgK) followed by its cleavage into pyruvate and glyceraldehyde-3-phosphate (G3P) using 2-keto-3-deoxy-D-6-phosphate-gluconate (KDPG) aldolase (KdgA). The expression of these enzymes concurrently with KDG

permease (*e.g.*, KdgT) is negatively regulated by KdgR and is almost none at basal level. The expression is dramatically (3-5-fold) induced upon the addition of hexuronates, and a similar result has been reported in *Pseudomonas* grown on alginate. Hence, to increase the conversion of KDG to pyruvate and G3P, the negative regulator KdgR may be removed. To further improve the pathway efficiency, exogenous copies of KdgK and KdgA may also be incorporated into a given recombinant microorganism.

In certain aspects, a recombinant microorganism that is able to grow on a polysaccharide (*e.g.*, alginate, pectin, etc) as a sole source of carbon may be capable of producing an increased amount of a given commodity chemical (*e.g.*, ethanol) while growing on that polysaccharide. For example, *E. coli* engineered to grown on alginate may be engineered to produced an increased amount of ethanol from alginate as compared to *E. coli* that is not engineered to grown on alginate (*see* Example 11). Thus, certain aspects include a recombinant microorganism that is capable of growing on alginate or pectin as a sole source carbon, and that is capable of producing an increased amount of ethanol, such as by comprising one or more genes encoding and expressing a pyruvate decarboxylase (*pdc*) and/or an alcohol dehydrogenase, including functional variants thereof. In certain aspects, such a recombinant microorganism may comprise a pyruvate decarboxylase (*pdc*) and two alcohol dehydrogenases (*adhA* and *adhB*) obtained from *Zymomonas mobilis*.

Embodiments of the present invention also include methods for converting polysaccharide to a suitable monosaccharide comprising, (a) obtaining a polysaccharide; (b) contacting the polysaccharide with a chemical catalysis or enzymatic pathway, thereby converting the polysaccharide to a first monosaccharide or oligosaccharide; and (c) contacting the first monosaccharide with a microbial system for a time sufficient to convert the first monosaccharide or oligosaccharide to the suitable monosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, and a polysaccharide transporter; and (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a

kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide.

In certain aspects of the present invention, aquatic or marine-biomass polysaccharides such as alginate may be chemically degraded using chemical catalysts such as acids. Similarly, biomass-derived pectin may be chemically degraded. For instance, the reaction catalyzed by chemical catalysts is typically through hydrolysis, as opposed to the β -elimination type of reactions catalyzed by enzymatic catalysts. Thus, certain embodiments may include boiling alginate or pectin with strong mineral acids to liberate carbon dioxide from D-mannuronate, thereby forming D-lyxose, a common sugar metabolite utilized by many microorganisms. Such embodiments may use, for example, formate, hydrochloric acid, sulfuric acid, in addition to other suitable acids known in the art as chemical catalysts.

An enzymatic pathway may utilize one or more enzymes described herein that are capable of catalyzing the degradation of polysaccharides, such as alginate or pectin.

Other embodiments may use variations of chemical catalysis similar to those described herein or known to a person skilled in the art, including improved or redesigned methods of chemical catalysis suitable for use with biomass related polysaccharides. Certain embodiments include those wherein the resulting monosaccharide uronate is D-mannuronate.

As noted above, the suitable monosaccharides or suitable oligosaccharides produced by the recombinant microorganisms and microbial systems of the present invention may be utilized as a feedstock in the production of commodity chemicals, such as biofuels, as well as commodity chemical intermediates. Thus, certain embodiments of the present invention relate generally to methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical, such as a biofuel, comprising, (a) obtaining a suitable monosaccharide or oligosaccharide; (b) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a

time sufficient to convert to the suitable monosaccharide to the biofuel, thereby converting the suitable monosaccharide to the biofuel.

Certain aspects include methods for converting a suitable monosaccharide to a first commodity chemical such as a biofuel, comprising, (a) obtaining a suitable monosaccharide; (b) contacting the suitable monosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the first commodity chemical, wherein the microbial system comprises one or more genes encoding a aldehyde or ketone biosynthesis pathway, thereby converting the suitable monosaccharide to the first commodity chemical.

In these and other related aspects, depending on the particular ketone or aldehyde biosynthesis pathway employed, the first commodity chemical may be further enzymatically and/or chemically reduced and dehydrated to a second commodity chemical. Examples of such second commodity chemicals include, but are not limited to, butene or butane; 1-phenylbutene or 1-phenylbutane; pentene or pentane; 2-methylpentene or 2-methylpentane; 1-phenylpentene or 1-phenylpentane; 1-phenyl-4-methylpentene or 1-phenyl-4-methylpentane; hexene or hexane; 2-methylhexene or 2-methylhexane; 3-methylhexene or 3-methylhexane; 2,5-dimethylhexene or 2,5-dimethylhexane; 1-phenylhexene or 1-phenylhexane; 1-phenyl-4-methylhexene or 1-phenyl-4-methylhexane; 1-phenyl-5-methylhexene or 1-phenyl-5-methylhexane; heptene or heptane; 2-methylheptene or 2-methylheptane; 3-methylheptene or 3-methylheptane; 2,6-dimethylheptene or 2,6-dimethylheptane; 3,6-dimethylheptene or 3,6-dimethylheptane; 3-methyloctene or 3-methyloctane; 2-methyloctene or 2-methyloctane; 2,6-dimethyloctene or 2,6-dimethyloctane; 2,7-dimethyloctene or 2,7-dimethyloctane; 3,6-dimethyloctene or 3,6-dimethyloctane; and cyclopentane or cyclopentene.

Certain embodiments of the present invention may also include methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical comprising (a) obtaining a suitable monosaccharide or oligosaccharide; (b) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time

sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises; (i) one or more genes encoding a biosynthesis pathway; (ii) one or more genes encoding and expressing a C-C ligation pathway; and (iii) one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical.

Certain aspects also include recombinant microorganism that comprise (i) one or more genes encoding a biosynthesis pathway; (ii) one or more genes encoding and expressing a C-C ligation pathway; and (iii) one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase. Certain aspects also include recombinant microorganisms that comprise the above pathways individually or in certain combinations, such as recombinant microorganism that comprises one or more genes encoding a biosynthesis pathway, as described herein. Certain aspects may also include recombinant microorganisms that comprise one or more genes encoding and expressing a C-C ligation pathway, as described herein. Certain aspects may also include include recombinant microorganisms that comprise one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, as described herein.

As for recombinant microorganisms that comprise combinations of the above-noted pathways, certain aspects may include recombinant microorganisms that comprise (i) one or more genes encoding a biosynthesis pathway; and (ii) one or more genes encoding and expressing a C-C ligation pathway. Certain aspects may also include recombinant microorganisms that comprise (i) one or more genes encoding and expressing a C-C ligation pathway; and (ii) one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase.

Certain aspects may also include recombinant microorganisms that comprise one or more individual components of a dehydration and reduction pathway, such as a recombinant microorganism that comprises a diol dehydrogenase, a diol dehydratase, or a secondary alcohol dehydrogenase. These and other microorganisms may be utilized, for example, to convert a suitable polysaccharide to a first commodity chemical, or an intermediate thereof, or to convert a first commodity chemical, or an intermediate thereof, to a second commodity chemical.

Merely by way of illustration, a recombinant microorganism comprising a C-C ligation pathway may be utilized to convert butanal into a first commodity chemical, or an intermediate thereof, such as 5-hydroxy-4-octanone, which can then be converted into a second commodity chemical, or intermediate thereof, by any suitable pathway. As a further example, a recombinant microorganism comprising a C-C ligation pathway and a diol hydrogenase may be utilized for the sequential conversion of butanal into 5-hydroxy-4-octanone and then 4,5-octanediol. Examples of recombinant microorganisms that comprise these and other various combinations of the individual pathways described herein, as well as various combinations of the individual components of those pathways, will be apparent to those skilled in the art, and may also be found in the Examples.

Also included are methods of converting a polysaccharide to a first commodity chemical, or an intermediate thereof, such as by utilizing a recombinant microorganism that comprises an aldehyde or ketone biosynthesis pathway. Also included are methods of converting a first commodity chemical, or intermediate thereof, to a second commodity chemical, such as by utilizing a recombinant microorganism that optionally comprises a biosynthesis pathway, optionally comprises C-C ligation pathway and/or optionally comprises one or more of the individual components of a dehydration and reduction pathway. Merely by way of illustration, a recombinant microorganism comprising an exogenous C-C ligase (*e.g.*, benzaldehyde lyase from *Pseudomonas fluorescens*) could be utilized in a method to convert a first commodity chemical such as 3-methylbutanal to a second commodity chemical such as 2,7-

dimethyl-5-hydroxy-4-octanone. Along this line of illustration, the same or different recombinant microorganism comprising a diol dehydrogenase could be utilized in a method to convert 2,7-dimethyl-5-hydroxy-4-octanone to another commodity chemical such as 2,7-dimethyl-4,5-octanediol (see Table 2 for other examples). As an additional illustrative example, a recombinant microorganism comprising an exogenous secondary alcohol dehydrogenase could be utilized in a method to convert a first commodity chemical such as 2,7-dimethyl-4-octanone to a second commodity chemical such as 2,7-dimethyloctanol.

Embodiments of a microbial system or isolated microorganism of the present application may include a naturally-occurring biosynthesis pathway, and/or an engineered, reconstructed, or re-designed biosynthesis pathway that has been optimized for improved functionality.

Embodiments of a microbial system or recombinant microorganism of the present invention may include a natural or reconstructed biosynthesis pathway, such as a butyraldehyde biosynthesis pathway, as found in such microorganisms as *Clostridium acetobutylicum* and *Streptomyces coelicolor*. In explanation, butyrate and butanol are the common fermentation products of certain bacterial species such as *Clostridia*, in which the production of butyrate and butanol is mediated by a synthetic thiolase dependent pathway characteristically similar to fatty acid degradation pathway. Such pathways may be initiated with the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA, which is catalyzed by thiolase. Acetoacetyl-CoA is then reduced to β -hydroxy butyryl-CoA, which is catalyzed by NAD(P)H dependent β -hydroxy butyryl-CoA dehydrogenase (HBDH). Crotonase catalyzes dehydration from β -hydroxy butyryl-CoA to form crotonyl-CoA. Further reduction catalyzed by NADH-dependent butyryl-CoA dehydrogenase (BCDH) saturates the double bond at C2 of crotonyl-CoA to form butyryl-CoA.

In certain embodiments, thiolase, the first enzyme in this pathway, may be overexpressed to maximize production. In certain embodiments, thiolase may over-expressed in *E. coli*. In this regard, all three enzymes (e.g., HBDH, crotonase, and

BCDH) catalyzing the following reaction steps are found in *Clostridium acetobutylicum* ATCC824. In certain embodiments, BDH, crotonase, and BCDH may be expressed or over-expressed in a suitable microorganism such as *E. coli*. Alternatively, a short-chain aliphatic acyl-CoA dehydrogenase derived from *Pseudomonas putida* KT2440 may be utilized in other embodiments of a microbial system or isolated microorganism of the present application.

Further to this end, butyryl-CoA in *Clostridia* may be readily converted to butanol and/or butyrate by at least a few different pathways. In one pathway, butyryl-CoA is directly reduced to butyraldehyde catalyzed by NADH dependent CoA-acylating aldehyde dehydrogenase (ALDH). Butyraldehyde may be further reduced to butanol by NADH-dependent butanol dehydrogenase. Although CoA-acylating ALDH catalyzes the one step reduction of butyryl-CoA to butyraldehyde, the incorporation of CoA-acylating ALDH to the microbial system may result in acetaldehyde formation because of its promiscuous acetyl-CoA deacylating activity. In certain embodiments, the formation of acetaldehyde may be minimized by functionally redesigning the relevant enzyme(s).

Butyryl-CoA in other biosynthesis pathways is deacylated to form butyryl phosphate catalyzed by phosphotransbutyrylase. Butyryl phosphate is then hydrolyzed by reversible butyryl phosphate kinase to form butyrate. This reaction is coupled with ATP generation from ADP. The butyrate formation through these enzymes is known to be significantly more specific. Certain embodiments may comprise phosphotransbutyrylase and butyryl phosphate kinase to the microbial system. In other embodiments, butyrate may be directly formed from butyryl-CoA by short chain acyl-CoA thioesterase.

Butyrate in *Clostridia* may also be sequentially reduced to butanol, which is catalyzed by a single alcohol/aldehyde dehydrogenase. Certain embodiments may comprise short chain aldehyde dehydrogenase from other bacteria such as *Pseudomonas putida* to complement the production of butyraldehyde in the microbial system. One potential concern in using short chain aldehyde dehydrogenase involves

the possible formation of acetaldehyde from acetate. Certain embodiments may be directed to minimizing the acetate formation in the microbial system, for example, by deleting several genes encoding enzymes involved in the acetate production.

Moreover, there are multiple routes in *E.coli* to form acetate, one of which is mediated by pyruvate oxygenase (POXB) from pyruvate, whereas another is mediated by phosphotransacetylase (PTA) and acetyl phosphate kinase (ACKA) from acetyl-CoA. The acetate production from *E. coli* mutant strains with *poxB*⁻, *pta*⁻, and *ackA*⁻ are significantly diminished. In addition, incorporation of acetyl-CoA synthase (ACS) which catalyses the acetyl-CoA formation from acetate is also known to significantly reduce the accumulation of acetate. Certain embodiments may comprise a microbial system or isolated microorganism with deleted POXB, PTA, and/or ACKA genes, and other embodiments may also comprise, separately or together with the deleted genes, one or more genes encoding and expressing ACS.

A microbial system or recombinant microorganism provided herein may also comprise a glutaraldehyde biosynthesis pathway. As one example, *Saccharomyces cerevisiae* has a lysine biosynthetic pathway in which acetyl-CoA is initially condensed to α -ketoglutarate, a common metabolite in citric acid cycle, to form homocitrate. This reaction is catalyzed by homocitrate synthase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans*. Homoaconitase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans* catalyzes the conversion between homocitrate and homoisocitrate. Homoisocitrate is then oxidatively decarboxylated to form 2-ketoadipate, which is catalyzed by homoisocitrate dehydrogenase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans*. Homoisocitrate is also oxidatively decarboxylated to form glutaryl-CoA, which may be catalyzed by homoisocitrate dehydrogenase. Thus, certain embodiments may comprise a homocitrate synthase, a homoaconitase, and/or a homoisocitrate dehydrogenase.

Further to this end, in synthesizing 2-keto-adipicsemialdehyde, 2-ketoadipate is reduced to 2-keto-adipicsemialdehyde. This reaction can be catalyzed by dialdehyde dehydrogenase, which, for example, may be isolated from *Agrobacterium*

tumefaciens C58. Thus, certain embodiments may incorporate dialdehyde dehydrogenases into a microbial system or recombinant microorganism.

In synthesizing glutaraldehyde, Acyl-CoA thioesterases (ACOT) may also catalyze the hydrolysis of glutaryl-CoA. The genes encoding ω -carboxylic acyl-CoA specific peroxisomal ACOTs are found in many mammalian species; both ACOT4 and ACOT8 derived from mice have been previously expressed in *E. coli* and shown that both enzymes are highly active on the hydrolysis of glutaryl-CoA to form glutarate. Certain embodiments may comprise one or more Acyl-CoA thioesterases.

Glutarate is sequentially reduced to glutaraldehyde. This reaction can be catalyzed by glutaraldehyde dehydrogenase (CpnE), which, for example, may be isolated from *Comomonas* sp. Strain NCIMB 9872. Certain embodiments may incorporate glutaraldehyde dehydrogenases such as CpnE into a microbial system or isolated microorganism. Other embodiments may comprise both ACOT and CpnE enzymes. Other embodiments may comprise CpnE enzymes redesigned to catalyze the reduction of 1-hydroxy propanoate and succinate to 1-hydroxy propanal and succinicaldehyde.

In certain aspects, the biosynthesis pathway may include an aldehyde biosynthesis pathway, a ketone biosynthesis pathway, or both. In certain aspects, the biosynthesis pathway may include one or more of an acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, 4-methylpentaldehyde, phenylacetaldehyde, 2-phenyl acetaldehyde, 2-(4-hydroxyphenyl) acetaldehyde, 2-Indole-3-acetaldehyde, glutaraldehyde, 5-amino-pentaldehyde, succinate semialdehyde, and/or succinate 4-hydroxyphenyl acetaldehyde biosynthesis pathway, including various combinations thereof.

With regard to combinations of biosynthesis pathways, a biosynthesis pathway may comprise an acetaldehyde biosynthesis pathway in combination with at least one of a propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetaldehyde biosynthesis pathway.

In certain aspects, a biosynthesis pathway may comprise a propionaldehyde biosynthesis pathway in combination with at least one of a butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a butyraldehyde biosynthesis pathway in combination with at least one of an isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise an isobutyraldehyde biosynthesis pathway in combination with at least one of a 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a 2-methyl-butyraldehyde biosynthesis pathway in combination with at least one of a 3-methyl-butyraldehyde or a phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a 3-methyl-butyraldehyde biosynthesis pathway in combination with a phenylacetoaldehyde biosynthesis pathway.

In certain aspects, a propionaldehyde biosynthesis pathway may comprise a threonine deaminase (*ilvA*) gene from an organism such as *Escherichia coli* and a keto-isovalerate decarboxylase (*kivd*) gene from an organism such as *Lactococcus lactis*, and/or functional variants of these enzymes, including homologs or orthologs thereof, as well as optimized variants. These enzymes may be utilized generally to convert L-threonine to propionaldehyde.

In certain aspects, a butyraldehyde biosynthesis pathway may comprise at least one of a thiolase (*atoB*) gene from an organism such as *E. coli*, a β -hydroxy butyryl-CoA dehydrogenase (*hbd*) gene, a crotonase (*crt*) gene, a butyryl-CoA dehydrogenase (*bcd*) gene, an electron transfer flavoprotein A (*etfA*) gene, and/or an electron transfer flavoprotein B (*etfB*) gene from an organism such as *Clostridium acetobutyricum* (e.g., ATCC 824), as well as a coenzyme A-linked butyraldehyde dehydrogenase (*ald*) gene from an organism such as *Clostridium beijerinckii acetobutyricum* ATCC 824. In certain aspects, a coenzyme A-linked alcohol

dehydrogenase (*adhE2*) gene from an organism such as *Clostridium acetobutyricum* ATCC 824 may be used as an alternative to an *ald* gene.

In certain aspects, an isobutyraldehyde biosynthetic pathway may comprise an acetolactate synthase (*alsS*) from an organism such as *Bacillus subtilis* or an *als* gene from an organism such as *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (codon usage may be optimized for *E. coli* protein expression). Such a pathway may also comprise acetolactate reductoisomerase (*ilvC*) and/or 2,3-dihydroxyisovalerate dehydratase (*ilvD*) genes from an organism such as *E. coli*, as well as a keto-isovalerate decarboxylase (*kivd*) gene from an organism such as *Lactococcus lactis*.

In certain aspects, a 3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathway may comprise an acetolactate synthase (*alsS*) gene from an organism such as *Bacillus subtilis* or an (*als*) gene from an organism such as *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (codon usage may be optimized for *E. coli* protein expression). Certain aspects of such a pathway may also comprise acetolactate reductoisomerase (*ilvC*), 2,3-dihydroxyisovalerate dehydratase (*ilvD*), isopropylmalate synthase (*LeuA*), isopropylmalate isomerase (*LeuC* and *LeuD*), and 3-isopropylmalate dehydrogenase (*LeuB*) genes from an organism such as *E. coli*, as well as a keto-isovalerate decarboxylase (*kivd*) from an organism such as *Lactococcus lactis*.

In certain aspects, a phenylacetaldehyde and 4-hydroxyphenylacetaldehyde biosynthesis pathway may comprise one or more of 3-deoxy-7-phosphoheptulonate synthase (*aroF*, *aroG*, and *aroH*), 3-dehydroquinate synthase (*aroB*), a 3-dehydroquinate dehydratase (*aroD*), dehydroshikimate reductase (*aroE*), shikimate kinase II (*aroL*), shikimate kinase I (*aroK*), 5-enolpyruvylshikimate-3-phosphate synthetase (*aroA*), chorismate synthase (*aroC*), fused chorismate mutase P/prephenate dehydratase (*pheA*), and/or fused chorismate mutase T/prephenate dehydrogenase (*tyrA*) genes from an organism such as *E. coli*, as well as a keto-isovalerate decarboxylase (*kivd*) from an organism such as *Lactococcus lactis*.

In certain aspects, such as for the ultimate production of 1,10-diamino-5-decanol and 1,10-dicarboxylic-5-decanol, a biosynthesis pathway may comprise one or more homocitrate synthase, homoaconitate hydratase, homoisocitrate dehydrogenase, and/or homoisocitrate dehydrogenase genes from an organism such as *Deinococcus radiodurans* and/or *Thermus thermophilus*, as well as a keto-adipate decarboxylase gene, a 2-aminoadipate transaminase gene, and a L-2-Aminoadipate-6-semialdehyde: NAD⁺ 6-oxidoreductase gene. Such a biosynthesis pathway would be able to convert α -ketoglutarate to 5-aminopentaldehyde.

In certain aspects, such as for one step in cyclopentanol production, a α -ketoadipate semialdehyde biosynthesis pathway may comprise homocitrate synthase (*hcs*), homoaconitate hydratase, and homoisocitrate dehydrogenase genes from an organism such as *Deinococcus radiodurans* and/or *Thermus thermophilus*, and an α -ketoadipate semialdehyde dehydrogenase gene. Such a biosynthesis pathway would be able to convert acetyl-CoA and α -ketoglutarate to α -ketoadipate semialdehyde.

For the production of certain commodity chemicals, such as 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol, among other similar chemicals, a biosynthesis pathway (*e.g.*, aldehyde biosynthesis pathway) may optionally or further comprise one or more genes encoding a carboxylase enzyme, such as an indole-3-pyruvate decarboxylase (IPDC). An IPDC may be obtained, for example, from such microorganisms as *Azospirillum brasilense* and *Paenibacillus polymyxa* E681. In this regard, an IPDC may be utilized to more efficiently catalyze the decarboxylation of various carboxylic acids to form the corresponding aldehyde, which can be further converted to a commodity chemical by a reductase or dehydrogenase, as detailed herein.

In certain aspects, a 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-)ethanol biosynthesis pathway may comprise a transketolase (*tktA*), a 3-deoxy-7-phosphoheptulonate synthase (*aroF*, *aroG*, and *aroH*), 3-dehydroquinate synthase (*aroB*), a 3-dehydroquinate dehydratase (*aroD*), a dehydroshikimate reductase (*aroE*), a shikimate kinase II (*aroL*), a shikimate kinase I (*aroK*), a 5-

enolpyruvylshikimate-3-phosphate synthetase (*aroA*), a chorismate synthase (*aroC*), a fused chorismate mutase P/prephenate dehydratase (*pheA*), and a fused chorismate mutase T/prephenate dehydrogenase (*tyrA*) genes from *E. coli*, keto-isovalerate decarboxylase (*kivd*) from *Lactococcus lactis*, alcohol dehydrogenase (*adh2*) from *Saccharomyces cerevisiae*, Indole-3-pyruvate decarboxylase (*ipdc*) from *Azospirillum brasilense*, phenylethanol reductase (*par*) from *Rhodococcus sp.* ST-10, and a benzaldehyde lyase (*bal*) from *Pseudomonas fluorescense*.

As for all other pathways described herein, the components for each of the biosynthesis pathways described herein may be present in a recombinant microorganism either endogenously or exogenously. To improve the efficiency of a given biosynthesis pathway, endogenous genes, for example, may be up-regulated or over-expressed, such as by introducing an additional (*i.e.*, exogenous) copy of that endogenous gene into the recombinant microorganism. Such pathways may also be optimized by altering via mutagenesis the endogenous version of a gene to improve functionality, followed by introduction of the altered gene into the microorganism. The expression of endogenous genes may be up or down-regulated, or even eliminated, according to known techniques in the art and described herein. Similarly, the expression levels of exogenously provided genes may be regulated as desired, such as by using various constitutive or inducible promoters. Such genes may also be "codon-optimized," as described herein and known in the art. Also included are functional naturally-occurring variants of the genes and enzymes described herein, including homologs or orthologs thereof.

Certain embodiments of a microbial system or isolated microorganism may comprise a CC-ligation pathway. In certain aspects, a CC-ligation pathway may comprise a ThDP-dependent enzyme, such as a C-C ligase, or an optimized C-C ligase. For example, eight-carbon unit molecules (butyroids) may be made from condensing together two four-carbon unit molecules (butyraldehydes). ThDP-dependent enzymes are a group of enzymes known to catalyze both breaking and formation of C-C bonds and have been utilized as catalysts in chemoenzymatic syntheses. The spectrum of

chemical reactions that these enzymes catalyze ranges from decarboxylation of α -keto acids, oxidative decarboxylation, carboligation, and to the cleavage of C-C bonds.

To provide a few examples, benzaldehyde lyase (BAL) from *Pseudomonas fluorescens*, benzoylformate decarboxylase (BFD) from *Pseudomonas putida*, and pyruvate decarboxylase (PDC) from *Zymomonas mobilis* may catalyze a carboligation reaction between two aldehydes. BAL accepts the broadest spectrum of aldehydes as substrates among these three enzymes ranging from substituted benzaldehyde to acetaldehyde, among others, as shown herein. BAL catalyzes stereospecific carboligation reaction between two aldehydes and forms α -hydroxy ketone with over 99 %ee for R-configuration. The benzoin formation from two benzaldehyde molecules is a favored reaction catalyzed by BAL and proceeds as fast as $320 \mu\text{mol (benzoin) mg (protein)}^{-1} \text{ min}^{-1}$. The formation of α -hydroxy ketone may be carried out using many different aldehydes, including butyraldehyde.

BFD and PCD may also catalyze the carboligation reactions between two aldehyde molecules. BFD and PCD accept relatively larger and smaller aldehyde molecules, respectively. With the presence of benzaldehyde and acetaldehyde, BFD catalyzes the formation of benzoin and (S)- α -hydroxy phenylpropanone (2S-HPP), whereas PCD catalyzes the formation of (R)- α -hydroxy phenylpropanone (2R-HPP) and (R)- α -hydroxy 2-butanone (acetoin). As detailed below, certain microbial systems or isolated microorganisms of the present application may comprise natural or optimized C-C ligases (ThDP-dependent enzymes) selected from benzaldehyde lyase (BAL) from *Pseudomonas fluorescens*, benzoylformate decarboxylase (BFD) from *Pseudomonas putida*, and pyruvate decarboxylase (PDC) from *Zymomonas mobilis*. Other embodiments may comprise a benzaldehyde lyase (BAL) from *Pseudomonas fluorescens* (see SEQ ID NOS:143-144, showing the nucleotide and polypeptide sequences, respectively) including biologically active variants thereof, such as optimized variants.

A C-C ligation pathway of the present invention typically comprises one or more C-C ligases, such as a lyase enzyme. Exemplary lyases include, but are not

limited to, acetoaldehyde lyases, propionaldehyde lyases, butyraldehyde lyases, isobutyraldehyde lyases, 2-methyl-butyraldehyde lyases, 3-methyl-butyraldehyde lyases (isoveraldehyde), phenylacetaldehyde lyases, α -keto adipate carboxylyases, pentaldehyde lyases, 4-methyl-pentaldehyde lyases, hexaldehyde lyases, heptaldehyde lyases, octaldehyde lyases, 4-hydroxyphenylacetaldehyde lyases, indoleacetaldehyde lyases, indolephenylacetaldehyde lyases. In certain aspects, a selected CC-ligase or lyase enzyme may have one or more of the above exemplified lyase activities, such as acetoaldehyde lyase activity, a propionaldehyde lyase activity, a butyraldehyde lyase activity, and/or an isobutyraldehyde lyase activity, among others.

As noted above, a C-C ligase may comprise a benzaldehyde lyase, such as a benzaldehyde lyase isolated from *Pseudomonas fluorescens* (SEQ ID NOS:143-144), as well as biologically active fragments or variants of this reference sequence, such as optimized variants of a benzaldehyde lyase. In this regard, certain aspects may comprise nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:143-144, and which are capable of catalyzing a carboligation reaction, or which possess C-C lyase activity, as described herein. In certain aspects, a BAL enzyme will comprise one or more conserved amino acid residues, including G27, E50, A57, G155, P162, P234, D271, G277, G422, G447, D448, and/or G512.

Pseudomonas fluorescens is able to grow on R-benzoin as the sole carbon and energy source because it harbours the enzyme benzaldehyde lyase that cleaves the acyloin linkage using thiamine diphosphate (ThDP) as a cofactor. In the reverse reaction, as utilized herein, benzaldehyde lyase catalyses the carboligation of two aldehydes with high substrate and stereospecificity. Structure-based comparisons with other proteins show that benzaldehyde lyase belongs to a group of closely related ThDP-dependent enzymes. The ThDP cofactors of these enzymes are fixed at their two ends in separate domains, suspending a comparatively mobile thiazolium ring between them. While the residues binding the two ends of ThDP are well conserved, the lining of the active centre pocket around the thiazolium moiety varies greatly within the

group. The active sites for BAL have been described, for example, in Kneen *et al.* (*Biochimica et Biophysica Acta* 1753:263-271, 2005) and Brandt *et al.* (*Biochemistry* 47:7734-43, 2008). Benzaldehyde lyase derived from *Pseudomonas fluorescens* has been demonstrated herein to at least have an acetaldehyde lyase activity, a propionaldehyde lyase activity, a butyraldehyde lyase activity, a 3-methyl-butyraldehyde lyase activity, a pentaldehyde lyase activity, a 4-methylpentaldehyde lyase activity, a hexaldehyde lyase activity, a phenylacetaldehyde lyase activity, and an octaldehyde lyase activity (*see* Table 2), among other *in vivo* lyase activities (*see* Figures 48-55).

In certain aspects, a C-C ligase, such as BAL derived from *Pseudomonas fluorescens*, BFD derived from *Pseudomonas putida*, or PDC derived from *Zymomonas mobilis* may comprise a lyase with a combination of lyase activities, such as a lyase having both a propionaldehyde lyase activity and a 3-methyl-butyraldehyde lyase activity, among other combinations and activities, such as those exemplary combinations detailed herein. Merely by way of illustration, a lyase having a combination of lyase activities may be referred to herein as a propionaldehyde/3-methyl-butyraldehyde lyase.

A dehydration and reduction pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, may be utilized to further convert an aldehyde, ketone, or corresponding alcohol, to a commodity chemical, such as a biofuel.

To this end, a dehydration and reduction pathway may comprise one or more diol dehydrogenases. A "diol dehydrogenase" refers generally to an enzyme that catalyzes the reversible reduction and oxidation of a α -hydroxy ketone and/or its corresponding diol. Certain embodiments of a microbial system or isolated microorganism may comprise genes encoding a diol dehydrogenase that specifically catalyzes the reduction of α -hydroxy-ketones, including, for example, a 4, 5, octanediol dehydrogenase. Diol dehydrogenases, such as 4, 5, octanediol dehydrogenase, may be isolated from a variety of organisms and incorporated into a microbial system or

isolated microorganism. A particular group of alcohol dehydrogenases has a characteristic ability to oxidize various α -hydroxy alcohols and reduce various α -hydroxy ketones and α -keto ketones. As such, the recitation "diol dehydrogenase" may also encompass such alcohol dehydrogenases.

By way of example regarding diol dehydrogenases from exemplary organisms, glycerol dehydrogenase isolated from *Hansenula ofunaensis* has broad substrate specificity and is capable of catalyzing the oxidation of various α -hydroxy alcohols, including 1, 2-octane, as well as the reduction of various α -hydroxy ketones and α -keto ketones, including 3-hydroxy-2-butanone and 3, 4-hexanedione, with the activity comparable to its native substrates, glycerol and dihydroxyacetone, respectively (40 - 200%). As one further example, glycerol dehydrogenase discovered in *Hansenula polymorpha* DI-1 works similarly. In certain embodiments, a microbial system or recombinant microorganism may comprise a glycerol dehydrogenase gene isolated from *Hansenula ofunaensis*, a glycerol dehydrogenase isolated from *Hansenula polymorpha* DI-1 and/or a meso-2,3-butane diol dehydrogenase from *Klebsiella pneumoniae*. In other embodiments, a microbial system or isolated microorganism may comprise a 4, 5, octanediol dehydrogenase, among others detailed herein. Diol dehydrogenases may also be obtained from *Lactobacillus brevis* ATCC 367, *Pseudomonas putida* KT2440, and *Klebsiella pneumoniae* MGH78578), as described herein (see Example 5).

Exemplary diol dehydrogenases include, but are not limited to, 2,3-butanediol dehydrogenase, 3,4-hexanediol dehydrogenase, 4,5-octanediol dehydrogenase, 5,6-decanediol dehydrogenase, 6,7-dodecanediol dehydrogenase, 7,8-tetradecanediol dehydrogenase, 8,9-hexadecanediol dehydrogenase, 2,5-dimethyl-3,4-hexanediol dehydrogenase, 3,6-dimethyl-4,5-octanediol dehydrogenase, 2,7-dimethyl-4,5-octanediol dehydrogenase, 2,9-dimethyl-5,6-decanediol dehydrogenase, 1,4-diphenyl-2,3-butanediol dehydrogenase, bis-1,4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, 1,4-diindole-2,3-butanediol dehydrogenase, 1, 2-cyclopentanediol dehydrogenase, 2,3-pentanediol dehydrogenase, 2,3-hexanediol dehydrogenase, 2,3-

heptanediol dehydrogenase, 2,3-octanediol dehydrogenase, 2,3-nonanediol dehydrogenase, 4-methyl-2,3-pentanediol dehydrogenase, 4-methyl-2,3-hexanediol dehydrogenase, 5-methyl-2,3-hexanediol dehydrogenase, 6-methyl-2,3-heptanediol dehydrogenase, 1-phenyl -2,3-butanediol dehydrogenase, 1-(4-hydroxyphenyl) -2,3-butanediol dehydrogenase, 1-indole -2,3-butanediol dehydrogenase, 3,4-heptanediol dehydrogenase, 3,4-octanediol dehydrogenase, 3,4-nonanediol dehydrogenase, 3,4-decanediol dehydrogenase, 3,4-undecanediol dehydrogenase, 2-methyl-3,4-hexanediol dehydrogenase, 5-methyl-3,4-heptanediol dehydrogenase, 6-methyl-3,4-heptanediol dehydrogenase, 7-methyl-3,4-octanediol dehydrogenase, 1-phenyl -2,3-pentanediol dehydrogenase, 1-(4-hydroxyphenyl) -2,3-pentanediol dehydrogenase, 1-indole-2,3-pentanediol dehydrogenase, 4,5-nonanediol dehydrogenase, 4,5-decanediol dehydrogenase, 4,5-undecanediol dehydrogenase, 4,5-dodecanediol dehydrogenase, 2-methyl-3,4-heptanediol dehydrogenase, 3-methyl-4,5-octanediol dehydrogenase, 2-methyl-4,5-octanediol dehydrogenase, 8-methyl-4,5-nonanediol dehydrogenase, 1-phenyl-2,3-hexanediol dehydrogenase, 1-(4-hydroxyphenyl) -2,3-hexanediol dehydrogenase, 1-indole-2,3-hexanediol dehydrogenase, 5,6-undecanediol dehydrogenase, 5,6-undecanediol dehydrogenase, 5,6-tridecanediol dehydrogenase, 2-methyl-3,4-octanediol dehydrogenase, 3-methyl-4,5-nonanediol dehydrogenase, 2-methyl-4,5-nonanediol dehydrogenase, 2-methyl-5,6-decanediol dehydrogenase, 1-phenyl-2,3-heptanediol dehydrogenase, 1-(4-hydroxyphenyl) -2,3-heptanediol dehydrogenase, 1-indole-2,3-heptanediol dehydrogenase, 6,7-tridecanediol dehydrogenase, 6,7-tetradecanediol dehydrogenase, 2-methyl-3,4-nonanediol dehydrogenase, 3-methyl-4,5-decanediol dehydrogenase, 2-methyl-4,5-decanediol dehydrogenase, 2-methyl-5,6-undecanediol dehydrogenase, 1-phenyl-2,3-octanediol dehydrogenase, 1-(4-hydroxyphenyl) -2,3-octanediol dehydrogenase, 1-indole-2,3-octanediol dehydrogenase, 7,8-pentadecanediol dehydrogenase, 2-methyl-3,4-decanediol dehydrogenase, 3-methyl-4,5-undecanediol dehydrogenase, 2-methyl-4,5-undecanediol dehydrogenase, 2-methyl-5,6-dodecanediol dehydrogenase, 1-phenyl-2,3-nonanediol dehydrogenase, 1-(4-hydroxyphenyl) -2,3-nonanediol dehydrogenase, 1-indole-2,3-nonanediol dehydrogenase, 2-methyl-3,4-undecanediol dehydrogenase, 3-

methyl-4,5-dodecanediol dehydrogenase, 2-methyl-4,5-dodecanediol dehydrogenase, 2-methyl-5,6-tridecanediol dehydrogenase, 1-phenyl-2,3-decanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-decanediol dehydrogenase, 1-indole-2,3-decanediol dehydrogenase, 2,5-dimethyl-3,4-heptanediol dehydrogenase, 2,6-dimethyl-3,4-heptanediol dehydrogenase, 2,7-dimethyl-3,4-octanediol dehydrogenase, 1-phenyl-4-methyl-2,3-pentanediol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydrogenase, 1-indole-4-methyl-2,3-pentanediol dehydrogenase, 2,6-dimethyl-4,5-octanediol dehydrogenase, 3,8-dimethyl-4,5-nonanediol dehydrogenase, 1-phenyl-4-methyl-2,3-hexanediol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydrogenase, 1-indole-4-methyl-2,3-hexanediol dehydrogenase, 2,8-dimethyl-4,5-nonanediol dehydrogenase, 1-phenyl-5-methyl-2,3-hexanediol dehydrogenase, 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydrogenase, 1-indole-5-methyl-2,3-hexanediol dehydrogenase, 1-phenyl-6-methyl-2,3-heptanediol dehydrogenase, 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydrogenase, 1-indole-6-methyl-2,3-heptanediol dehydrogenase, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydrogenase, 1-indole-4-phenyl-2,3-butanediol dehydrogenase, 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, 1,10-diamino-5,6-decanediol dehydrogenase, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 2,3-hexanediol-1,6-dicarboxylic acid dehydrogenase, and the like.

In certain aspects, a selected diol dehydrogenase enzyme may have one or more of the above exemplified diol dehydrogenase activities, such as a 2,3-butanediol dehydrogenase activity, a 3,4-hexanediol dehydrogenase activity, and/or a 4,5-octanediol dehydrogenase activity, among others.

In certain aspects, a recombinant microorganism may comprise a diol dehydrogenase encoded by a nucleotide reference sequence selected from SEQ ID NO:97, 99, and 101, or an enzyme having a polypeptide sequence selected from SEQ ID NO:98, 100, and 102, including biologically active fragments or variants thereof, such as optimized variants. Certain aspects may also comprises nucleotide sequences or

polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:97-102.

Other embodiments may comprise re-designed diol dehydrogenases for reduction of 1-hydroxy propanal, succinaldehyde, and glutaraldehyde to 1,3-propanediol, 1,4-butanediol, and 1,5 pentanediol, respectively, among others.

A dehydration and reduction pathway, as described herein, may comprise one or more diol dehydratases. A "diol dehydratase" refers generally to an enzyme that catalyzes the irreversible dehydration of diols. For instance, this enzyme may serve to dehydrate octanediol to form 4-octane. It has been recognized that there are at least two different types of diol dehydratases: a group dependent on and independent of coenzyme B12 for its catalysis. Coenzyme B12 dependent diol dehydratases are known to catalyze a radical mediated dehydration reaction from α -hydroxy alcohol to aldehydes or ketones. For example, a diol dehydratase from *Klebsiella pneumoniae* catalyzes the dehydration of glycerol to form β -hydroxypropyl aldehyde, accepts 2,3-butanediol as a substrate, and catalyzes the dehydration reaction to form 2-butanone.

As a further example, *Clostridium butylicum* contains coenzyme B12 independent diol dehydratases. Figure 46 shows the *in vivo* biological activity of coenzyme B12 independent diol dehydratase (*dhaB1*) and activator (*dhaB2*) isolated from *Clostridium butylicum* (see Example 9). 46A shows the *in vivo* production of 1-propanol from 1,2-propanediol, Figure 46B shows the *in vivo* production of 2-butanol from meso-2,3 butanediol, and Figure 46C shows the *in vivo* production of cyclopentanone from trans-1,2-cyclopentanediol.

Thus, certain embodiments of the present invention may comprise optimized or redesigned diol dehydratases that accommodate various substrates, such as 4,5-octanediol as a substrate, and may include diol dehydratases isolated and/or optimized from *Klebsiella pneumoniae* and *Clostridium butylicum*, among other organisms described herein and known in the art.

Exemplary diol dehydratases include, but are not limited to, 2,3-butanediol dehydratase, 3,4-hexanediol dehydratase, 4,5-octanediol dehydratase, 5,6-decanediol dehydratase, 6,7-dodecanediol dehydratase, 7,8-tetradecanediol dehydratase, 8,9-hexadecanediol dehydratase, 2,5-dimethyl-3,4-hexanediol dehydratase, 3,6-dimethyl-4,5-octanediol dehydratase, 2,7-dimethyl-4,5-octanediol dehydratase, 2,9-dimethyl-5,6-decanediol dehydratase, 1,4-diphenyl-2,3-butanediol dehydratase, bis-1,4-(4-hydroxyphenyl) -2,3-butanediol dehydratase, 1,4-diindole-2,3-butanediol dehydratase, 1, 2-cyclopentanediol dehydratase, 2,3-pentanediol dehydratase, 2,3-hexanediol dehydratase, 2,3-heptanediol dehydratase, 2,3-octanediol dehydratase, 2,3-nonanediol dehydratase, 4-methyl-2,3-pentanediol dehydratase, 4-methyl-2,3-hexanediol dehydratase, 5-methyl-2,3-hexanediol dehydratase, 6-methyl-2,3-heptanediol dehydratase, 1-phenyl -2,3-butanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-butanediol dehydratase, 1-indole -2,3-butanediol dehydratase, 3,4-heptanediol dehydratase, 3,4-octanediol dehydratase, 3,4-nonanediol dehydratase, 3,4-decanediol dehydratase, 3,4-undecanediol dehydratase, 2-methyl-3,4-hexanediol dehydratase, 5-methyl-3,4-heptanediol dehydratase, 6-methyl-3,4-heptanediol dehydratase, 7-methyl-3,4-octanediol dehydratase, 1-phenyl -2,3-pentanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-pentanediol dehydratase, 1-indole-2,3-pentanediol dehydratase, 4,5-nonanediol dehydratase, 4,5-decanediol dehydratase, 4,5-undecanediol dehydratase, 4,5-dodecanediol dehydratase, 2-methyl-3,4-heptanediol dehydratase, 3-methyl-4,5-octanediol dehydratase, 2-methyl-4,5-octanediol dehydratase, 8-methyl-4,5-nonanediol dehydratase, 1-phenyl-2,3-hexanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-hexanediol dehydratase, 1-indole-2,3-hexanediol dehydratase, 5,6-undecanediol dehydratase, 5,6-undecanediol dehydratase, 5,6-tridecanediol dehydratase, 2-methyl-3,4-octanediol dehydratase, 3-methyl-4,5-nonanediol dehydratase, 2-methyl-4,5-nonanediol dehydratase, 2-methyl-5,6-decanediol dehydratase, 1-phenyl-2,3-heptanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-heptanediol dehydratase, 1-indole-2,3-heptanediol dehydratase, 6,7-tridecanediol dehydratase, 6,7-tetradecanediol dehydratase, 2-methyl-3,4-nonanediol dehydratase, 3-methyl-4,5-decanediol dehydratase, 2-methyl-4,5-decanediol dehydratase, 2-methyl-5,6-undecanediol

dehydratase, 1-phenyl-2,3-octanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-octanediol dehydratase, 1-indole-2,3-octanediol dehydratase, 7,8-pentadecanediol dehydratase, 2-methyl-3,4-decanediol dehydratase, 3-methyl-4,5-undecanediol dehydratase, 2-methyl-4,5-undecanediol dehydratase, 2-methyl-5,6-dodecanediol dehydratase, 1-phenyl-2,3-nonanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-nonanediol dehydratase, 1-indole-2,3-nonanediol dehydratase, 2-methyl-3,4-undecanediol dehydratase, 3-methyl-4,5-dodecanediol dehydratase, 2-methyl-4,5-dodecanediol dehydratase, 2-methyl-5,6-tridecanediol dehydratase, 1-phenyl-2,3-decanediol dehydratase, 1-(4-hydroxyphenyl) -2,3-decanediol dehydratase, 1-indole-2,3-decanediol dehydratase, 2,5-dimethyl-3,4-heptanediol dehydratase, 2,6-dimethyl-3,4-heptanediol dehydratase, 2,7-dimethyl-3,4-octanediol dehydratase, 1-phenyl-4-methyl-2,3-pentanediol dehydratase, 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydratase, 1-indole-4-methyl-2,3-pentanediol dehydratase, 2,6-dimethyl-4,5-octanediol dehydratase, 3,8-dimethyl-4,5-nonanediol dehydratase, 1-phenyl-4-methyl-2,3-hexanediol dehydratase, 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydratase, 1-indole-4-methyl-2,3-hexanediol dehydratase, 2,8-dimethyl-4,5-nonanediol dehydratase, 1-phenyl-5-methyl-2,3-hexanediol dehydratase, 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydratase, 1-indole-5-methyl-2,3-hexanediol dehydratase, 1-phenyl-6-methyl-2,3-heptanediol dehydratase, 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydratase, 1-indole-6-methyl-2,3-heptanediol dehydratase, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydratase, 1-indole-4-phenyl-2,3-butanediol dehydratase, 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydratase, 1,10-diamino-5,6-decanediol dehydratase, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 2,3-hexanediol-1,6-dicarboxylic acid dehydratase, and the like.

In certain aspects, a selected diol dehydratase enzyme may have one or more of the above exemplified diol dehydratase activities, such as a 2,3-butanediol dehydratase activity, a 3,4-hexanediol dehydratase activity, and/or a 4,5-octanediol dehydratase activity, among others.

In certain aspects, diol dehydratases may be obtained from *Klebsiella pneumoniae* MGH 78578, including from the *pduCDE* gene of this and other microorganisms. In certain aspects, a recombinant microorganism may comprise one or more diol dehydratases encoded by a nucleotide reference sequence selected from SEQ ID NO:103, 105, and 107, or an enzyme having a polypeptide sequence selected from SEQ ID NO:104, 106, and 108, including biologically active fragments or variants thereof, such as optimized variants. Certain aspects may also comprises nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:103-108. In certain aspects, polypeptides of SEQ ID NO:104 may comprise certain conserved amino acid residues, including those chosen from D149, P151, A155, A159, G165, E168, E170, A183, G189, G196, Q200, E208, G215, Y219, E221, T222, S224, Y226, G227, T228, F232, G235, D236, D237, T238, P239, S241, L245, Y249, S251, R252, G253, K255, R257, S260, E265, M268, G269, S275, Y278, L279, E280, C283, G291, Q293, G294, Q296, N297, G298, G312, E329, S341, R344, G356, D371, N372, F374, S377, R392, D393, R412, L477, A486, G499, D500, S516, N522, D523, Y524, G526, and G530.

In certain aspects, a diol dehydratase may include a polypeptide that comprises an amino acid sequence having 0%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:308-311. SEQ ID NO:308 shows the polypeptide sequence of PduG, a diol dehydratase reactivation large subunit derived from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578. SEQ ID NO:309 shows the polypeptide sequence of PduH, diol dehydratase reactivation small subunit derived from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578. SEQ ID NO:310 shows the polypeptide sequence of a B12-independent glycerol dehydratase from *Clostridium Butyricum*. SEQ ID NO:311 shows the polypeptide sequence of a glycerol dehydratase activator from *Clostridium Butyricum*. In certain aspects, a B12-independent glycerol dehydratase may comprise conserved amino acid residues, such as T36, G74, P87, E88, E97, W126, R221, A263, Q265, R287, D289, E309, R317, G335, G345, G346, N356, P374, R379, G399, G401, P403, D408, G432, C433, N452, C529, G533, G539, G540, S559, G603, N604, A654, G658, R659, D676, N702, Q735, N737, A747, P751, R760,

V761, A762, G763, Q776, I780, and/or R782. In certain aspects, a B12-independent glycerol dehydratase activator may comprise certain conserved amino acid residues, including D19, G20, G22, R24, F28, G31, C32, C36, W38, C39, N41, P42, C58, C64, C96, G129, T132, G135, G136, D185, R187, N208, R222, and/or R264.

A dehydration and reduction pathway, as described herein, may comprise one or more alcohol dehydrogenases or secondary alcohol dehydrogenases. An "alcohol dehydrogenase" or "secondary alcohol dehydrogenase" that is part of a dehydration and reduction pathway refers generally to an enzyme that catalyzes the conversion of aldehyde or ketone substituents to alcohols. For instance, 4-octanone may be reduced to 4-octanol by a secondary alcohol dehydrogenase one enzymatic step for the conversion of butyrolin to a biofuel. *Pseudomonads* express at least one secondary alcohol dehydrogenase that oxidizes 4-octanol to 4-octanone using NAD^+ as a co-factor. As another example, *Rhodococcus erythropolis* ATCC4277 catalyzes oxidation of medium to long chain secondary fatty alcohols using NADH as a co-factor, using an enzyme that also catalyzes the oxidation of 3-decanol and 4-decanol. In addition, *Norcadia fusca* AKU2123 contains an (S)-specific secondary alcohol dehydrogenase.

Genes encoding secondary alcohol dehydrogenases may be isolated from these and other organisms according to known techniques in the art and incorporated into the microbial systems recombinant organisms as described herein. In certain embodiments, a microbial system or isolated microorganism may comprise natural or optimized secondary alcohol dehydrogenases from *Pseudomonads*, *Rhodococcus erythropolis* ATCC4277, *Norcadia fusca* AKU2123, or other suitable organisms.

Examples of secondary alcohol dehydrogenases include, but are not limited to, 2-butanol dehydrogenase, 3-hexanol dehydrogenase, 4-octanol dehydrogenase, 5-decanol dehydrogenase, 6-dodecanol dehydrogenase, 7-tetradecanol dehydrogenase, 8-hexadecanol dehydrogenase, 2,5-dimethyl-3-hexanol dehydrogenase, 3,6-dimethyl-4-octanol dehydrogenase, 2,7-dimethyl-4-octanol dehydrogenase, 2,9-dimethyl-4-decanol dehydrogenase, 1,4-diphenyl-2-butanol dehydrogenase, bis-1,4-(4-

hydroxyphenyl)-2-butanol dehydrogenase, 1,4-diindole-2-butanol dehydrogenase, cyclopentanol dehydrogenase, 2(or 3)-pentanol dehydrogenase, 2(or 3)-hexanol dehydrogenase, 2(or 3)-heptanol dehydrogenase, 2(or 3)-octanol dehydrogenase, 2(or 3)-nonanol dehydrogenase, 4-methyl-2(or 3)-pentanol dehydrogenase, 4-methyl-2(or 3)-hexanol dehydrogenase, 5-methyl-2(or 3)-hexanol dehydrogenase, 6-methyl-2(or 3)-heptanol dehydrogenase, 1-phenyl -2(or 3)-butanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase, 1-indole-2(or 3)-butanol dehydrogenase, 3(or 4)-heptanol dehydrogenase, 3(or 4)-octanol dehydrogenase, 3(or 4)-nonanol dehydrogenase, 3(or 4)-decanol dehydrogenase, 3(or 4)-undecanol dehydrogenase, 2-methyl-3(or 4)-hexanol dehydrogenase, 5-methyl-3(or 4)-heptanol dehydrogenase, 6-methyl-3(or 4)-heptanol dehydrogenase, 7-methyl-3(or 4)-octanol dehydrogenase, 1-phenyl -2(or 3)-pentanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-pentanol dehydrogenase, 1-indole-2(or 3)-pentanol dehydrogenase, 4(or 5)-nonanol dehydrogenase, 4(or 5)-decanol dehydrogenase, 4(or 5)-undecanol dehydrogenase, 4(or 5)-dodecanol dehydrogenase, 2-methyl-3(or 4)-heptanol dehydrogenase, 3-methyl-4(or 5)-octanol dehydrogenase, 2-methyl-4(or 5)-octanol dehydrogenase, 8-methyl-4(or 5)-nonanol dehydrogenase, 1-phenyl -2(or 3)-hexanol dehydrogenase, 1-(4-hydroxyphenyl) -2(or 3)-hexanol dehydrogenase, 1-indole-2(or 3)-hexanol dehydrogenase, 4(or 5)-undecanol dehydrogenase, 5(or 6)-undecanol dehydrogenase, 5(or 6)-tridecanol dehydrogenase, 2-methyl-3(or 4)-octanol dehydrogenase, 3-methyl-4(or 5)-nonanol dehydrogenase, 2-methyl-4(or 5)-nonanol dehydrogenase, 2-methyl-5(or 6)-decanol dehydrogenase, 1-phenyl -2(or 3)-heptanol dehydrogenase, 1-(4-hydroxyphenyl) -2(or 3)-heptanol dehydrogenase, 1-indole-2(or 3)-heptanol dehydrogenase, 6(or 7)-tridecanol dehydrogenase, 6(or 7)-tetradecanol dehydrogenase, 2-methyl-3(or 4)-nonanol dehydrogenase, 3-methyl-4(or 5)-decanol dehydrogenase, 2-methyl-4(or 5)-decanol dehydrogenase, 2-methyl-5(or 6)-undecanol dehydrogenase, 1-phenyl -2(or 3)-octanol dehydrogenase, 1-(4-hydroxyphenyl) -2(or 3)-octanol dehydrogenase, 1-indole-2(or 3)-octanol dehydrogenase, 7(or 8)-pentadecanol dehydrogenase, 2-methyl-3(or 4)-decanol dehydrogenase, 3-methyl-4(or 5)-undecanol dehydrogenase, 2-methyl-4(or 5)-undecanol dehydrogenase, 2-methyl-5(or 6)-

dodecanol dehydrogenase, 1-phenyl -2(or 3)-nonanol dehydrogenase, 1-(4-hydroxyphenyl) -2 (or 3)-nonanol dehydrogenase, 1-indole-2(or 3)-nonanol dehydrogenase, 2-methyl-3(or 4)-undecanol dehydrogenase, 3-methyl-4(or 5)-dodecanol dehydrogenase, 2-methyl-4(or 5)-dodecanol dehydrogenase, 2-methyl-5(or 6)-tridecanol dehydrogenase, 1-phenyl -2(or 3)-decanol dehydrogenase, 1-(4-hydroxyphenyl) -2 (or 3)-decanol dehydrogenase, 1-indole-2(or 3)-decanol dehydrogenase, 2,5-dimethyl-3(or 4)-heptanol dehydrogenase, 2,6-dimethyl-3(or 4)-heptanol dehydrogenase, 2,7-dimethyl-3(or 4)-octanol dehydrogenase, 1-phenyl-4-methyl-2(or 3)-pentanol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2(or 3)-pentanol dehydrogenase, 1-indole-4-methyl-2(or 3)-pentanol dehydrogenase, 2,6-dimethyl-4(or 5)-octanol dehydrogenase, 3,8-dimethyl-4(or 5)-nonanol dehydrogenase, 1-phenyl-4-methyl-2(or 3)-hexanol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2(or 3)-hexanol dehydrogenase, 1-indole-4-methyl-2(or 3)-hexanol dehydrogenase, 2,8-dimethyl-4(or 5)-nonanol dehydrogenase, 1-phenyl-5-methyl-2(or 3)-hexanol dehydrogenase, 1-(4-hydroxyphenyl)-5-methyl-2(or 3)-hexanol dehydrogenase, 1-indole-5-methyl-2(or 3)-hexanol dehydrogenase, 1-phenyl-6-methyl-2(or 3)-heptanol dehydrogenase, 1-(4-hydroxyphenyl)-6-methyl-2(or 3)-heptanol dehydrogenase, 1-indole-6-methyl-2(or 3)-heptanol dehydrogenase, 1-(4-hydroxyphenyl)-4-phenyl-2(or 3)-butanol dehydrogenase, 1-indole-4-phenyl-2(or 3)-butanol dehydrogenase, 1-indole-4-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase, 1,10-diamino-5-decanol dehydrogenase, 1,4-di(4-hydroxyphenyl)-2-butanol dehydrogenase, 2-hexanol-1,6-dicarboxylic acid dehydrogenase, phenylethanol dehydrogenase, 4-hydroxyphenylethanol dehydrogenase, Indole-3-ethanol dehydrogenase, and the like.

In certain aspects, a selected alcohol dehydrogenase or secondary alcohol dehydrogenase may have one or more of the above exemplified alcohol dehydrogenase activities, such as a 2-butanol dehydrogenase activity, 3-hexanol dehydrogenase activity, and/or a 4-octanol dehydrogenase activity, among others.

In certain aspects, a recombinant microorganism may comprise one or more secondary alcohol dehydrogenases encoded by a nucleotide reference sequence

selected from SEQ ID NO:109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, and 141, or an enzyme having a polypeptide sequence selected from SEQ ID NO:110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, and 142, including biologically active fragments or variants thereof, such as optimized variants. Certain aspects may also comprises nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:109-142.

For the secondary alcohol dehydrogenase sequences referred to above, SEQ ID NO:109 is the nucleotide sequence and SEQ ID NO:110 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-1*: PP_1946) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:111 is the nucleotide sequence and SEQ ID NO:112 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-2*: PP_1817) isolated from *Pseudomonas putida* KT2440.

SEQ ID NO:113 is the nucleotide sequence and SEQ ID NO:114 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-3*: PP_1953) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:115 is the nucleotide sequence and SEQ ID NO:116 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-4*: PP_3037) isolated from *Pseudomonas putida* KT2440.

SEQ ID NO:117 is the nucleotide sequence and SEQ ID NO:118 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-5*: PP_1852) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:119 is the nucleotide sequence and SEQ ID NO:120 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-6*: PP_2723) isolated from *Pseudomonas putida* KT2440.

SEQ ID NO:121 is the nucleotide sequence and SEQ ID NO:122 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-7*: PP_2002) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:123 is the nucleotide sequence and SEQ ID NO:124 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-8*: PP_1914) isolated from *Pseudomonas putida* KT2440.

SEQ ID NO:125 is the nucleotide sequence and SEQ ID NO:126 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-9*: PP_1914) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:127 is the nucleotide sequence and SEQ ID NO:128 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-10*: PP_3926) isolated from *Pseudomonas putida* KT2440.

SEQ ID NO:129 is the nucleotide sequence and SEQ ID NO:130 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-11*: PFL_1756) isolated from *Pseudomonas fluorescens* Pf-5. SEQ ID NO:131 is the nucleotide sequence and SEQ ID NO:132 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-12*: KPN_01694) isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578.

SEQ ID NO:133 is the nucleotide sequence and SEQ ID NO:134 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-13*: KPN_02061) isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578. SEQ ID NO:135 is the nucleotide sequence and SEQ ID NO:136 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-14*: KPN_00827) isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578.

SEQ ID NO:137 is the nucleotide sequence and SEQ ID NO:138 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-16*: KPN_01350) isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578. SEQ ID NO:139 is the nucleotide sequence and SEQ ID NO:140 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-17*: KPN_03369) isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578. SEQ ID NO:141 is the nucleotide sequence and SEQ ID NO:142 is the polypeptide sequence of a secondary alcohol dehydrogenase (*2adh-18*: KPN_03363) isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578.

In certain aspects, an alcohol dehydrogenase (*e.g.*, DEHU hydrogenase), a secondary alcohol dehydrogenase (2ADH), a fragment, variant, or derivative thereof,

or any other enzyme that utilizes such an active site, may comprise at least one of a nicotinamide adenine dinucleotide (NAD⁺), NADH, nicotinamide adenine dinucleotide phosphate (NADP⁺), or NADPH binding motif. In certain embodiments, the NAD⁺, NADH, NADP⁺, or NADPH binding motif may be selected from the group consisting of Y-X-G-G-X-Y, Y-X-X-G-G-X-Y, Y-X-X-X-G-G-X-Y, Y-X-G-X-X-Y, Y-X-X-G-G-X-X-Y, Y-X-X-X-G-X-X-Y, Y-X-G-X-Y, Y-X-X-G-X-Y, Y-X-X-X-G-X-Y, and Y-X-X-X-X-G-X-Y; wherein Y is independently selected from alanine, glycine, and serine, wherein G is glycine, and wherein X is independently selected from a genetically encoded amino acid.

As one example of a step in a reduction and dehydration pathway, α -hydroxy cyclopentanone may be reduced to 1,2-cyclopentanediol. For example, the glycerol dehydrogenase isolated from *Hansenula ofunaensis* favors the reduction of α -hydroxy ketones and α -keto ketones, and has very broad substrate specificity. The similar alcohol dehydrogenase derived from *Hansenula polymorpha* and *meso*-2,3-butanediol dehydrogenase has similar properties. Certain embodiments may incorporate a 1,2-cyclopentanediol dehydrogenase to the microbial system or isolated microorganism. Other embodiments may incorporate a glycerol dehydrogenase from *Hansenula ofunaensis*, *Hansenula polymorpha*, *Klebsiella pneumonia*, or any other suitable organism.

By way of example, a chemical or hydrocarbon such as 1,2-cyclopentanediol may be dehydrated to form cyclopentanone as one enzymatic step in a reduction and dehydration pathway. There are at least two different types of diol dehydratases that may catalyze dehydration of chemicals such as 1,2-cyclopentanediol. Certain embodiments of microbial system comprising a reduction and dehydration pathway will comprise diol dehydratases such as 1,2-cyclopentanediol dehydratase.

In the last enzymatic step for a reduction and dehydration pathway, the conversion of such exemplary chemicals as α -hydroxy cyclopentanone to cyclopentanol may include the reduction of cyclopentanone to cyclopentanol. This step may be catalyzed by cyclopentanol dehydrogenase, which is found in *Comomonas sp.* strain

NCIMB 9872 and its gene (*cpnA*) has been isolated. Certain embodiments of a microbial system or isolated microorganism may comprise a cyclopentanol dehydrogenase, such as that expressed by *cpnA* in *Comomonas sp.* strain NCIMB 9872, among others described herein.

As detailed below, in certain embodiments, selected C-C ligation pathways may be utilized in combination with selected components or enzymes of a reduction and dehydration pathway to produce a commodity chemical, or intermediate thereof.

For example, certain embodiments include a method wherein the C-C ligation pathway may comprise an acetoaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-butanediol dehydrogenase, a 2,3-butanediol dehydratase, and a 2-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a propionaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-hexanediol dehydrogenase, a 3,4-hexanediol dehydratase, and a 3-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-octanediol dehydrogenase, a 4,5-octanediol dehydratase, and a 4-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-decanediol dehydrogenase, a 5,6-decanediol dehydratase, and a 5-decanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6,7-dodecanediol dehydrogenase, a 6,7-dodecanediol dehydratase, and a 6-dodecanol dehydrogenase. Additional

embodiments include a method wherein the C-C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 7,8-tetradecanediol dehydrogenase, a 7,8-tetradecanediol dehydratase, and a 7-tetradecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 8,9-hexadecanediol dehydrogenase, a 8,9-hexadecanediol dehydratase, and a 8-hexadecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise an isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,5-dimethyl-3,4-hexanediol dehydrogenase, a 2,5-dimethyl-3,4-hexanediol dehydratase, and a 2,5-dimethyl-3-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a 2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,6-dimethyl-4,5-octanediol dehydrogenase, a 3,6-dimethyl-4,5-octanediol dehydratase, and a 3,6-dimethyl-4-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a 3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,7-dimethyl-4,5-octanediol dehydrogenase, a 2,7-dimethyl-4,5-octanediol dehydratase, and a 2,7-dimethyl-4-octanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a 3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,9-dimethyl-5,6-decanediol dehydrogenase, a 2,9-dimethyl-4,5-decanediol dehydratase, and a 2,9-dimethyl-4-decanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1,4-diphenyl-2,3-butanediol

dehydrogenase, a 1,4-diphenyl-2,3-butanediol dehydratase, and a 1,4-diphenyl-2-butanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a bis-1,4-(4-hydroxyphenyl) -2,3-butanediol dehydrogenase, a bis-1,4-(4-hydroxyphenyl) -2,3-butanediol dehydratase, and a bis-1,4-(4-hydroxyphenyl)-2-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1,4-diindole-2,3-butanediol dehydrogenase, a 1,4-diindole-2,3-butanediol dehydratase, and a 1,4-diindole-2-butanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise an α -keto adipate carboxylase, and wherein the reduction and dehydration pathway may comprise at least one of a 1, 2-cyclopentanediol dehydrogenase, a 1, 2-cyclopentanediol dehydratase, and a cyclopentanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/propiondehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-pentanediol dehydrogenase, a 2,3-pentanediol dehydratase, and a 2(or 3)-pentanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-hexanediol dehydrogenase, a 2,3-hexanediol dehydratase, and a 2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-heptanediol dehydrogenase, a 2,3-heptanediol dehydratase, and a 2(or 3)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/hexaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-octanediol dehydrogenase, a 2,3-octanediol dehydratase, and a 2(or 3)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-nonanediol dehydrogenase, a 2,3-nonanediol dehydratase, and a 2(or 3)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4-methyl-2,3-pentanediol dehydrogenase, a 4-methyl-2,3-pentanediol dehydratase, and a 4-methyl-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4-methyl-2,3-hexanediol dehydrogenase, a 4-methyl-2,3-hexanediol dehydratase, and a 4-methyl-2(or 3)-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5-methyl-2,3-hexanediol dehydrogenase, a 5-methyl-2,3-hexanediol dehydrogenase, and a 5-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6-methyl-2,3-heptanediol dehydrogenase, a 6-methyl-2,3-heptanediol dehydrogenase, and a 6-methyl-2(or 3)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl

-2,3-butanediol dehydrogenase, a 1-phenyl -2,3-butanediol dehydratase, and a 1-phenyl -2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl) -2,3-butanediol dehydrogenase, a 1-(4-hydroxyphenyl) -2,3-butanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an acetoaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole -2,3-butanediol dehydrogenase, a 1-indole -2,3-butanediol dehydratase, and a 1-indole-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-heptanediol dehydrogenase, a 3,4-heptanediol dehydratase, and a 3(or 4)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-octanediol dehydrogenase, a 3,4-octanediol dehydratase, and a 3(or 4)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/hexaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-nonanediol dehydrogenase, a 3,4-nonanediol dehydratase, and a 3(or 4)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-decanediol dehydrogenase, a 3,4-decanediol dehydratase, and a 3(or 4)-decanol

dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-undecanediol dehydrogenase, a 3,4-undecanediol dehydratase, and a 3(or 4)-undecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/ isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-hexanediol dehydrogenase, a 2-methyl-3,4-hexanediol dehydratase, and a 2-methyl-3(or 4)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5-methyl-3,4-heptanediol dehydrogenase, a 5-methyl-3,4-heptanediol dehydratase, and a 5-methyl-3(or 4)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6-methyl-3,4-heptanediol dehydrogenase, a 6-methyl-3,4-heptanediol dehydratase, and a 6-methyl-3(or 4)-heptanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 7-methyl-3,4-octanediol dehydrogenase, a 7-methyl-3,4-octanediol dehydratase, and a 7-methyl-3(or 4)-octanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde and a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl -2,3-pentanediol dehydrogenase, a 1-phenyl -2,3-pentanediol dehydratase, and a 1-phenyl -2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a

propionaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-pentenediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-pentenediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-pentanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a propionaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-pentenediol dehydrogenase, a 1-indole-2,3-pentenediol dehydratase, and a 1-indole-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/pentanaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-nonenediol dehydrogenase, a 4,5-nonenediol dehydratase, and a 4(or 5)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/hexaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-decanediol dehydrogenase, a 4,5-decanediol dehydratase, and a 4(or 5)-decanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-undecanediol dehydrogenase, a 4,5-undecanediol dehydratase, and a 4(or 5)-undecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-dodecanediol dehydrogenase, a 4,5-dodecanediol dehydratase, and a 4(or 5)-dodecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-

methyl-3,4-heptanediol dehydrogenase, a 2-methyl-3,4-heptanediol dehydratase, and a 2-methyl-3(or 4)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-octanediol dehydrogenase, a 3-methyl-4,5-octanediol dehydratase, and a 3-methyl-4(or 5)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-octanediol dehydrogenase, a 2-methyl-4,5-octanediol dehydratase, and a 2-methyl-4(or 5)-octanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of an 8-methyl-4,5-nonanediol dehydrogenase, an 8-methyl-4,5-nonanediol dehydratase, and an 8-methyl-4(or 5)-nonanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-hexanediol dehydrogenase, a 1-phenyl -2,3-hexanediol dehydratase, and a 1-phenyl -2(or 3)-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl) -2,3-hexanediol dehydrogenase, a 1-(4-hydroxyphenyl) -2,3-hexanediol dehydratase, and a 1-(4-hydroxyphenyl) -2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a butyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-

hexanediol dehydrogenase, a 1-indole-2,3-hexanediol dehydratase, and a 1-indole-2(or 3)-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/hexaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-undecanediol dehydrogenase, a 4,5-undecanediol dehydratase, and a 4(or 5)-undecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-undecanediol dehydrogenase, a 5,6-undecanediol dehydratase, and a 5(or 6)-undecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-tridecanediol dehydrogenase, a 5,6-tridecanediol dehydratase, and a 5(or 6)-tridecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-octanediol dehydrogenase, a 2-methyl-3,4-octanediol dehydratase, and a 2-methyl-3(or 4)-octanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-nonanediol dehydrogenase, a 3-methyl-4,5-nonanediol dehydratase, and a 3-methyl-4(or 5)-nonanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-nonanediol dehydrogenase, a 2-methyl-4,5-nonanediol dehydratase, and a 2-methyl-4(or 5)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-decanediol dehydrogenase, a 2-methyl-5,6-decanediol dehydratase, and a 2-methyl-5(or 6)-decanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-heptanediol dehydrogenase, a 1-phenyl -2,3-heptanediol dehydratase, and a 1-phenyl -2(or 3)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl) -2,3-heptanediol dehydrogenase, a 1-(4-hydroxyphenyl) -2,3-heptanediol dehydratase, and a 1-(4-hydroxyphenyl) -2(or 3)-heptanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a pentaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-heptanediol dehydrogenase, a 1-indole-2,3-heptanediol dehydratase, and a 1-indole-2(or 3)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6,7-tridecanediol dehydrogenase, a 6,7-tridecanediol dehydratase, and a 6(or 7)-tridecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6,7-tetradecanediol dehydrogenase, a 6,7-tetradecanediol dehydratase, and a 6(or 7)-tetradecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-nonanediol dehydrogenase, a 2-methyl-3,4-nonanediol dehydratase, and a 2-methyl-3(or 4)-nonanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-decanediol dehydrogenase, a 3-methyl-4,5-decanediol dehydratase, and a 3-methyl-4(or 5)-decanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-decanediol dehydrogenase, a 2-methyl-4,5-decanediol dehydratase, and a 2-methyl-4(or 5)-decanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-undecanediol dehydrogenase, a 2-methyl-5,6-undecanediol dehydratase, and a 2-methyl-5(or 6)-undecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-octanediol dehydrogenase, a 1-phenyl -2,3-octanediol dehydratase, and a 1-phenyl -2(or 3)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl) -2,3-octanediol dehydrogenase, a 1-(4-hydroxyphenyl) -2,3-octanediol dehydratase, and a 1-(4-hydroxyphenyl) -2(or 3)-octanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a hexaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-octanediol dehydrogenase, a 1-indole-2,3-octanediol dehydratase, and a 1-indole-2(or 3)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 7,8-pentadecanediol dehydrogenase, a 7,8-pentadecanediol dehydratase, and a 7(or 8)-pentadecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-decanediol dehydrogenase, a 2-methyl-3,4-decanediol dehydratase, and a 2-methyl-3(or 4)-decanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-undecanediol dehydrogenase, a 3-methyl-4,5-undecanediol dehydratase, and a 3-methyl-4(or 5)-undecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-undecanediol dehydrogenase, a 2-methyl-4,5-undecanediol dehydratase, and a 2-methyl-4(or 5)-undecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-dodecanediol dehydrogenase, a 2-methyl-5,6-dodecanediol dehydratase, and a 2-methyl-5(or 6)-dodecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-nonanediol dehydrogenase, a 1-phenyl-2,3-nonanediol dehydratase, and a 1-phenyl-2(or 3)-nonanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-nonanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-nonanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a heptaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-nonanediol dehydrogenase, a 1-indole-2,3-nonanediol dehydratase, and a 1-indole-2(or 3)-nonanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-undecanediol dehydrogenase, a 2-methyl-3,4-undecanediol dehydratase, and a 2-methyl-3(or 4)-undecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-dodecanediol dehydrogenase, a 3-methyl-4,5-dodecanediol dehydratase, and a 3-methyl-4(or 5)-dodecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-dodecanediol dehydrogenase, a 2-methyl-4,5-dodecanediol dehydratase, and a 2-methyl-4(or 5)-dodecanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-tridecanediol dehydrogenase, a 2-methyl-5,6-tridecanediol dehydratase, and a 2-methyl-5(or 6)-tridecanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-decanediol dehydrogenase, a 1-phenyl-2,3-decanediol dehydratase, and a 1-phenyl-2(or 3)-decanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-decanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-decanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-decanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an octaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-decanediol dehydrogenase, a 1-indole-2,3-decanediol dehydratase, and a 1-indole-2(or 3)-decanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an isobutyraldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,5-dimethyl-3,4-heptanediol dehydrogenase, a 2,5-dimethyl-3,4-heptanediol dehydratase, and a 2,5-dimethyl-3(or 4)-heptanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an isobutyraldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,6-dimethyl-3,4-heptanediol dehydrogenase, a 2,6-dimethyl-3,4-heptanediol dehydratase, and a 2,6-dimethyl-3(or 4)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an isobutyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,7-dimethyl-3,4-octanediol dehydrogenase, a 2,7-dimethyl-3,4-octanediol dehydratase, and a 2,7-dimethyl-3(or 4)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an isobutyraldehyde/ phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-4-methyl-2,3-pentanediol dehydrogenase, a 1-phenyl-4-methyl-2,3-pentanediol dehydratase, and a 1-phenyl-4-methyl-2(or 3)-pentanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an isobutyraldehyde/ 4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydrogenase, a 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydratase, and a 1-(4-hydroxyphenyl)-4-methyl-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of an isobutyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-methyl-2,3-pentanediol dehydrogenase, a 1-indole-4-methyl-2,3-pentanediol dehydratase, and a 1-indole-4-methyl-2(or 3)-pentanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,6-dimethyl-4,5-octanediol dehydrogenase, a 2,6-dimethyl-4,5-octanediol dehydratase, and a 2,6-dimethyl-4(or 5)-octanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,8-dimethyl-4,5-

nonanediol dehydrogenase, a 3,8-dimethyl-4,5-nonanediol dehydratase, and a 3,8-dimethyl-4(or 5)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-4-methyl-2,3-hexanediol dehydrogenase, a 1-phenyl-4-methyl-2,3-hexanediol dehydratase, and a 1-phenyl-4-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydrogenase, a 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydratase, and a 1-(4-hydroxyphenyl)-4-methyl-2 (or 3)-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-methyl-2,3-hexanediol dehydrogenase, a 1-indole-4-methyl-2,3-hexanediol dehydratase, and a 1-indole-4-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,8-dimethyl-4,5-nonanediol dehydrogenase, a 2,8-dimethyl-4,5-nonanediol dehydratase, and a 2,8-dimethyl-4(or 5)-nonanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-5-methyl-2,3-hexanediol dehydrogenase, a 1-phenyl-5-methyl-2,3-hexanediol dehydratase, and a 1-phenyl-5-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may

comprise at least one of a 3-methyl-butyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydrogenase, a 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydratase, and a 1-(4-hydroxyphenyl)-5-methyl-2(or 3)-hexanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-5-methyl-2,3-hexanediol dehydrogenase, a 1-indole-5-methyl-2,3-hexanediol dehydratase, and a 1-indole-5-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 4-methyl-pentaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-6-methyl-2,3-heptanediol dehydrogenase, a 1-phenyl-6-methyl-2,3-heptanediol dehydratase, and a 1-phenyl-6-methyl-2(or 3)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 4-methyl-pentaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydrogenase, a 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydratase, and a 1-(4-hydroxyphenyl)-6-methyl-2(or 3)-heptanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 4-methyl-pentaldehyde/Indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-6-methyl-2,3-heptanediol dehydrogenase, a 1-indole-6-methyl-2,3-heptanediol dehydratase, and a 1-indole-6-methyl-2(or 3)-heptanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a phenylacetaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway

may comprise at least one of a 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydrogenase, a 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydratase, and a 1-(4-hydroxyphenyl)-4-phenyl-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a phenylacetaldehyde/indolephenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-phenyl-2,3-butanediol dehydrogenase, a 1-indole-4-phenyl-2,3-butanediol dehydratase, and a 1-indole-4-phenyl-2(or 3)-butanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise at least one of a 4-hydroxyphenylacetaldehyde/indolephenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, a 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydratase, and a 1-indole-4-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a 5-amino-pantaldehyde lyase, and wherein the reduction and dehydration pathway may comprise at least one of a 1,10-diamino-5,6-decanediol dehydrogenase, a 1,10-diamino-5,6-decanediol dehydratase, and a 1,10-diamino-5-decanol dehydrogenase.

Additional embodiments include a method wherein the C-C ligation pathway may comprise a 4-hydroxyphenyl acetaldehyde lyase, and wherein the reduction and dehydration pathway may comprise at least one of a 1,4-di(4-hydroxyphenyl)-2,3-butanediol, a 1,4-di(4-hydroxyphenyl)-2,3-butanediol dehydratase, and a 1,4-di(4-hydroxyphenyl)-2-butanol dehydrogenase. Additional embodiments include a method wherein the C-C ligation pathway may comprise a succinate semialdehyde lyase, and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-hexanediol-1,6-dicarboxylic acid dehydrogenase, a 2,3-hexanediol-1,6-dicarboxylic acid dehydratase, and a 2-hexanol-1,6-dicarboxylic dehydrogenase.

Certain embodiments of a microbial system or recombinant microorganism may comprise genes encoding enzymes that are able to catalyze (*e.g.*, reduction and dehydration) the conversion of 4-octanol to octene or octane. Other embodiments may comprise redesigned or *de novo* designed enzymes for this reduction and dehydration pathway. For example, three redesigned enzymes could convert 4-octanone to either 3- and 4-octene. The first step could be catalyzed by redesigned isocitrate dehydrogenase. This enzyme could catalyze the formation of 4-hydroxy-3(or 5)-carboxylic octane. The 4-hydroxy group could be phosphorylated by redesigned kinase. Finally, redesigned mevalonate diphosphate decarboxylase catalyzes the formation of 3(or 4)-octene.

In other embodiments, several redesigned enzymes could convert 4-octanone to octane. For example, the 4-hydroxy-3(or 5)-carboxylic octane is sequentially reduced and dehydrated to form 3(or 5)-carboxylic octane. Redesigned enzymes involved in fatty acid metabolism can catalyze these reactions. The 3(or 5)-carboxylic octane can be reduced to corresponding aldehyde by aldehyde dehydrogenase and the product may be decarbonylated to form octane catalyzed by a redesigned decarbonylase.

As noted above, for the production of certain commodity chemicals, such as 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol, among other similar chemicals, a biosynthesis pathway (*e.g.*, aldehyde biosynthesis pathway) may optionally or further comprise one or more genes encoding a decarboxylase enzyme, such as an indole-3-pyruvate decarboxylase (IPDC), to produce an aldehyde. In certain aspects, an IPDC may comprise an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO:312. An IDPC enzyme may comprise certain conserved amino acid residues, such as G24, D25, E48, A55, R60, G75, E89, H113, G252, G405, G413, G428, G430, and/or N456.

In these and other embodiments, a recombinant microorganism may comprise an aldehyde reductase, such as a phenylacetaldehyde reductase (PAR), to

convert an aldehyde to a commodity chemical. In certain aspects, a PAR may comprise an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO:313, which shows the sequence of a PAR enzymed derived from *Rhodococcus sp.* ST-10. In certain aspects, a PAR enzyme may comprise at least one of a nicotinamide adenine dinucleotide (NAD⁺), NADH, nicotinamide adenine dinucleotide phosphate (NADP⁺), or NADPH binding motif. In certain embodiments, the NAD⁺, NADH, NADP⁺, or NADPH binding motif may be selected from the group consisting of Y-X-G-G-X-Y, Y-X-X-G-G-X-Y, Y-X-X-X-G-G-X-Y, Y-X-G-X-X-Y, Y-X-X-G-G-X-X-Y, Y-X-X-X-G-X-X-Y, Y-X-G-X-Y, Y-X-X-G-X-Y, Y-X-X-X-G-X-Y, and Y-X-X-X-X-G-X-Y; wherein Y is independently selected from alanine, glycine, and serine, wherein G is glycine, and wherein X is independently selected from a genetically encoded amino acid.

In certain embodiments, such a recombinant microorganism may also or alternatively comprise a secondary alcohol dehydrogenase having an activity selected from at least one of a phenylethanol dehydrogenase activity, a 4-hydroxyphenylethanol dehydrogenase activity, and an Indole-3-ethanol dehydrogenase activity, to reduce the aldehyde to its corresponding alcohol (*e.g.* 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol).

Embodiments of the present invention also include methods for converting a suitable monosaccharide to a commodity chemical comprising, (a) obtaining a suitable monosaccharide; (b) contacting the suitable monosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the biofuel, wherein the microbial system comprises, (i) one or more genes encoding and expressing a fatty acid biosynthesis pathway, an amino acid biosynthetic pathway, and/or a short chain alcohol biosynthetic pathway; (ii) one or more genes encoding and expressing a *keto*-acid decarboxylase, aldehyde dehydrogenase, and/or alcohol dehydrogenase; and (iii) an enzymatic reduction pathway selected from (1) an enzymatic long chain alcohol reduction pathway, (2) an enzymatic decarbonylation pathway, (3) an enzymatic decarboxylation pathway, and (4) an enzymatic reduction

pathway comprising (1), (2), and/or (3), thereby converting the suitable monosaccharide to the commodity chemical.

Embodiments of the present invention may comprise one or more genes encoding and expressing enzymes in a fatty acid synthesis pathway, which may be used, as one example, to produce biofuels in the form of alkanes, such as medium to long chain alkanes. In certain embodiments, the specificity of the fatty acid biosynthesis pathway in the microbial system may be recalibrated or redesigned. Merely by way of example, microorganisms generally produce a mixture of long chain fatty acids (*e.g.*, *E. coli* naturally produce large quantities of long chain fatty acids (C16-C19: < 95% in whole cells) and small quantity of medium chain fatty acids (C12: 2 % and C14: 5% in whole cells)).

In certain embodiments, the recalibration or re-engineering may be directed to increasing production of medium chain alkanes, including, but not limited to, caprylate (C8), caprate (C10), laurate (C12), myristate (C14), and palmitate (C16), as alkanes produced from these fatty acids are major components of gasoline, diesels, and kerosene. In addition to these fatty acids, other embodiments may be directed to increased production of long chain fatty acids, including, but not limited to, stearate (C18), arachidonate (C20), behenate (C22) and longer fatty acids, as *n*-alkanes produced from these fatty acids are one of major components in heavy oils.

For example, *Cuphea* mainly accumulate medium chain fatty acids as major components in their seed oils, and these compositions alter depending on species. In particular, *Cuphea pulcherrima* accumulates caprylate (C8:0) 96 %, *Cuphea koehneana* accumulates caprate (C10:0) 95.3 %, and *Cuphea polymorpha* accumulates laurate (C12:0) 80.1%. Embodiments of the microbial systems or isolated microorganisms according to the present application may incorporate genes from various *Cuphea* species encoding enzymes involved in a fatty acid biosynthesis pathway, and these microorganisms may be directed in part to the production of middle chain fatty acids.

In other embodiments, acyl-acyl carrier protein (ACP) thioesterases (TEs) derived from various species including *Cuphea hookeriana*, *Cuphea palustris*, *Umbellularia californica*, and *Cinnamomum camphorum* may be over-expressed in such microorganisms as *E. coli*, wherein the specific activity for the formation of each medium chain fatty acids, caprylate (C8), caprate (C10), laurate (C12), myristate (C14), and palmitate (C16) is improved over the wild type. Certain embodiments may include other enzyme components involved in fatty acid biosynthesis as known to a person skilled in the arts, including, but not limited to, ACP and β -ketoacyl ACP synthase (KAS) IV.

Microbial systems and isolated microorganisms of the present application may also incorporate fatty aldehyde dehydrogenases to reduce fatty acids to fatty aldehydes. Merely by way of explanation, the conversion of fatty acids to fatty aldehydes may be catalyzed by medium and/or long chain fatty aldehyde dehydrogenases isolated from various suitable organisms. Certain embodiments may incorporate, for example, a fatty aldehyde dehydrogenase derived from *Vibrio harveyi*.

Microbial systems and isolated microorganisms of the present application may also incorporate one or more enzymes that catalyze the conversion of fatty aldehydes to biofuels such as *n*-alkanes, including, for example, enzymes comprising an enzymatic long chain alcohol reduction pathway. Certain embodiments may incorporate genes from various other sources that encode enzymes capable of catalyzing the reduction and dehydration of fatty acids to biofuels, such as alkanes. For example, bacterial strain HD-1 is able to produce biofuels, such as *n*-alkanes, with various chain lengths, and also produces both odd and even numbered alkanes. Certain embodiments of the microbial systems and recombinant microorganisms provided herein may incorporate the HD-1 genes encoding the enzymes involved in this pathway.

Other embodiments may incorporate redesigned or *de novo* designed enzymes for this reduction pathway. For example, embodiments of the present invention may include a redesigned isocitrate dehydrogenase, which may catalyze the formation of 2-carboxy-1-alcohols. In certain embodiments, the 2-carboxy-1-alcohols

may be sequentially reduced and dehydrated to form 2-carboxy-alkanes, which may be catalyzed by redesigned enzymes involved in fatty acid metabolism. The 2-carboxy-alkanes can be reduced to corresponding aldehyde by aldehyde dehydrogenase and then decarbonylated to form *n*-alkanes catalyzed by the redesigned decarbonylase as discussed below. Certain embodiments of these microbial systems may produce either even numbered *n*-alkanes, odd numbered *n*-alkanes, or both.

Certain embodiments of the present application may incorporate the genes encoding enzymes catalyzing decarbonylation, or an enzymatic decarbonylation pathway. Merely by way of example, green colonial alga *Botryococcus braunii*, race A, produces linear odd-numbered C27, C29, and C31 hydrocarbons that total up to 32 % of the alga's dry weight. Microsomal preparations of this organism have decarbonylation activity. This decarbonylase from *B. braunii* culture is a cobalt-protoporphyrin IX containing enzyme. Certain microbial systems of isolated microorganisms may incorporate the gene encoding fatty aldehyde decarbonylase from *Botryococcus braunii*.

Other embodiments may include redesigned decarbonylase enzymes, for example, wherein the N-terminal membrane sequence is substituted. By way of explanation, the functional activity of a similar enzyme, cytochrome P450 containing Fe-protoporphyrin IX (heme), is improved by substituting N-terminal membrane associated sequence, and the functional activity of decarbonylases of the present microbial systems may comprise similar substitutions or improvements.

Other embodiments may incorporate the genes encoding a Co-porphyrin synthase. In explanation, decarbonylase enzymes may use Co-protoporphyrin IX as a co-factor, and *Clostridium tetranomorphum* is able to incorporate cobalt into incubated protoporphyrin IX. Certain embodiments may incorporate the Co-porphyrin synthase from *Clostridium tetranomorphum*, or from other suitable microorganisms. Other embodiments may incorporate *de novo* designed decarbonylation enzymes using inorganic metals such as Co^{2+} , Fe^{2+} , and Ni^{2+} as catalysts.

Certain embodiments may comprise genes encoding the enzymes responsible for the formation of alkenes, or an enzymatic decarboxylation pathway. These genes may be derived or isolated from various sources, such as higher plants and insects. For example, higher plants such as germinating safflower (*Carthamus tinctorius* L.) produce a number of odd numbered 1-alkenes, including 1-pentadecene, 1-heptadecene, 1,8-heptadecadiene and 1,8,11-heptadecatriene besides about 80-90 % 1,8,11,14-heptadecatetraene by decarboxylation from their corresponding fatty acids. Certain embodiments may incorporate the genes from higher plants such as *Carthamus tinctorius*.

Other embodiments may incorporate the genes encoding the enzymes responsible for the formation of alkenes (*e.g.*, an enzymatic decarboxylation pathway) from microorganisms, including, but not limited to, such as bacterial strain DH-1. By way of explanation, bacterial strain DH-1 produces *n*-alkenes in addition to *n*-alkanes.

Other embodiments may incorporate the genes from *de novo* designed enzymes for an enzymatic decarboxylation pathway. For example, these redesigned enzymes convert β -hydroxy fatty acids to *n*-alkenes. The first step is catalyzed by a redesigned kinase, which catalyzes the phosphorylation of a β -hydroxy group. A redesigned mevalonate diphosphate decarboxylase then catalyzes the formation of *n*-alkenes, such as *n*-1-alkene.

Any microorganism may be utilized according to the present invention. In certain aspects, a microorganism is a eukaryotic or prokaryotic microorganism. In certain aspects, a microorganism is a yeast, such as *S. cerevisiae*. In certain aspects, a microorganism is a bacteria, such as a gram-positive bacteria or a gram-negative bacteria. Given its rapid growth rate, well-understood genetics, the variety of available genetic tools, and its capability in producing heterologous proteins, genetically modified *E. coli* may be used in certain embodiments of a microbial system as described herein, whether for the degradation and metabolism of a polysaccharide, such as alginate or pectin, or the formation or biosynthesis of commodity chemicals, such as biofuels.

Other microorganisms may be used according to the present invention, based in part on the compatibility of enzymes and metabolites to host organisms. For example, other organisms such as *Acetobacter aceti*, *Achromobacter*, *Acidiphilium*, *Acinetobacter*, *Actinomadura*, *Actinoplanes*, *Aeropyrum pernix*, *Agrobacterium*, *Alcaligenes*, *Ananas comosus* (M), *Arthrobacter*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus melleus*, *Aspergillus pulverulentus*, *Aspergillus saitoi*, *Aspergillus sojae*, *Aspergillus usamii*, *Bacillus alcalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bifidobacterium*, *Brevibacillus brevis*, *Burkholderia cepacia*, *Candida cylindracea*, *Candida rugosa*, *Carica papaya* (L), *Cellulosimicrobium*, *Cephalosporium*, *Chaetomium erraticum*, *Chaetomium gracile*, *Clostridium*, *Clostridium butyricum*, *Clostridium acetobutylicum*, *Clostridium thermocellum*, *Corynebacterium (glutamicum)*, *Corynebacterium efficiens*, *Escherichia coli*, *Enterococcus*, *Erwinia chrysanthemi*, *Gliconobacter*, *Gluconacetobacter*, *Haloarcula*, *Humicola insolens*, *Humicola insolens*, *Kitasatospora setae*, *Klebsiella*, *Klebsiella oxytoca*, *Kluyveromyces*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Kocuria*, *Lactolactis*, *Lactobacillus*, *Lactobacillus fermentum*, *Lactobacillus sake*, *Lactococcus*, *Lactococcus lactis*, *Leuconostoc*, *Methylocystis*, *Methanobacterium bryantii*, *Methanobacterium imperiale*, *Micrococcus lysodeikticus*, *Micrococcus lysodeikticus*, *Mucor javanicus*, *Mycobacterium*, *Myrothecium*, *Nitrobacter*, *Nitrosomonas*, *Nocardia*, *Papaya carica*, *Pediococcus*, *Pediococcus halophilus*, *Penicillium*, *Penicillium camemberti*, *Penicillium citrinum*, *Penicillium emersonii*, *Penicillium roqueforti*, *Penicillium lilactinum*, *Penicillium multicolor*, *Paracoccus pantotrophus*, *Propionibacterium*, *Pseudomonas*, *Pseudomonas fluorescens*, *Pseudomonas denitrificans*, *Pyrococcus*, *Pyrococcus furiosus*, *Pyrococcus horikoshii*, *Rhizobium*, *Rhizomucor miehei*, *Rhizomucor pusillus* Lindt, *Rhizopus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Rhizopus niveus*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Rhodococcus*, *Saccharomyces cerevisiae*, *Sclerotinia libertina*, *Sphingobacterium multivorum*, *Sphingobium*, *Sphingomonas*, *Streptococcus*, *Streptococcus thermophilus* Y-1, *Streptomyces*,

Streptomyces griseus, *Streptomyces lividans*, *Streptomyces murinus*, *Streptomyces rubiginosus*, *Streptomyces violaceoruber*, *Streptoverticillium mobaraense*, *Tetragenococcus*, *Thermus*, *Thiosphaera pantotropha*, *Trametes*, *Trichoderma*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma viride*, *Trichosporon penicillatum*, *Vibrio alginolyticus*, *Xanthomonas*, yeast, *Zygosaccharomyces rouxii*, *Zymomonas*, and *Zymomonas mobilis*, may be utilized as recombinant microorganisms provided herein, and, thus, may be utilized according to the various methods of the present invention.

The following Examples are offered by way of illustration, not limitation.

Examples

Example 1

Engineering *E. coli* to Grow on Alginate as a Sole Source of Carbon

Wild type *E. coli* cannot use alginate polymer or degraded alginate as its sole carbon source (see Figure 4). *Vibrio splendidus*, however, is known to be able to metabolize alginate to support growth. To generate recombinant *E. coli* that use degraded alginate as its sole carbon source, a *Vibrio splendidus* fosmid library was constructed and cloned into *E. coli*.

To prepare the *Vibrio splendidus* fosmid library, genomic DNA was isolated from *Vibrio Splendidus* B01 (gift from Dr. Martin Polz, MIT) using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). A fosmid library was then constructed using Copy Control Fosmid Library Production Kit (Epicentre, Madison, WI). This library consisted of random genomic fragments of approximately 40kb inserted into the vector pCC1FOS (Epicentre, Madison, WI).

The fosmid library was packaged into phage, and *E. coli* DH10B cells harboring a pDONR221 plasmid (Invitrogen, Carlsbad, CA) carrying certain *Vibrio splendidus* genes (V12B01_02425 to V12B01_02480; encoding a type II secretion apparatus; see SEQ ID NO:1) were transfected with the phage library. This secretome region encodes a type II secretion apparatus derived from *Vibrio splendidus*, which was cloned into a pDONR221 plasmid and introduced into *E. coli* strain DH10B (see Example 1).

Transformants were selected for chloroamphenicol resistance and then screened for their ability to grow on degraded alginate. The resultant transformants were screened for growth on degraded alginate media. Degraded alginate media was prepared by incubating 2% Alginate (Sigma-Aldrich, St. Louis, MO) 10 mM Na-Phosphate buffer, 50 mM KCl, 400 mM NaCl with alginate lyase from *Flavobacterium* sp. (Sigma-Aldrich, St. Louis, MO) at room temperature for at least one week. This

degraded alginate was diluted to a concentration of 0.8% to make growth media that had a final concentration of 1X M9 salts, 2 mM MgSO₄, 100 µM CaCl₂, 0.007% Leucine, 0.01% casamino acids, 1.5% NaCl (this includes all sources of sodium: M9, diluted alginate and added NaCl).

One fosmid-containing *E. coli* clone was isolated that grew well on this media. The fosmid DNA from this clone was isolated and prepared using FosmidMAX DNA Purification Kit (Epicentre, Madison, WI). This isolated fosmid was transferred back into DH10B cells, and these cells were tested for the ability to grown on alginate.

The results are illustrated in Figure 4, which shows that certain fosmid-containing *E. coli* clones are capable of growing on alginate as a sole source of carbon. *Agrobacterium tumefaciens* provides a positive control (*see* hatched circles). As a negative control, *E. coli* DH10B cells are not capable of growing on alginate (*see* immediate left of positive control).

These results also demonstrate that the sequences contained within this *Vibrio splendidus* derived fosmid clone are sufficient to confer on *E. coli* the ability to grow on degraded alginate as a sole source of carbon. Accordingly, the type II secretion machinery sequences contained within the pDONR221 vector (*i.e.*, SEQ ID NO:1), which was harbored by the original DH10B cells, were not necessary for growth on degraded alginate.

The isolated fosmid sufficient to confer growth alginate as a sole source of carbon was sequenced by Elim Biopharmaceuticals (Hayward, CA) using the following primers: Uni R3 – GGGCGGCCGCAAGGGGTTCGCGTTGGCCGA (SEQ ID NO:147) and PCC1FOS_uni_F –GGAGAAAATACCGCATCAGGCG (SEQ ID NO:148). Sequencing showed that the vector contained a genomic DNA section that contained the full length genes V12B01_24189 to V12B01_24249 (*see* SEQ ID NOS:2-64). SEQ ID NO:2 shows the nucleotide sequence of entire region between V12B01_24189 to V12B01_24249. SEQ ID NOS:3-64 show the individual putative genes contained within SEQ ID NO:2. In this sequence, there is a large gene before

V12B01_24189 that is truncated in the fosmid clone. The large gene V12B01_24184 is a putative protein with similarity to autotransporters and belongs to COG3210, which is a cluster of orthologous proteins that include large exoproteins involved in heme utilization or adhesion. In the fosmid clone, V12B01_24184 is N-terminally truncated such that the first 5893 bp are missing from the predicted open reading frame (which is predicted to contain 22889 bp in total).

Example 2

Engineering *E. coli* to Grow on Pectin as a Sole Source of Carbon

Wild type *E. coli* is not capable of growing on pectin, di-, or tri-galacturonates as a sole source of carbon. To identify the minimal components to confer on *E. coli* the capability of growing on pectin, di- and/or tri-galacturonates as a sole source of carbon, an *E. coli* strain BL21(DE3) harboring both the pBBRGal3P plasmid and the pTrcogl-kdgR plasmid was engineered and tested for the ability to grown on these polysaccharides.

The pBBRGal3P plasmid was engineered to contain certain genomic region of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043, comprising several genes (*kdgF*, *kduI*, *kduD*, *pelW*, *togM*, *togN*, *togA*, *togB*, *kdgM*, and *paeX*) encoding certain enzymes (*kduI*, *kduD*, *ogl*, *pelW*, and *paeX*), transporters (*togM*, *togN*, *togA*, *togB*, and *kdgM*), and regulatory proteins (*kdgR*) responsible for the degradation of di- and trigalacturonate. SEQ ID NO:65 shows the nucleotide sequence of the *kdgF*-*PaeX* region from *Erwinia carotovora* subsp. *Atroseptica* SCRI1043.

To construct this plasmid, the DNA sequence encoding *kdgF*, *kduI*, *kduD*, *pelW*, *togM*, *togN*, *togA*, *togB*, *kdgM*, *paeX*, *ogl*, and *kdgR* of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043 was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 6 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CGGGATCC AAGTTGCAGGATATGACGAAAGCG-3') (SEQ ID NO:149) and reverse (5'-GCTCTAGA AGATTATCCCTGTCTGCGGAAGCGG-3') (SEQ ID

NO:150) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043 genome (ATCC) in 50 µl.

The vector pBBR1MCS-2 was then amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 2.5 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'- GCTCTAGA GGGGTGCCTAATGAGTGAGCTAAC-3') (SEQ ID NO:151) and reverse (5'- CGGGATCC GCGTTAATATTTTGTAAATTCGC-3') (SEQ ID NO:152) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBBR1MCS-2 in 50 µl. Both amplified DNA fragments were digested with BamHI and XbaI and ligated.

The pTrcogl-kdgR plasmid was engineered to contain certain genomic regions of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043, comprising two genes (*ogl* and *kdgR*) encoding an enzyme (*ogl*) and a regulatory protein (*kdgR*) responsible for degradation of di- and trigalacturonate. SEQ ID NO:66 shows the nucleotide sequence of *ogl-kdgR* from *Erwinia carotovora* subsp. *Atroseptica* SCRI1043.

To prepare this construct, the DNA sequence encoding *ogl* and *kdgR* of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043 was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'- GCTCTAGA GTTTATGTTCGCACCCGCCGTTGG-3') (SEQ ID NO:153) and reverse (5'- CCCAAGC TTAGAAAGGGAAATTGTGGTAGCCC-3') (SEQ ID NO:154) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043 genome (ATCC) in 50 µl. The amplified DNA fragment was digested with XbaI and HindIII and ligated into pTrc99A pre-digested with the same restriction enzymes.

The plasmids pBBRGal3P and pTrcogl-kdgR were co-transformed into *E. coli* strain BL21(DE3). A single colony was inoculated into LB media containing 50 µg/ml kanamycin and 100 µg/ml ampicillin, and the culture was grown in incubation shaker with 200rpm at 37°C. When culture reached OD 600nm of 0.6, 500 µl of culture

was transferred to eppendorf tube and centrifuged to pellet the cells. The cells were resuspended into 50 μ l of M9 media containing 2 mM MgSO_4 , 100 μ M CaCl_2 , 0.4 % di- or trigalacturonate, and 5 μ l of this solution was inoculated into 500 μ l of fresh M9 media containing 2 mM MgSO_4 , 100 μ M CaCl_2 , 0.4 % di- or trigalacturonate. The culture was grown in incubation shaker with 200 rpm at 37 C.

The results in Figure 5A show that these two plasmids were sufficient to provide *E. coli* ability to grow on di- and trigalacturonate as sole source of carbon, but not pectin. In particular, these results show that the regions *kdgF-paeX* and *ogl-kdgR* were sufficient to confer this ability on *E. coli*.

Based on the information obtained from the above experiments, it was considered whether the introduction of pectate lyase, pectate acetyltransferase, and methyltransferase might confer *E. coli* capability of growing on pectin. To test this hypothesis, *E. coli* strain DH5 α bacterial cells were engineered to contain both the pROU2 plasmid and the pPEL74 plasmid.

The pROU2 plasmid contains certain genomic regions of *Erwinia chrysanthemi*, comprising several genes (*kdgF*, *kduI*, *kduD*, *pelW*, *togM*, *togN*, *togA*, *togB*, *kdgM*, *paeX*, *ogl*, and *kdgR*) encoding enzymes (*kduI*, *kduD*, *ogl*, *pelW*, and *paeX*), transporters (*togM*, *togN*, *togA*, *togB*, and *kdgM*), and regulatory proteins (*kdgR*) responsible for degradation of di- and trigalacturonate.

The pPEL74 plasmid contains certain genomic regions of *Erwinia chrysanthemi*, comprising several genes (*pelA*, *pelE*, *paeY*, and *pem*) encoding pectate lyases (*pelA* and *pelE*), pectin acetyltransferases (*paeY*), and pectin methyltransferase (*pem*).

As shown in Figure 5B, *E. coli* DH5 α engineered with pROU2 and pPEL74 was able to grow on pectin as a sole source of carbon, showing that the genes contained within these plasmids are sufficient to confer this property on an organism that is otherwise incapable of growing on pectin as a sole source of carbon.

Example 3

In vitro Conversion of Alginate to Pyruvate and Glyceraldehyde-3-Phosphate

The ability of an enzyme mixture containing all required enzymes for alginate degradation and metabolism was investigated for its ability to produce pyruvate from alginate. In addition, various novel alcohol dehydrogenases (ADHs), such as ADH1-12 (*see* SEQ ID NOS:69-92), isolated from *Agrobacterium tumefaciens*, were tested for their ability to catalyze either DEHU or mannuronate hydrogenation.

A simplified metabolic pathway for alginate degradation and metabolism is shown in Figure 2. Alginate can be degraded by at least two different methodologies: enzymatic and chemical methodologies.

In enzymatic degradation, the degradation of alginate is catalyzed by a family of enzymes called alginate lyases. For this experiment, Atu3025 was used. Atu3025 is an exolytically acting enzyme and yields DEHU from alginate polymer. DEHU is converted to the common hexuronate metabolite, KDG. This reaction is catalyzed by alcohol dehydrogenases (*e.g.*, DEHU hydrogenases).

Chemical degradation catalyzed by acid solution, such as formate, yields a monosaccharide mannuronate. Mannuronate is then converted to mannonate, which is catalyzed by enzymes with mannonate dehydrogenase (mannuronate reductase) activity. In bacteria, mannonate dehydratase (UxuA) catalyzes dehydration from mannuronate to form KDG.

KDG is readily metabolized to form of pyruvate and glyceraldehydes-3-phosphate (G3P). KDG is first phosphorylated to KDG-6-phosphate (KDGP), which is catalyzed by KDG kinase, and then broken down to pyruvate and G3P, which is catalyzed by KDGP aldolase.

Preparation of oligoalginate lyase Atu3025 derived from *Agrobacterium tumefaciens* C58. pETAtu3025 was constructed based on pET29 plasmid backbone (Novagen). The oligoalginate lyase Atu3025 was amplified by PCR: 98°C for 10 sec, 55°C for 15 sec, and 72°C for 60 sec, repeated for 30 times. The reaction mixture

contained 1 x Phusion buffer, 2 mM dNTP, 0.5 μ M forward (5'-GGAATTCATATGCGTCCCTCTGCCCCGGCC-3') (SEQ ID NO:155) and reverse (5'-CGGGATCCTTAGAACTGCTTGGGAAGGGAG-3') (SEQ ID NO:156) primers, 2.5 U Phusion DNA polymerase (Finezyme), and an aliquot of *Agrobacterium tumefaciens* C58 (gift from Professor Eugene Nester, University of Washington) cells as a template in total volume of 100 μ l. The amplified fragment was digested with NdeI and BamHI and ligated into pET29 pre-digested with the same enzymes using T4 DNA ligase to form pETAtu3025. The constructed plasmid was sequenced (Elim Biopharmaceuticals) and the DNA sequence of the insert was confirmed. The nucleotide sequence of the Atu3025 insert is provided in SEQ ID NO:67. The polypeptide sequence encoded by the Atu3025 insert is provided in SEQ ID NO:68.

The pETAtu3025 was transformed into *Escherichia coli* strain BL21(DE3). A colony of BL21(DE3) containing pETAtu3025 was inoculated into 50 ml of LB media containing 50 μ g/ml kanamycin (Km^{50}). This strain was grown in an orbital shaker with 200 rpm at 37 °C. The 0.2 mM IPTG was added to the culture when the OD_{600nm} reached 0.6, and the induced culture was grown in an orbital shaker with 200 rpm at 20 °C. 24 hours after the induction, the cells were harvested by centrifugation at 4,000 rpm x g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 μ l of Lysonase™ Bioprocessing Reagent (Novagen). The solution was again centrifuged at 4,000 rpm x g for 10 min and the supernatant was obtained.

Construction of pETADH1 through pETADH12. DNA sequences of ADH1-12 of *Agrobacterium tumefaciens* C58 were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (Table 1) and reverse (Table 1) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Agrobacterium tumefaciens* C58 genome in 50 μ l. Amplified DNA fragment was digested with NdeI and BamHI and ligated into pET28 pre-digested with the same restriction enzymes. For DNA sequences with internal NdeI

or BamHI site, front and bottom half sequences of each ADH were first amplified using described method. The resulting two DNA fragments were gel purified and spliced by overlapping PCR.

Table 1: Primers used to amplify ADH1-12 from *Agrobacterium tumefaciens* C58.

Name	A. tumefaciens C58	Forward Primer	Reverse Primer
ADH1	Atu1557	GGAATTCCATATGTTTACAACGTCCGCCTA (SEQ ID NO:276)	GCTTGACGGCCATGTGGCCGAGGCCGC (SEQ ID NO:277)
		GCGGCCTCGGCCACATGGCCGTCAAGC (SEQ ID NO:278)	CGGGATCCTTAGGCGCCTTCTGGCGCG (SEQ ID NO:279)
ADH2	Atu2022	GGAATTCCATATGGCTATTGCAAGAGGTTA (SEQ ID NO:280)	CGGGATCCTTAAGCGTCGAGCGAGGCCA (SEQ ID NO:281)
ADH3	Atu0626	GGAATTCCATATGACTAAAACAATGAAGGC (SEQ ID NO:282)	CACCGGGGCCGGGGTCCGGTATTGCCA (SEQ ID NO:283)
		TGGCAATACCGGACCCCGCCCCGGTG (SEQ ID NO:284)	CGGGATCCTTAGGCGCGAGATCCACGA (SEQ ID NO:285)
ADH4	Atu5240	GGAATTCCATATGACCGGGGCGAACCAGCC (SEQ ID NO:286)	ATAGCCGCTCATACGCTCGGTTGCCT (SEQ ID NO:287)
		AGGCAACCGAGGCGTATGAGCGGCTAT (SEQ ID NO:288)	CGGGATCCTTAAGCGCCGTGCGGAAGGA (SEQ ID NO:289)
ADH5	Atu3163	GGAATTCCATATGACCATGCATGCCATTCA (SEQ ID NO:290)	CGGGATCCTTATTCGGCTGCAAATTGCA (SEQ ID NO:291)
ADH6	Atu2151	GGAATTCCATATGCGCGCGCTTATTACGA (SEQ ID NO:292)	CGGGATCCTTATTCGAACCGGTGATGA (SEQ ID NO:293)
ADH7	Atu2814	GGAATTCCATATGCTGGCGATTTTCTGTGA (SEQ ID NO:294)	CGGGATCCTTATGCGACCTCCACCATGC (SEQ ID NO:295)
ADH8	Atu5447	GGAATTCCATATGAAAGCCTTCGTCGTCGA (SEQ ID NO:296)	CGGGATCCTTAGGATGCGTATGTAACCA (SEQ ID NO:297)
ADH9	Atu4087	GGAATTCCATATGAAAGCGATTGTCGCCCCA (SEQ ID NO:298)	CGGGATCCTTAGGAAAAGGCGATCTGCA (SEQ ID NO:299)
ADH10	Atu4289	GGAATTCCATATGCCGATGGCGCTCGGGCA (SEQ ID NO:300)	CGGGATCCTTAGAATTCGATGACTTGCC (SEQ ID NO:301)
ADH11	Atu3027	GGAATTCCATATGAAACATTCTCAGGACAA (SEQ ID NO:302)	GGGCGCCGATCATGTGGTGC GTTTCCG (SEQ ID NO:303)
		CGGAAACGCACCACATGATCGGCGCCC (SEQ ID NO:304)	CGGGATCCTTATGCCATACGTTCCATAT (SEQ ID NO:305)
ADH12	Atu3026	GGAATTCCATATGCAGCGTTTTACCAACAG (SEQ ID NO:306)	CGGGATCCTTAGGAAAACAGGACGCCGC (SEQ ID NO:307)

Expression and Purification of ADH1-10. All plasmids were transformed into *Escherichia coli* strain BL21(DE3). The single colonies of BL21(DE3) containing respective alcohol dehydrogenase (ADH) genes were inoculated into 50 ml of LB media containing 50 µg/ml kanamycin (Km⁵⁰). These strains were grown in an orbital shaker with 200 rpm at 37 °C. The 0.2 mM IPTG was added to each culture when the OD_{600nm} reached 0.6, and the induced culture was grown in an orbital

shaker with 200 rpm at 20 °C. 24 hours after the induction, the cells were harvested by centrifugation at 4,000 rpm x g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 µl of Lysonase™ Bioprocessing Reagent (Novagen). The solution was again centrifuged at 4,000 rpm x g for 10 min and the supernatant was obtained.

Preparation of ~2% DEHU solution by enzymatic degradation.

DEHU solution was enzymatically prepared. A 2% alginate solution was prepared by adding 10 g of low viscosity alginate into the 500 ml of 20 mM Tris-HCl (pH7.5) solution. An approximately 10 mg of alginate lyase derived from *Flavobacterium sp.* (purchased from Sigma-aldrich) was added to the alginate solution. 250 ml of this solution was then transferred to another bottle and the *E. coli* cell lysate containing Atu3025 prepared above section was added. The alginate degradation was carried out at room temperature over night. The resulting products were analyzed by thin layer chromatography, and DEHU formation was confirmed.

Preparation of D-mannuronate solution by chemical degradation.

D-mannuronate solution was chemically prepared based on the protocol previously described by Spoehr (*Archive of Biochemistry*, 14: pp153-155). Fifty milligram of alginate was dissolved into 800 µL of ninety percent formate. This solution was incubated at 100 °C for over night. Formate was then evaporated and the residual substances were washed with absolute ethanol twice. The residual substance was again dissolved into absolute ethanol and filtrated. Ethanol was evaporated and residual substances were resuspended into 20 mL of 20 mM Tris-HCl (pH 8.0) and the solution was filtrated to make a D-mannuronate solution. This D-mannuronate solution was diluted 5-fold and used for assay.

Assay for DEHU hydrogenase. To identify DEHU hydrogenase, a NADPH dependent DEHU hydrogenation assay was performed. 20 µl of prepared cell lysate containing each ADH was added to 160 µl of 20-fold deluted DEHU solution prepared in the above section. 20 µl of 2.5 mg/ml of NADPH solution (20 mM Tris-HCl, pH 8.0) was added to initiate the hydrogenation reaction, as a preliminary study

using cell lysate of *A. tumefaciens* C58 have shown that DEHU hydrogenation requires NADPH as a co-factor. The consumption of NADPH was monitored an absorbance at 340 nm for 30 min using the kinetic mode of ThermoMAX 96 well plate reader (Molecular Devises). *E. coli* cell lysate containing alcohol dehydrogenase (ADH) 10 lacking a portion of N-terminal domain was used in a control reaction mixture.

Assay for D-mannuronate hydrogenase. To identify D-mannuronate hydrogenase, a NADPH dependent D-mannuronate hydrogenation assay was performed. 20 µl of prepared cell lysate containing each ADH was added to 160 µl of D-mannuronate solution prepared in the above section. 20 µl of 2.5 mg/ml of NADPH solution (20 mM Tris-HCl, pH 8.0) was added to initiate the hydrogenation reaction. The consumption of NADPH was monitored an absorbance at 340 nm for 30 min using the kinetic mode of ThermoMAX 96 well plate reader (Molecular Devises). *E. coli* cell lysate containing alcohol dehydrogenase (ADH) 10 lacking a portion of N-terminal domain was used in a control reaction mixture.

Construction of pETkdgK. DNA sequence of *kdgK* of *Escherichi coli* encoding 2-keto-deoxy gluconate kinase was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'- AGGTACGGTGAAATAA AGGAGG ATATACCAT ATGTCCAAAAAGATTGCCGT -3') (SEQ ID NO:157) and reverse (5'- TTTTCCTTTTGCGGCCGCCCCGCTGGCATCGCCTCAC -3') (SEQ ID NO:158) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 µl. Amplified DNA fragment was digested with NdeI and NotI and ligated into pET29 pre-digested with the same restriction enzymes.

Construction of pETkdgA. DNA sequence of *kdgA* *Escherichi coli* encoding 2-keto-deoxy gluconate-6-phosphate aldolase was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'- GGCGATGCCAGCGTAA AGGAGG ATATACCAT

ATGAAAACTGGAAAACAAG -3') (SEQ ID NO:159) and reverse (5'-TTTTCCTTTTGCGGCCGCCCCAGCTTAGCGCCTTCTA -3') (SEQ ID NO:160) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 µl. Amplified DNA fragment was digested with NdeI and NotI and ligated into pET29 pre-digested with the same restriction enzymes.

Protein expression and purification. All plasmids (pETAtu3025, pETADH11, pETADH12, pETkdgA, pETkdgK, and pETuxuA) were transformed into *Escherichia coli* strain BL21(DE3). The single colonies of BL21(DE3) containing respective plasmids were inoculated into 50 ml of LB media containing 50 µg/ml kanamycin (Km⁵⁰). These strains were grown in an orbital shaker with 200 rpm at 37 °C. The 0.2 mM IPTG was added to each culture when the OD_{600nm} reached 0.6, and the induced culture was grown in an orbital shaker with 200 rpm at 20 °C. 24 hours after the induction, the cells were harvested by centrifugation at 4,000 rpm x g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 µl of Lysonase™ Bioprocessing Reagent (Novagen) and suggested amount of protease inhibitor cocktail (SIGMA). The solution was again centrifuged at 4,000 rpm x g for 10 min and the supernatant was obtained. The supernatant was applied to Nickel-NTA spin column (Qiagen) to purify His-tagged proteins.

The results of the assays for DEHU hydrogenase activity and D-mannuronate hydrogenase activity of ADH1-10 are shown in Figure 7A and 7B. These results demonstrate that the novel enzymes ADH1 and ADH2 showed significant DEHU hydrogenase activity (Figure 7A), and that the novel enzymes ADH3, ADH4, and ADH9 showed significant mannuronate hydrogenase activity (Figure 7B).

In vitro pyruvate formation. The reaction mixture contained 1 % alginate or ~0.5 % mannuronate, ~5µg of purified Atu3026 (ADH12) or Atu3027 (ADH11), and ~5µg of purified oligoalginate lyase (Atu3025), UxuA, KdgK, and KdgA, 2 mM of ATP, and 0.6 mM of NADPH in 20mM Tris-HCl pH7.0. The reaction was carried out over night and the pyruvate formation was monitored by the pyruvate assay kit (BioVision, Inc).

The results of *in vitro* pyruvate formation from alginate mediated by enzymatic and chemical degradation are shown in Figure 6B and Figure 6C, respectively. As can be seen in these figures, alginate was converted to pyruvate via the isolated enzymes. These results also show that each of Atu3026 (ADH12) and Atu3027 (ADH11) are capable of catalyzing both DEHU hydrogenase and mannuronate hydrogenase reactions.

Example 4

Construction and Biological Activity of Biosynthesis Pathways

Construction of Pathways:

A propionaldehyde biosynthetic pathway comprising a threonine deaminase (*ilvA*) gene from *Escherichia coli* and keto-isovalerate decarboxylase (*kivd*) from *Lactococcus lactis* is constructed and tested for the ability to convert L-threonine to propionaldehyde.

A butyraldehyde biosynthetic pathway comprising a thiolase (*atoB*) gene from *E. coli*, β -hydroxy butyryl-CoA dehydrogenase (*hbd*), crotonase (*crt*), butyryl-CoA dehydrogenase (*bcd*), electron transfer flavoprotein A (*etfA*), and electron transfer flavoprotein B (*etfB*) genes from *Clostridium acetobutyricum* ATCC 824, and a coenzyme A-linked butyraldehyde dehydrogenase (*ald*) gene from *Clostridium beijerinckii acetobutyricum* ATCC 824 was constructed in *E. coli* and tested for the ability to produce butyraldehyde. Also, a coenzyme A-linked alcohol dehydrogenase (*adhE2*) gene from *Clostridium acetobutyricum* ATCC 824 was used as an alternative to *ald* and tested for the ability to produce butanol.

An isobutyraldehyde biosynthetic pathway comprising an acetolactate synthase (*alsS*) from *Bacillus subtilis* or (*als*) from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (codon usage was optimized for *E. coli* protein expression) and acetolactate reductoisomerase (*ilvC*) and 2,3-dihydroxyisovalerate dehydratase

(*ilvD*), genes from *E. coli* and keto-isovalerate decarboxylase (*kivd*) from *Lactococcus lactis* was constructed and tested for the ability to produce isobutyraldehyde, as measured by isobutanol production.

3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathways comprising an acetolactate synthase (*alsS*) from *Bacillus subtilis* or (*als*) from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 (codon usage was optimized for *E. coli* protein expression), acetolactate reductoisomerase (*ilvC*), 2,3-dihydroxyisovalerate dehydratase (*ilvD*), isopropylmalate synthase (*LeuA*), isopropylmalate isomerase (*LeuC* and *LeuD*), and 3-isopropylmalate dehydrogenase (*LeuB*) genes from *E. coli* and keto-isovalerate decarboxylase (*kivd*) from *Lactococcus lactis* were constructed and tested for the ability to produce 3-isovaleraldehyde and 2-isovaleraldehyde.

Phenylacetaldehyde and 4-hydroxyphenylacetaldehyde biosynthesis pathways comprising a transketolase (*tktA*), a 3-deoxy-7-phosphoheptulonate synthase (*aroF*, *aroG*, and *aroH*), 3-dehydroquinate synthase (*aroB*), a 3-dehydroquinate dehydratase (*aroD*), a dehydroshikimate reductase (*aroE*), a shikimate kinase II (*aroL*), a shikimate kinase I (*aroK*), a 5-enolpyruvylshikimate-3-phosphate synthetase (*aroA*), a chorismate synthase (*aroC*), a fused chorismate mutase P/prephenate dehydratase (*pheA*), and a fused chorismate mutase T/prephenate dehydrogenase (*tyrA*) genes from *E. coli*, keto-isovalerate decarboxylase (*kivd*) from *Lactococcus lactis* were constructed and tested for the ability to produce phenylacetaldehyde and/or 4-hydroxyphenylacetaldehyde.

A 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-yl)ethanol biosynthesis pathway comprising a transketolase (*tktA*), a 3-deoxy-7-phosphoheptulonate synthase (*aroF*, *aroG*, and *aroH*), 3-dehydroquinate synthase (*aroB*), a 3-dehydroquinate dehydratase (*aroD*), a dehydroshikimate reductase (*aroE*), a shikimate kinase II (*aroL*), a shikimate kinase I (*aroK*), a 5-enolpyruvylshikimate-3-phosphate synthetase (*aroA*), a chorismate synthase (*aroC*), a fused chorismate mutase P/prephenate dehydratase (*pheA*), and a fused chorismate mutase T/prephenate

dehydrogenase (*tyrA*) genes from *E. coli*, keto-isovalerate decarboxylase (*kivd*) from *Lactococcus lactis*, alcohol dehydrogenase (*adh2*) from *Saccharomyces cerevisiae*, Indole-3-pyruvate decarboxylase (*ipdc*) from *Azospirillum brasilense*, phenylethanol reductase (*par*) from *Rhodococcus* sp. ST-10, and benzaldehyde lyase (*bal*) from *Pseudomonas fluorescens* was constructed and tested for the ability to produce 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol and/or 2-(indole-3)ethanol.

Construction of pBADButP. The DNA sequence encoding *hbd*, *crt*, *bcd*, *etfA*, and *etfB* of *Clostridium acetobutyricum* ATCC 824 was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 3 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCTTAGGAGGATTAGTCATGGAAC-3') (SEQ ID NO:161) and reverse (5'-GCTTCTAGATTATTTTGAATAATCGTAGAAACC-3') (SEQ ID NO:162) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Clostridium acetobutyricum* ATCC 824 genome (ATCC) in 50 µl. Amplified DNA fragment was digested with BamHI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADButP-atoB. The DNA sequence encoding *atoB* of *Escherichia coli* DH10B was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-GCTTCTAGAGGAGGATATATATATGAAAAATTGTGTCATCGTC-3') (SEQ ID NO:163) and reverse (5'-AA CTGCAGTTAAATTCAACCGTTCAATCACC-3') (SEQ ID NO:164) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 µl. Amplified DNA fragment was digested with XbaI and PstI and ligated into pBADButP pre-digested with the same restriction enzymes.

Construction of pBADatoB-ald. The DNA sequence encoding *atoB* of *Escherichia coli* DH10B and *ald* from *Clostridium beijerinckii* were amplified separately by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and

72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CGAGCTC AGGAGGATATATATATGAAAAATTGTGTCATCGTCAGTG-3') (SEQ ID NO:165) for *atoB* and 5'-GGTTGAATTAAGGAGGATATATATATGAATAAAGACACACTAATACCTAC-3' for *ald*) (SEQ ID NO:166) and reverse (5'-GTCTTTATTCATATATATATCCTCCTTAATTCAACCGTTCAATCACCATC-3' (SEQ ID NO:146) for *atoB* and 5'-CCCAAGCTTAGCCGGCAAGTACACATCTTC-3' for *ald*) (SEQ ID NO:167) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B and *Clostridium beijerinckii* genome (ATCC) in 50 µl, respectively. The amplified DNA fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 2 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CGAGCTC AGGAGGATATATATATGAAAAATTGTGTCATCGTCAGTG-3') (SEQ ID NO:168) and reverse (5'-CCCAAGCTTAGCCGGCAAGTACACATCTTC-3') (SEQ ID NO:169) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with *SacI* and *HindIII* and ligated into pBADButP pre-digested with the same restriction enzymes.

Construction of pBADButP-*atoB*-ALD. The DNA fragment 1 encoding chloramphenicol acetyltransferase (CAT), P15 origin of replication, *araBAD* promoter, *atoB* of *Escherichia coli* DH10B and *ald* of *Clostridium beijerinckii* and the DNA fragment 2 encoding *araBAD* promoter, *hbd*, *crt*, *bcd*, *etfA*, and *etfB* of *Clostridium acetobutyricum* ATCC 824 were amplified separately by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-AAGGAAAAAAGCGGCCGCCCTGAACCGACGACCGGGTTCG-3') (SEQ ID NO:170) for fragment 1 and 5'-CGGGGTACCACTTTTCATACTCCCGCCATTCAG-3' (SEQ ID NO:274) for

fragment 2, and reverse (5'-

CGGGGTACCGCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:171) for

fragment 1 and (5'-

AAGGAAAAAAGCGGCCGCGGATACATATTTGAATGTATTTAG-3') (SEQ

ID NO:172) for fragment 2) primers, 1U Phusion High Fidelity DNA polymerase

(NEB), and 50 ng pBADatoB-ald and pBADButP in 50 µl, respectively. Amplified

DNA fragments were digested with NotI and KpnI and ligated each other.

Construction of pBADilvCD. The DNA fragments encoding *ilvC* and *ilvD* of *Escherichia coli* DH10B were amplified separately by polymerase chain

reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30

times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM

forward (5'-GCTCTAGAGGAGGATATATATATGGCTAACTACTTCAATACAC-

3') (SEQ ID NO:173) for *ilvC* and 5'-

TGCTGTTGCGGGTTAAGGAGGATATATATATGCCTAAGTACCGTTCCGCC-3'

for *ilvD*) (SEQ ID NO:174) and reverse (5'-

AACGGTACTTAGGCATATATATATCCTCCTTAACCCGCAACAGCAATACG-

3') (SEQ ID NO:175) for *ilvC* and 5'-

ACATGCATGCTTAACCCCCCAGTTTCGATT-3') (SEQ ID NO:176) for *ilvD*)

primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli*

DH10B genome (ATCC) in 50 µl. The amplified DNA fragments were gel purified and

eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and

each DNA fragment was spliced by another round of PCR: 98°C for 10 sec, 60°C for 15

sec, and 72°C for 2 min, repeated 30 times. The reaction mixture contained 1 x Phusion

buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-

GCTCTAGAGGAGGATATATATATGGCTAACTACTTCAATACAC-3') (SEQ ID

NO:177) and reverse (5'- ACATGCATGCTTAACCCCCCAGTTTCGATT-3') (SEQ

ID NO:178) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced

fragment was digested with XbaI and SphI and ligated into pBAD33 pre-digested with

the same restriction enzymes.

Construction of pBADals-ilvCD. The DNA fragment encoding *als* of *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 of its codon usage optimized for over-expression in *E.coli* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCAGGAGGATATATATATGGATAAACAGTATCCGGT-3') (SEQ ID NO:179) and reverse (5'-GCTTCTAGATTACAGAATTTGACTCAGGT-3') (SEQ ID NO:180) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETals in 50 µl. The amplified DNA fragment was digested with SacI and XbaI and ligated into pBADilvCD pre-digested with the same restriction enzymes.

Construction of pBADalsS-ilvCD. The DNA fragments encoding front and bottom halves of *alsS* of *Bacillus subtilis B26* were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 0.5 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCAGGAGGATATATATATGTTGACAAAAGCAACAAAAG-3') (SEQ ID NO:181) for front and 5'-CGGTACCCTTTCCAGAGATTAGAG-3' (SEQ ID NO:275) for back halves, and reverse (5'-CTCTAAATCTCTGGAAAGGGTACCG-3') (SEQ ID NO:182) for front and (5'-GCTTCTAGATTAGAGAGCTTTCGTTTTTCATG-3' for back halves) (SEQ ID NO:183) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Bacillus subtilis B26* genome (ATCC) in 50 µl. The amplified DNA fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCAGGAGGATATATATATGTTGACAAAAGCAACAAAAG-3') (SEQ ID NO:184) and reverse (5'-GCTTCTAGATTAGAGAGCTTTCGTTTTTCATG-3') (SEQ ID NO:185) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The

spliced fragment was internal XbaI site free and thus was digested with SacI and XbaI and ligated into pBADilvCD pre-digested with the same restriction enzymes.

Construction of pBADLeuABCD. The DNA fragment encoding *leuA*, *leuB*, *leuC*, and *leuD* of *Escherichia coli* BL21(DE3) was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 3 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-
CGAGCTCAGGAGGATATATATATGAGCCAGCAAGTCATTATTTTCG-3')
 (SEQ ID NO:186) and reverse (5'-
 AAAACTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:187)
 primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 µl. The amplified DNA fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADLeuABCD2. The DNA fragment 1 encoding *leuA* and *leuB* and the DNA fragment 2 encoding *leuC* and *leuD* of *Escherichia coli* BL21(DE3) were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-
CGAGCTCAGGAGGATATATATATGAGCCAGCAAGTCATTATTTTCG-3')
 (SEQ ID NO:188) for fragment 1 and (5'-
 AGGGGTGTAAGGAGGATATATATATGGCTAAGACGTTATACGAAAAATTG -
 3') (SEQ ID NO:189) for fragment 2 and reverse (5'-
 CGTCTTAGCCATATATATATCCTCCTTACACCCCTTCTGCTACATAGCGG-3')
 (SEQ ID NO:190) for fragment 1 and (5'-
 AAAACTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:191) for
 fragment 2 primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 µl, respectively. The amplified DNA fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of

PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 3 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CGAGCTCAGGAGGATATATATATGAGCCAGCAAGTCATTATTTTCG-3') (SEQ ID NO:192) and reverse (5'-AAAACTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:193) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADLeuABCD4. The DNA fragments encoding *leuA*, *leuB*, *leuC* and *leuD* of *Escherichia coli* BL21(DE3) were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-

CGAGCTCAGGAGGATATATATATGAGCCAGCAAGTCATTATTTTCG-3')

(SEQ ID NO:194) for *leuA*, (5'-

GAAACCGTGTGAGGAGGATATATATATGTCGAAGAATTACCATATTGCCG-3') (SEQ ID NO:195) for *leuB*, (5'-

AGGGGTGTAAGGAGGATATATATATGGCTAAGACGTTATACGAAAAATTG

-3') (SEQ ID NO:196) for *leuC*, and (5'-

ACATTAAATAAGGAGGATATATATATGGCAGAGAAATTTATCAAACACAC-3') (SEQ ID NO:197) for *leuD* and reverse (5'-

ATTCTTCGACATATATATATCCTCCTCACACGGTTTCCTTGTTGTTTTCG-3')

(SEQ ID NO:198) for *leuA*, (5'-

CGTCTTAGCCATATATATATCCTCCTTACACCCCTTCTGCTACATAGCGG-3') (SEQ ID NO:199) for *leuB*, (5'-

TTTCTCTGCCATATATATATCCTCCTTATTTAATGTTGCGAATGTCGGCG-3') (SEQ ID NO:200) for *leuC*, and (5'-

AAAACTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:201) for *leuD* primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng

Escherichia coli BL21(DE3) genome in 50 µl, respectively. The amplified DNA fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 3 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CGAGCTCAGGAGGATATATATATGAGCCAGCAAGTCATTATTTTCG-3') (SEQ ID NO:202) and reverse (5'-AAAACTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:203) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADals-ilvCD-leuABCD, pBADals-ilvCD-leuABCD2, pBADals-ilvCD-leuABCD4, pBADalsS-ilvCD-leuABCD, pBADalsS-ilvCD-leuABCD2, pBADalsS-ilvCD-leuABCD4. The DNA fragments 1 (for als) and 2 (for alsS) encoding chloramphenicol acetyltransferase (CAT), P15 origin of replication, *araBAD* promoter, *als* of *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 of its codon usage optimized for over-expression in *E.coli* or *alsS* of *Bacillus subtilis* B26 and *ilvC* and *ilvD* of *E. coli* DH10B were amplified separately by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-AAGGAAAAAGCGGCCGCCCCTGAACCGACGACCGGGTTCG-3') (SEQ ID NO:204) and reverse (5'-CGGGGTACCGCGGATACATATTTGAATGTATTAG-3') (SEQ ID NO:205) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADals-ilvCD and pBADalsS-ilvCD in 50 µl, respectively.

To remove an internal SphI restriction enzyme site from *leuC*, overlap PCR was carried out. The front and bottom halves of DNA fragment 3 (for *leuABCD*), fragment 4 (for *leuABCD2*), and fragment 5 (for *leuABCD4*) encoding *araBAD* promoter, *leuA*, *leuB*, *leuC*, and *leuD* of *E. coli* BL21(DE3) were amplified separately

by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-AAGGAAAAAGCGGCCGCACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:206) for front and (5'-CAAAGGCCGTCTGCACGCGCCGAAAGGCAAA-3') (SEQ ID NO:207) for back halves) and reverse (5'-TTTGCCTTTTCGGCGCGTGCAGACGGCCTTTG-3') (SEQ ID NO:208) for front and (5'-ACATGCATGCCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:209) for bottom halves, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADleuABCD, pBADleuABCD2, and pBADleuABCD4 in 50 μ l, respectively. The amplified DNA fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-AAGGAAAAAGCGGCCGCACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:210) and reverse (5'-ACATGCATGCCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:211) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The resulting fragment 3, 4, and 5 were digested with SphI and NotI and ligated into both fragment 1 and 2 pre-digested with the same restriction enzymes.

Construction of pBADaroG-tktA-aroBDE. The DNA fragments encoding *aroG*, *tktA*, *aroB*, *aroD*, and *aroE* of *Escherichia coli* BL21(DE3) were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCCGAGCTCAGGAGGATATATATATGAATTATCAGAACGACGATTTAC-3') (SEQ ID NO:212) for *aroG*, (5'-GCGTCGCGGGTAAGGAGGAAAATTTTATGTCCTCACGTAAAGAGCTTGCC-3') (SEQ ID NO:213) for *tktA*, (5'-GAACTGCTGTAAGGAGGTAAAATTATGGAGAGGATTGTCGTTACTCTCG-3') (SEQ ID NO:214) for *aroB*, } (5'-

CAATCAGCGTAAGGAGGTATATATAATGAAAACCGTAACTGTAAAAGATC-3') (SEQ ID NO:215) for *aroD*, and (5'-TACACCAGGCATAAGGAGGAATTAATTATGGAAACCTATGCTGTTTTTGG-3') (SEQ ID NO:216) for *aroE* and reverse (5'-TACGTGAGGACATAAAATTTTCCTCCTTACCCGCGACGCGCTTTTACTGC-3') (SEQ ID NO:217) for *aroG*, (5'-CAATCCTCTCCATAATTTTAACCTCCTTACAGCAGTTCTTTTGCTTTCGC-3') (SEQ ID NO:218) for *tktA*, (5'-CAATCAGCGTAAGGAGGTATATATAATGAAAACCGTAACTGTAAAAGATC-3') (SEQ ID NO:219) for *aroB*, (5'-TACGGTTTTTCATTATATATACCTCCTTACGCTGATTGACAATCGGCAATG-3') (SEQ ID NO:220) for *aroD*, and (5'-ACATGCATGCTTACGCGGACAATTCCTCCTGCAA-3') (SEQ ID NO:221) for *aroE*, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 µl, respectively. The amplified DNA fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 3 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCAGGAGGATATATATATGAATTATCAGAACGACGATTTAC-3') (SEQ ID NO:222) and reverse (5'-ACATGCATGCTTACGCGGACAATTCCTCCTGCAA-3') (SEQ ID NO:223) primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with *SacI* and *SphI* and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADp*heA*-*aroLAC*. The DNA fragments encoding *pheA*, *aroL*, *aroA*, and *aroC* of *Escherichia coli* DH10 were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-

CCCGAGCTCAGGAGGATATATATATGACATCGGAAAACCCGTTACTGG-3')
 (SEQ ID NO:224) for *pheA*, (5'-
 GATCCAACCTAAGGAGGAAAATTTTATGACACAACCTCTTTTTCTGATCG-3')
 (SEQ ID NO:225) for *aroL*, (5'-
 GATCAATTGTTAAGGAGGTATATATAATGGAATCCCTGACGTTACAACCC-
 3') (SEQ ID NO:226) for *aroA*, and (5'-
 CAGGCAGCCTAAGGAGGAATTAATTATGGCTGGAAACACAATTGGACAAC-
 3') (SEQ ID NO:227) for *aroC* and reverse (5'-
 AGGTTGTGTCATAAAATTTTCCTCCTTAGGTTGGATCAACAGGCACTACG-3')
 (SEQ ID NO:228) for *pheA*, (5'-
 CAGGGATTCCATTATATATACCTCCTTAACAATTGATCGTCTGTGCCAGG-3')
 (SEQ ID NO:229) for *aroL*, (5'-
 GTTTCAGCCATAATTAATTCCTCCTTAGGCTGCCTGGCTAATCCGCGCC-3')
 (SEQ ID NO:230) for *aroA*, and (5'-
 ACATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:231) for
aroC primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng
Escherichia coli BL21(DE3) genome in 50 µl, respectively. The amplified DNA
 fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each
 DNA solution was combined and each DNA fragment was spliced by another round of
 PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The
 reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward
 (5'-CCCGAGCTCAGGAGGATATATATATGACATCGGAAAACCCGTTACTGG-
 3') (SEQ ID NO:232) and reverse (5'-
 ACATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:233)
 primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was
 digested with SacI and SphI and ligated into pBAD33 pre-digested with the same
 restriction enzymes.

Construction of pBAD_{tyrA}-aroLAC. The DNA fragments encoding
pheA, *aroL*, *aroA*, and *aroC* of *Escherichia coli* DH10 were amplified by polymerase
 chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated

30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-

CCCGAGCTCAGGAGGATATATATATGGTTGCTGAATTGACCGCATTAC-3')

(SEQ ID NO:234) for *tyrA*, (5'-

AATCGCCAGTAAGGAGGAAAATTTTATGACACAACCTCTTTTTCTGATCG-

3') (SEQ ID NO:235) for *aroL*, (5'-

GATCAATTGTTAAGGAGGTATATATAATGGAATCCCTGACGTTACAACCC-

3') (SEQ ID NO:236) for *aroA*, and (5'-

CAGGCAGCCTAAGGAGGAATTAATTATGGCTGGAAACACAATTGGACAAC-

3') (SEQ ID NO:237) for *aroC*, and reverse (5'-

GAGGTTGTGTCATAAAATTTTCCTCCTTACTGGCGATTGTCATTCGCCTG-3')

(SEQ ID NO:238) for *tyrA*, (5'-

CAGGGATTCCATTATATATACCTCCTTAACAATTGATCGTCTGTGCCAGG-3')

(SEQ ID NO:239) for *aroL*, (5'-

GTTTCCAGCCATAATTAATTCCTCCTTAGGCTGCCTGGCTAATCCGCGCC-3')

(SEQ ID NO:240) for *aroA*, and (5'-

ACATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:241) for

aroC, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli*

BL21(DE3) genome in 50 μ l, respectively. The amplified DNA fragments were gel

purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l from each DNA solution was

combined and each DNA fragment was spliced by another round of PCR: 98°C for 10

sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture

contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-

CCCGAGCTCAGGAGGATATATATATGGTTGCTGAATTGACCGCATTAC-3')

(SEQ ID NO:242) and reverse (5'-

ACATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:243)

primers, 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was

digested with *SacI* and *SphI* and ligated into pBAD33 pre-digested with the same

restriction enzymes.

Construction of pBADpheA-aroLAC-aroG-tktA-aroBDE and pBADtyrA-aroLAC-aroG-tktA-aroBDE. A DNA fragment 1 (for *pheA*) and 2 (for *tyrA*) encoding chloramphenicol acetyltransferase (CAT), P15 origin of replication, *araBAD* promoter, *pheA* or *tyrA*, *aroL*, *aroA*, *aroC* of *Escherichia coli* DH10B and a DNA fragment 3 encoding *araBAD* promoter, *aroG*, *tktA*, *aroB*, *aroD*, and *aroE* of *Escherichia coli* DH10B were amplified separately by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 4 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-AAGGAAAAAAGCGGCCGCCCTGAACCGACGACCGGGTCG-3') (SEQ ID NO:244) for fragment 1 and 2 and (5'-GCTCTAGAACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:245) for fragment 3, and reverse (5'-GCTCTAGAGCGGATACATATTTGAATGTATTAG-3') (SEQ ID NO:246) for fragment 1 and 2 and (5'-AAGGAAAAAAGCGGCCGCCGCGGATACATATTTGAATGTATTAG-3') (SEQ ID NO:247) for fragment 3, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADpheA-aroLAC, pBADtyrA-aroLAC, and pBADaroG-tktA-aroBDE in 50 µl, respectively. Amplified DNA fragments 1 and 2 were digested with NotI and XbaI and ligated into fragment 3 pre-digested with the same restriction enzymes.

Construction of pTrcBAL. A DNA sequence encoding benzaldehyde lyase (*bal*) of *Pseudomonas fluorescens* of its codon usage optimized for over-expression in *E. coli* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CATGCCATGGCTATGATTACTGGTGG-3') (SEQ ID NO:248) and reverse (5'-CCCCGAGCTCTTACGCGCCGGATTGGAAATACA-3') (SEQ ID NO:249) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 µl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

Construction of pTrcAdhE2. A DNA sequence encoding Co-A linked alcohol/aldehyde dehydrogenase (*adhE2*) of *Clostridium acetobutyricum* ATCC824 was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CATGCCATGGCCAAAGTTACAAATCAAAAAG-3') (SEQ ID NO:250) and reverse (5'-CGAGCTCTTAAAATGATTTTATATAGATATCC-3') (SEQ ID NO:251) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Clostridium acetobutyricum* ATCC824 genome in 50 µl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

Construction of pTrcAdh2. A DNA sequence encoding alcohol dehydrogenase (*adh2*) of *Saccharomyces cerevisiae* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CATGCCATGGGTATTCCAGAACTCAAAAAG-3') (SEQ ID NO:252) and reverse (5'-CCCGAGCTCTTATTTAGAAGTGTCACAACG-3') (SEQ ID NO:253) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng genome of *Saccharomyces cerevisiae* in 50 µl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

Construction of pTrcBALD. A DNA sequence encoding CoA-linked aldehyde dehydrogenase (*ald*) of *Clostridium beijerinckii* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCCGAGCTCAGGAGGATATACATATGAATAAAGACACACTAATACC-3') (SEQ ID NO:254) and reverse (5'-CCCAAGCTTAGCCGGCAAGTACACATCTTC-3') (SEQ ID NO:255) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 µl.

Amplified DNA fragment was digested with SacI and HndIII and ligated into pTrcBAL pre-digested with the same restriction enzymes.

Construction of pTrcBALK. A DNA sequence encoding ketoisovalerate decarboxylase (kivd) of *Lactococcus lactis* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCAGGAGGATATATATATGTATACAGTAGGAGATTACC-3') (SEQ ID NO:256) and reverse (5'-GCTTCTAGATTATGATTTATTTTGTTTCAGCAAAT-3') (SEQ ID NO:257) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 µl. Amplified DNA fragment was digested with SacI and XbaI and ligated into pTrcBAL pre-digested with the same restriction enzymes.

Construction of pTrcAdh-Kivd. A DNA sequence encoding ketoisovalerate decarboxylase (kivd) of *Lactococcus lactis* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-CCCGAGCTCAGGAGGATATATATATGTATACAGTAGGAGATTACC-3') (SEQ ID NO:258) and reverse (5'-GCTTCTAGATTATGATTTATTTTGTTTCAGCAAAT-3') (SEQ ID NO:259) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 µl. Amplified DNA fragment was digested with SacI and XbaI and ligated into pTrcAdh2 pre-digested with the same restriction enzymes.

Construction of pTrcBAL-DDH-2ADH. To remove internal NcoI site, overlap PCR was carried out. DNA fragments encoding front and bottom halves of meso-2,3-butanedioldehydrogenase (ddh) of *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 and secondary alcohol dehydrogenase (2adh) of *Pseudomonas fluorescens* were amplified separately by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-

CGAGCTCAGGAGGATATATATATGAAAAAAGTCGCACTTGTTACCG-3')
 (SEQ ID NO:260) for front half of *ddh*, (5'-
 GGCCGGCGGCCGCGCGATGGCGGTGAAAGTG-3') (SEQ ID NO:261) for bottom
 half of *ddh*, (5'-
 AACTAATCTAGAGGAGGATATATATATGAGCATGACGTTTTCCGGCCAGG-
 3') (SEQ ID NO:262) for front half of *2adh*, and (5'-
 CCTTGCGGAGGGCTCGATGGATGAGTTCGAC-3') (SEQ ID NO:263) for bottom
 half of *2adh*, and reverse (5'-CACTTTCACCGCCATCGCGCGGCCGCGGCC-3')
 (SEQ ID NO:264) for front half of *ddh*, (5'-
 GCTCATATATATATCCTCCTCTAGATTAGTTAAACACCATCCCGCCGTCG-3')
 (SEQ ID NO:265) for bottom half of *ddh*, (5'-
 GTCGAACTCATCCATCGAGCCCTCCGCAAGG-3') (SEQ ID NO:266) for front
 half of *2adh*, and (5'-CCC**AAGCTT**AGATCGCGGTGGCCCCGCCGTCG-3') (SEQ
 ID NO:267) for bottom half of *2adh*, 1U Phusion High Fidelity DNA polymerase
 (NEB), and 50 ng *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 for *ddh* and
Pseudomonas fluorescens genome for *2adh* in 50 µl, respectively. The amplified DNA
 fragments were gel purified and eluted into 30ul of EB buffer (Qiagen). 5 ul from each
 DNA solution was combined and each DNA fragment was spliced by another round of
 PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 2 min, repeated 30 times. The
 reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward
 (5'- **CGAGCTC**AGGAGGATATATATATGAAAAAAGTCGCACTTGTTACCG-3')
 (SEQ ID NO:268) and reverse (5'-
 CCC**AAGCTT**AGATCGCGGTGGCCCCGCCGTCG-3') (SEQ ID NO:269) primers,
 1U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested
 with SacI and HindIII and ligated into pTrcBAL pre-digested with the same restriction
 enzymes.

Construction of pBBRPduCDEGH. A DNA sequence encoding
 propanediol dehydratase medium (pduD) and small (pduE) subunits and propanediol
 dehydratase reactivation large (pduG) and small (pduH) subunits of *Klebsiella*
pneumoniae subsp. pneumoniae MGH 78578 was amplified by polymerase chain

reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 2 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-

GCTCTAGAGGAGGATTAAAAATGGAAATTAACGAAACGCTGC-3') (SEQ ID NO:270) and reverse (5'-

TCCCGCGGTTAAGCATGGCGATCCCGAAATGGAATCCCTTTGAC-3') (SEQ ID NO:271) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 in 50 µl. Amplified DNA fragment was digested with SacII and XbaI and ligated into pTrc99A pre-digested with the same restriction enzymes to form pBBRPduDEGH.

A DNA sequence encoding propanediol dehydratase large subunit (pduC) of *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward (5'-

CCGCTCGAGGAGGATATATATATGAGATCGAAAAGATTTGAAGC-3') (SEQ ID NO:272) and reverse (5'- **GCTCTAGATTAGCCAAGTTCATTGGGATCG-3'**)

(SEQ ID NO:273) primers, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 in 50 µl. Amplified DNA fragment was digested with XhoI and XbaI and ligated into pBBRPduDEGH pre-digested with the same restriction enzymes.

Construction of pTrcIpdc-Par. A DNA sequence encoding indole-3-pyruvate (ipdc) of *Azospirillum brasilense* and phenylethanol reductase (par) of *Rhodococcus sp.* ST-10 were amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 1 min, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 µM forward primers (5'- **CATGCCATGGGACTGGCTGAGGCACTGCTGC-3'** (SEQ ID NO:314) for ipdc and 5'-**CGAGCTCAGGAGGATATATATATGAAAGCTATCCAGTACACCCGTAT-3'** (SEQ ID NO:315) for par, and reverse primers (5'-

CGAGCTCTTATTCGCGCGGTGCCGCGTGCAGG-3' (SEQ ID NO:316) for ipdc and 5'-GCTCTAGATTACAGGCCCGGAACCACAACGGCGC-3' (SEQ ID NO:317) for par, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pTrcIpdc and pTrcPar, respectively, in 50 µl. Amplified DNA fragment of ipdc and par were digested with NcoI/SacI and SacI/XbaI, respectively, and were ligated into pTrc99A pre-digested with NcoI and XbaI.

Testing and Results:

To test the butyraldehyde biosynthesis pathway, DH10B harboring pBADButP-atoB/pTrcBALD and pBADButP-atoB-ALD/pTrcB2DH/pBBRpduCDEGH were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and Amp¹⁰⁰ and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS.

To test the isobutyraldehyde biosynthesis pathway, DH10B cells harboring pBADals-ilvCD/pTrcBALK or pBADalsS-ilvCD/pTrcBALK were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and Amp¹⁰⁰ and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS for the production of isobutyraldehyde. Figure 8B shows the production of isobutanol from these cultures.

To test the 3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathways, DH10B harboring pBADals-ilvCD-LeuABCD/pTrcBALK,

pBADals-ilvCD-LeuABCD2/pTrcBALK, pBADals-ilvCD-LeuABCD/pTrcBALK4, pBADalsS-LeuABCD/pTrcBALK, pBADalsS-LeuABCD2/pTrcBALK, or pBADalsS-LeuABCD4/pTrcBALK were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS. The production of 2-isovaleralcohol (2-methylpentanal) and 3-isovaleralcohol (3-methylpentanal) was monitored because 3-isovaleraldehyde and 2-isovaleraldehyde are spontaneously converted to their corresponding alcohols. Figure 8B shows the production of 2-methylpentanal and 3-methylpentanal from these cultures.

To test the phenylacetaldehyde and 4-hydroxyphenylacetaldehyde biosynthesis pathways, DH10B cells harboring pBADpheA-aroLAC/pTrcBALK, pBADtyrA-aroLAC/pTrcBALK, pBADaroG-tktA-aroBDE/pTrcBALK, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK, and pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS. The production of phenylacetaldehyde, 4-hydroxyphenylaldehyde and their corresponding alcohols were monitored using GC-MS. Figure 9B shows the production of 4-hydroxyphenylethanol from these cultures.

To test the 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3) ethanol biosynthesis pathways, DH10B harboring pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK,

pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcAdh2-Kivd, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcAdh2-Kivd, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcIpdC-Par, and pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcIpdC-Par were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight to a week. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS. The results are detailed below.

The production of 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol and/or 2-(indole-3-)ethanol was monitored using GC-MS. Figure 42A shows the production of 2-phenylethanol from these cultures at 24 hours. Figure 42B shows the production of 2-(4-hydroxyphenyl)ethanol from these cultures at 24 hours. Figure 42C shows the production of 2-(indole-3-)ethanol from these cultures at 24 hours.

Figure 43A shows the GC-MS chromatogram for control (pBAD33 and pTrc99A) at one week. Figure 43B shows the GC-MS chromatogram for 2-phenylethanol (5.97 min) production from pBADpheA-aroLAC-aroG-tktA-aroBDE and pTrcBALK at one week. Figure 44 shows the GC-MS chromatogram for 2-(4-hydroxyphenyl) ethanol (9.36 min) and 2-(indole-3) ethanol (10.32 min) production from pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK at one week.

Example 5

Isolation and Biological Activity of Diol Dehydrogenases

Available substrates such as 3-hydroxy-2-butanone (acetoin), 4-hydroxy-3-hexanone (propionin), 5-hydroxy-4-octanone (butyrolin), 6-hydroxy-5-decanone (valeroin), and 1,2-cyclopentanediol were used to measure the ability of diol

dehydrogenases (*ddh*) to catalyze the reduction of large saturated α -hydroxyketones to produce a diol. All reagents were purchased from Sigma-Aldrich Co. and TCI America, unless otherwise stated.

For cloning and isolation of DDH polypeptides, genomic DNA from several species of bacteria were obtained from ATCC (*Lactobacillus brevis* ATCC 367, *Pseudomonas putida* KT2440, and *Klebsiella pneumoniae* MGH78578), PCR-amplified (using Phusion with polymerase with 1X Phusion buffer, 0.2 mM dNTP, 0.5 μ L Phusion enzyme, 1.5 μ M primers, and 20 pg template DNA in a 50 μ L reaction) utilizing the following protocol: 30 cycles, 98 °C/10 secs (denaturing), 60 °C/15 secs (annealing), 72 °C/30 secs (elongation). Polymerase chain reaction products were then digested using restriction enzymes NdeI and BamHI, then ligated into NdeI/BamHI digested pET28 vectors. Vectors containing *ddh* clones were transformed into BL21(DE3) competent cells for protein expression. Single colony was inoculated into LB media, and expression of 6X His-tagged proteins of interest was induced at OD₆₀₀ = 0.6 with 0.1 mM IPTG. Expression was allowed to proceed for 15 hours at 22 °C. The 6X His-tagged enzymes were purified using Ni-NTA spin columns following suggested protocols by QIAGEN, yielding purified protein concentrations in the range of 1.1 – 6.5 mg/mL (determined by Bradford assay).

Diol dehydrogenase *ddh1* was isolated from *Lactobacillus brevis* ATCC 367, diol dehydrogenase *ddh2* was isolated from *Pseudomonas putida* KT2440, and diol dehydrogenase *ddh3* was isolated from *Klebsiella pneumoniae* MGH78578. The nucleotide sequence encoding and polypeptide sequence of *ddh1* are shown in SEQ ID NOS:97 and 98, respectively; nucleotide sequence encoding and polypeptide sequence of *ddh2* are shown in SEQ ID NOS:99 and 100, respectively; and nucleotide sequence encoding and polypeptide sequence of *ddh3* are shown in SEQ ID NOS: 101 and 102, respectively.

Reactions to measure biological activity of DDH polypeptides were performed in a final volume of 200 μ L as follows: 25mM substrate, 0.04 mg/mL DDH polypeptide, 0.25 mg/mL nicotinamide cofactor, 200 mM imidazole, 14 mM Tris-HCl,

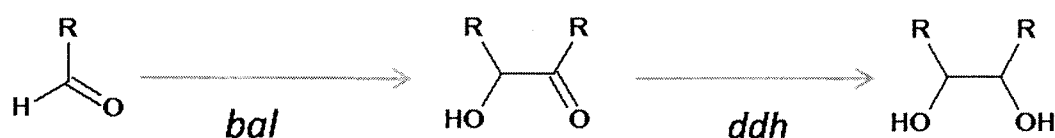
and 1.5% by volume DMSO. Biological activity was assayed using a Molecular Devices Thermomax 96 well plate reader, monitoring absorbance at 340 nm, which corresponds to NADH or NADPH concentration. For the kinetic studies, 0.04 mg/mL DDH polypeptide, 0.25 mg/mL NADH, 20mM Tris HCl Buffer pH 6.5(red) or 9.0(ox), T=25 C, 100 uL total volume was used.

Figure 12A shows the biological activity of *ddh1*, *ddh2*, and *ddh3* using butyoin as a substrate (triangles represent *ddh3* activity). Figure 12B shows the oxidation activity of *ddh3* towards 1,2-cyclopentanediol and 1,2-cyclohexanediol as measured by NADH production. Figure 13 summarizes the results of kinetic studies for various substrates in the oxidation reactions catalyzed by the DDH polypeptides. These reactions were NAD⁺ dependent.

Example 6

Sequential *In Vivo* Biological Activity of CC-Ligases (Lyases) and Diol Dehydrogenases

The ability of a C-C lyase and a diol hydrogenase to perform the following sequential reaction was tested in *E. coli*:



For α -hydroxyketone and diol production, a pathway comprising a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and meso-2,3-butanediol dehydrogenase (*ddh*) gene isolated from *Klebsiella pneumoniae subsp. pneumoniae* MGH 78578 was constructed in *E. coli* and tested for its ability to condensate the substrates detailed below in Table 2 (e.g., acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, phenylacetaldehyde, and 4-hydroxyphenylacetaldehyde, or their corresponding

alcohols) to form α -hydroxyketone and the corresponding diol *in vivo*. The production of various α -hydroxyketones and diols was monitored by gas chromatography-mass spectrometry (GC-MS).

Table 2: Summary of substrates and products.

Substrate	Produced α -hydroxyketone	Produced diol	Figures
Butanal	5-Hydroxy-4-octanone	4,5-Octanediol	17A & B
<i>n</i> -Pentanal	6-Hydroxy-5-decanone	5,6-Decanediol	18A & B
3-Methylbutanal	2,7-Dimethyl-5-hydroxy-4-octanone	2,7-Dimethyl-4,5-octanediol	19A & B
<i>n</i> -Hexanal	7-Hydroxy-6-dodecanone	6,7-dodecanediol	20A & B
4-Methylpentanal	2,9-Dimethyl-6-hydroxy-5-decanone	2,9-Dimethyl-5,6-decanediol	21A & B
<i>n</i> -Octanal	9-Hydroxy-8-hexadecanone	8,9-hexadecanediol	22
Acetaldehyde	3-Hydroxy-2-butanone	2,3-Butanediol	23
<i>n</i> -Propanal	4-Hydroxy-3-hexanone	3,4-Hexanediol	24A & B
Phenylacetaldehyde	1,4-Diphenyl-3-hydroxy-2-butanone	1,4-Diphenyl-2,3-butanediol	25

For analysis of $\leq$ C10. *E. coli* harboring pTrcBAL-DDH-2ADH was grown for overnight in LB media containing 50 ug/ml Kanamycine (Km). This seed culture was inoculated into M9 media containing 3 % (v/v) glycerol, 0.5 % (g/v) and 50 ug/ml Km. 10mL cultures were grown to O.D.₆₀₀ = 0.7, then cultures were induced with 0.5 mM IPTG. The cells were allowed to express the enzymes of interest for 3 hours before various aldehydes were added to a concentration of 5-10 mM. After addition of aldehydes, the cultures were capped and incubated at 37 °C with shaking for 72 hours. Cultures were extracted with 2 mL ethyl acetate, and analyzed on GC-MS using the following protocol:

1 μ L injection w/ 50:1 split
 Inlet temperature – 150 °C
 Initial oven temperature – 50 °C
 Temperature Ramp 1 – 10 °C/min to 150 °C
 Temperature Ramp 2 – 50 °C/min to 300 °C
 GC to MS transfer temp – 250 °C
 MS detection- full scan MW 35-200

For analysis of $\geq$ C12. *E. coli* DH10B strains harboring pTrc99A (Ctrl vector) or pTrcBAL were inoculated into 0.75 x M9/ 0.5% LB containing 0.1mM CaCl₂, 2 mM MgSO₄, 1mM KCl, 1% galacturonate, 5 μ g/mL thiamine, Amp. The

cultures were grown up to an optical density (600nm) of 0.8 and induced with 0.25 mM IPTG. The cells were allowed to express the proteins for 2.5 hours at 37 °C, then aldehyde substrate was added to a concentration of 5mM, the culture vial was capped tightly and incubated for 72 hours at 37 °C w/ shaking 200 rpm. 1 mL of the final culture was extracted with 0.75 mL of ethyl acetate, centrifuged facilitate phase separation, then analyzed via GCMS using the following method.

1 μ L injection w/ 50:1 split
Inlet temperature – 250 °C
Initial oven temperature – 50 °C
Temperature Ramp 1 – 10 °C/min to 125 °C
Temperature Ramp 2 – 30 °C/min to 300 °C
Final Temperature 300 °C – 1 minute
GC to MS transfer temp – 250 °C
MS detection- full scan MW 40-260.

The results are depicted in Figures 17 through 25. Figure 17 shows the sequential conversion of butanal into 5-hydroxy-4-octanone and then 4,5-octanediol. Figure 18 shows the sequential conversion of *n*-pentanal into 6-hydroxy-5-decanone and then 5,6-decanediol. Figure 19 shows the conversion of 3-methylbutanal into 2,7-dimethyl-5-hydroxy-4-octanone and then 2,7-Dimethyl-4,5-octanediol. Figure 20 shows the sequential conversion of *n*-hexanal into 7-hydroxy-6-dodecanone and then 6,7-dodecanediol. Figure 21 shows the conversion of 4-methylpentanal into 2,9-dimethyl-6-hydroxy-5-decanone and then 2,9-dimethyl-5,6-decanediol. Figure 22 shows the conversion of *n*-octanal into 9-hydroxy-8- hexadecanone. Figure 23 shows the conversion of acetaldehyde into 3-hydroxy-2-butanone. Figure 24 shows the sequential conversion of *n*-propanal into 4-hydroxy-3-hexanone and then 3,4-hexanediol. Figure 25 shows the conversion of phenylacetoaldehyde into 1,4-diphenyl-3-hydroxy-2-butanone.

Similar to above, a pathway comprising a benzaldehyde lyase (*bal*) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) was constructed in *E. coli* and tested for its ability to catalyze the production of various α -hydroxyketones. The results, which show the broad spectrum

of C-C ligase activity for the *bal* gene tested, are set forth in Figure 48 through Figure 55.

Example 7

Sequential Biological Activity of Diol Dehydrogenases and Diol Dehydratases

To test the sequential biological activity of diol dehydrogenases and diol dehydratases in a dehydration and reduction pathway, butyrolin was used as a substrate in a sequential reaction to produce 4-octanone. The enzyme diol dehydrogenase (*e.g.*, *ddh*) catalyzes the reversible reduction and oxidation of α -hydroxy ketones and its corresponding diol, such as 5-hydroxy-4-octanone and 4,5-octanediol, and the enzyme diol dehydratase (*e.g.*, *pduCDE*) catalyzes the irreversible dehydration of diols, such as 4,5-octanediol.

Diol dehydrogenase *ddh* from *Klebsiella pneumoniae* MGH 78578 and diol dehydratase *pduCDE* from *Klebsiella pneumoniae* MGH 78578 were cloned into a bacterial expression vector and expressed and purified on a Ni-NTA column, as described in Example X except that 1 mM of 1,2-propanediol was added at all time during the expression and purification of diol dehydratase. The large, medium, and small subunits of the *pduCDE* polypeptide are encoded by the nucleotide sequences of SEQ ID NOs:103, 105, and 107, respectively, and the polypeptide sequence are set forth in SEQ ID NOs: 104, 106, and 108, respectively.

The *ddh3* and *pduCDE* polypeptides were incubated with butyrolin and their appropriate cofactors, then assayed using gas chromatography-mass spectrometry (GC-MS) for their ability to perform sequential reactions resulting in the product 4-octanone. Reaction conditions are given in Table 3 below. The reaction mixture was incubated at 37 °C for 40 hours in a 0.6 mL eppendorf tube with minimal head space. The reaction product was extracted with an equivalent volume of ethyl acetate, stored in a glass vial, and sent to Thermo Fischer Scientific Instruments Division for compositional analysis by GC-MS.

Table 3 – Reaction Conditions	

Rxn Component	Concentration
5-hydroxy-4-octanone (butyrolin)	8.4 mM
Adenosylcobalamin (coenzyme B ₁₂)	33.5 μ M
KCl	9.6 mM
NADH	18 mM
dDH3 enzyme	0.19 mg/mL
dDOH1 enzyme mix	0.15 mg/mL
Reaction Buffer	10 mM Tris HCl pH 7.0

Figure 26A shows GC-MS data which confirms the presence of 4,5-octanediol in the sample extraction. The mass-spectra of the peaks, retention time, at 5.36 was identified as butyrolin (substrate), and at 6.01, 6.09, and 6.12 min were identified as different isomers of 4,5-octanediol. This compound is the expected product resulting from the reduction of butyrolin by *ddh3*.

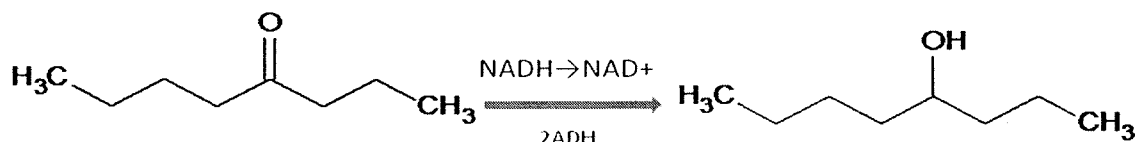
Figure 26B shows GC-MS data confirming the presence of 4-octanone in the sample extraction. The mass-spectra of the peak, retention time, at 4.55 was identified as 4-octanone. This compound is the expected product resulting from the sequential dehydrogenation of butyrolin and dehydration of 4,5-octanediol by *ddh3* and *pduCDE*, respectively.

Figures 27A and 27B show comparisons between the sample extraction gas chromatograph/mass spectrum and the 4-octanone standard gas chromatograph/mass spectrum. These results demonstrate that 4-octanone was produced from butyrolin using the enzymes diol dehydrogenase (*ddh3*) and a diol dehydratase (*pduCDE*). GC-MS analysis of the incubated reaction mixture confirmed starting material, intermediate and product, demonstrating that these enzymes can be reappropriated for these specific substrates.

Example 8

Isolation and Biological Activity of Secondary Alcohol Dehydrogenases

Substrates such as 4-octanone, 2,7-dimethyl-4-octanone, cyclopentanone and corresponding alcohols were utilized to measure the ability of secondary alcohol dehydrogenases (2ADHs) to catalyze the reduction of large saturated ketones to secondary alcohols. An example of a reaction catalyzed by secondary alcohol dehydrogenases is illustrated below (reduction of 4-octanone to 4-octanol is shown):



All enzymes and reagents were purchased from New England Biolabs and Sigma, respectively, unless otherwise stated.

Various secondary alcohol dehydrogenases (2ADHs) were isolated from *Pseudomonas putida* KT2440, *Pseudomonas fluorescens* Pf-5, and *Klebsiella pneumoniae* MGH 78578. All vectors were transformed in BL21(DE3) competent cells and expression of the genes encoding the proteins of interest was induced with IPTG (via the T7 promoter). The cells were lysed, proteins were extracted and then purified on Ni-NTA columns. Final protein concentration in the Ni-NTA eluate was diluted to 0.15 mg/mL prior to assays.

NADPH/NADPH consumption and production assays were performed using a THERMOMax microplate reader in the kinetic mode, monitoring the NADPH absorbance peak at 340 nm until the reaction reached equilibrium. In the assay described in Table 2, 2ADH-2, 2ADH-5, 2ADH-8, and 2ADH-10 were tested for their ability to either catalyze the oxidation of 4-octanol or catalyze the reduction of 4-octanone. These reaction conditions are found in Table 4 below.

Table 4 – Reaction Conditions for Various Enzyme Assays	
NADH Production Assay (30 °C)	
Reaction Component	Final Concentration
2ADH enzyme	Approx. 0.058 µg/µL
4-octanol	5.55 mM
NAD ⁺	Approx. 1.4 µg/µL
Imidazole (from Elution Buffer)	Approx. 280 mM

NADH Consumption Assay (30 °C)	
Reaction Component	Final Concentration
2ADH enzyme	Approx. 0.075 µg/µL
4-octanone	5.0 mM
NADH	Approx. 0.25 µg/µL
Imidazole (from Elution Buffer)	Approx. 250 mM
NADPH Production Assay (30 °C)	
Reaction Component	Final Concentration
2ADH enzyme	Approx. 0.058 µg/µL
4-octanol	5.55 mM
NADP+	Approx. 1.4 µg/µL
Imidazole (from Elution Buffer)	Approx. 280 mM

Further testing was performed, as described in Tables 5 below, in which 2ADH-2, 2ADH-11, 2ADH-12, 2ADH-13, 2ADH-14, 2ADH-15, 2ADH-16, 2ADH-17, and 2ADH-18 were tested for their ability to either catalyze the oxidation of 4-octanol, 2,7-dimethyl-4-octanol, or cyclopentanol, or catalyze the reduction of 4-octanone, 2,7-dimethyl-4-octanone, or cyclopentanone.

Table 5:

Rxn Components for NADPH Consumption Assays (Reduction)	
Rxn Component	Final Concentration
Substrate	25 mM
Enzyme	0.04 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidazole	200 mM
Tris HCl	14 mM
DMSO	1.5% by volume
Total Volume	200 µL
Rxn Components for NAD(P)H Production Assays (Oxidation)	
Rxn Component	Final Concentration
Substrate	5 mM
Enzyme	0.04 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidazole	200 mM
Tris HCl	14 mM
Rxn Components for NAD(P)H Production Assay using 2,7-dimethyl-4-octanone as a substrate	

Rxn Component	Final Concentration
Substrate	50 mM
Enzyme	0.08 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidazole	200 mM
Tris HCl	14 mM
DMSO	3% by volume

Figure 30A shows the results from the NADH Production Assay of Table 3, in which 2ADH-2 catalyzes the oxidation of 4-octanol in the presence of NAD⁺, as measured by NADH production. Figure 30B shows the results of the NADPH Production Assay of Table 3, in which 2ADH-5, 2ADH-8, and 2ADH-10 catalyze the oxidation of 4-octanol in the presence of NADP⁺, as measured by NADPH production.

Figure 31 shows the oxidation of 4-octanol by 2ADH-11 (Figure 31A) and 2ADH-16 (Figure 31B), as measured by NADH and NADPH production, respectively. Figure 32 shows the oxidation of 2,7-dimethyloctanol by 2ADH-11 and others (Figure 32A) and 2ADH-16 (Figure 32B), as measured by NADH and NADPH production, respectively.

Figure 33A shows the reduction of 2,7-dimethyl octanol by 2ADH 11 and 2ADH16 as monitored by NADPH consumption. Figure 33B shows the reduction activity of both 2ADH11 and 2ADH16 towards various substrates. Figure 34 shows the oxidation (Figure 34A) and reduction (Figure 34B) of cyclopentanol by 2ADH-16.

Similar to above, kinetic testing for both oxidation and reduction reactions was performed on various substrates using 2ADH-16. The conditions for these studies were as follows: 0.04 mg/mL enzyme, 0.25 mg/mL cofactor, 20mM Tris HCl Buffer pH 6.5(red) or 9.0(ox), T=25 C, 100 uL total volume was used. The calculated rate constants for the reduction reactions, along with the structures of the substrates, are summarized in Figure 35. The calculated rate constants for the oxidation reactions, along with the structures of the substrates, are summarized in Figure 36.

These results show that 2ADH-16 is capable of catalyzing both the oxidation and reduction of a wide variety of substrates.

Example 9

Isolation and *In Vitro* and *In Vivo* Activity of Coenzyme B12 Independent Diol Dehydratases

Substrates such as 1,2-propanediol, meso-2,3-butanediol, and *trans*-1,2-cyclopentanediol were utilized to test both the *in vitro* and *in vivo* biological activity of a B12 independent diol dehydratase in a dehydration and reduction pathway. Diol dehydratases catalyzes the irreversible dehydration of diols, such as 1,2-propanediol.

For *in vitro* activity, *E. coli* BL21(DE3) harboring pETPduCDE (diol dehydratase subunits) was inoculated into 100 mL LB media, grown to to OD₆₀₀ = 0.7, induced with 0.15 mM IPTG, and incubated for 22 hours at 22 °C. The cells were lysed and proteins of interest were purified on a Ni-NTA spin column. Purification of all three dehydratase subunits was accomplished by adding 5 mM 1,2-propanediol to the lysis and wash buffers. The Ni-NTA purification yielded approximately 660 µL of protein mixture at a concentration of 2.2 mg/mL. Protein concentration assays were conducted using a Bradford reagent protocol.

The purified PduCDE was used to set up *in vitro* diol dehydratase reactions. Three assays were conducted with 1,2-propanediol and meso-2,3-butanediol. Control reactions were also set up with elution buffer added in place of purified PduCDE. *In vitro* reactions were conducted under semi-anaerobic conditions in 2 mL screw cap glass vials. Reaction components and concentrations are given in Table 6.

Table 6 – Reaction conditions for B ₁₂ dependent DDOH <i>in vitro</i> assay	
Rxn Component	Concentration
Diol substrate	10 mM
Adenosylcobalamin (B ₁₂)	100 µg/mL
KCl	10 mM
dOH1 enzyme mix	0.08 mg/mL

Reaction Buffer	10 mM Tris HCl pH 7.5

After 48 hours, 1 mL of the reaction mixture was extracted with 0.5 mL of either ethylacetate or hexanol and analyzed by GCMS.

The following GCMS protocol was used for all experiments:

1 μ L injection w/ 50:1 split
 Inlet temperature – 250 °C
 Initial oven temperature – 50 °C
 Temperature Ramp 1 – 10 °C/min to 125 °C
 Temperature Ramp 2 – 30 °C/min to 300 °C
 Final Temperature 300 °C – 1 minute
 GC to MS transfer temp – 250 °C
 MS detection- full scan MW 40-260

The results are shown in Figure 45. Figure 45A confirms the formation of 1-propanal from 1,2-propanediol, and Figure 45B confirms the formation of 2-butanone from meso-2,3-butanediol, both of which were catalyzed by B12 independent diol dehydratase.

For *in vivo* activity, the pBBRDhaB1/2 plasmid was constructed as follows: the DNA sequence encoding B12-independent glycerol dehydratase (*dhaB1*) and activator (*dhaB2*) of *Clostridium butyricum* was amplified by polymerase chain reaction (PCR): 98°C for 10 sec, 60°C for 15 sec, and 72°C for 2 min for *dhaB1* and 1min for *dhaB2*, repeated 30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward primers (5'-CCGCTCGAGGAGGATATATATATGATTCTAAAGGCTTTAGCACCC-3' (SEQ ID NO:318) for *dhaB1* and 5'-ACGTGATGTAATTCTAGAGGAGGATATATATATGAGCAAAGAAATTAAAGG-3' (SEQ ID NO:319) for *dhaB2*, and reverse primers (5'-TCTTTGCTCATATATATATCCTCCTCTAGATTACATCACGTGTTTCAGTAC-3' (SEQ ID NO:320) for *dhaB1* and 5'-CGAGCTCTTATTCGGCGCCAATGGTGCACGGG-3' (SEQ ID NO:321) for *dhaB2*, 1U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETdhaB1 and pETdhaB2, respectively, in 50 μ L. Amplified fragments were gel purified and spliced by another round of PCR: 98°C for 10 sec, 60°C for 15 sec, and 72°C for 2.5 min, repeated

30 times. The reaction mixture contained 1 x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-

CCGCTCGAGGGAGGATATATATATGATTTCTAAAGGCTTTAGCACCC-3')

(SEQ ID NO:322) and reverse primers (5'-

CGAGCTCTTATTCGGCGCCAATGGTGCACGGG-3') (SEQ ID NO:323), 1U

Phusion High Fidelity DNA polymerase (NEB), and 50 ng each fragment in 50 μ l.

Amplified DNA fragment was digested with XhoI and SacI and ligated into

pBBR1MCS-2 pre-digested with the same restriction enzymes.

Two strains of *E. coli* DH10B harboring pBBR1MCS-2 or pBBRDhaB1/2 into TB media without glycerol were inoculated. Cultures were grown to OD₆₀₀ = 0.5 and the substrates 1,2-propanediol, meso-2,3-butanediol, and *trans*-1,2-cyclopentanediol were added to separate cultures to a concentration of 10 mM. 5 μ g/ml of co-enzyme S-adenosylmethionine was added before the culture is transferred to anaerobic environment. The cultures were incubated at 37C for 48 hrs.

After 48 hours, 1 mL of culture was extracted with 0.5 mL of ethylacetate or hexanol and analyzed by GCMS, as described above. The results are shown in Figure 46. Figure 46A shows the *in vivo* production of 1-propanol from 1,2-propanediol. Figure 46B shows the *in vivo* production of 2-butanol from meso-2,3-butanediol. Figure 46C shows the *in vivo* production of cyclopentanone from *trans*-1,2-cyclopentanediol.

Example 10

Identification of Secreted Alginate Lyase and Genomic Regions Sufficient For Growth on Alginate as a Sole Source of Carbon

To identify secreted or external alginate lyases, and to identify genomic regions from *Vibrio splendidus* that are sufficient to confer growth in alginate as a sole source of carbon, the following clones were made using the gateway system from Invitrogen (Carlsbad, CA). First, entry vectors were made by TOPO cloning PCR fragments into pENTR/D/TOPO. PCR fragments were generated using *Vibrio*

splendidus B01 genomic DNA as a template and amplified with the following primer pairs:

Vs24214-24249: genomic region corresponding to gene id between V12B01_24214 and V12B01_24249 (*see* Example 1).

Table 7

24214 F	cacc caagcgatagtttatatagcgt (SEQ ID NO:324)
24249R	gaaatgaacggatattacgt (SEQ ID NO:325)

Vs24189-24209: genomic region corresponding to gene id between V12B01_24189 and V12B01_24209 (*see* Example 1).

Table 8

24189 R	cggaacaggtgattgtggt (SEQ ID NO:326)
24209 F	cacc gccacttcaagatgaagctgt (SEQ ID NO:327)

Vs24214-24239: genomic region corresponding to gene id between V12B01_24214 and V12B01_24239 (*see* Example 1).

Table 9

24214 F	cacc caagcgatagtttatatagcgt (SEQ ID NO:328)
24239 R_1	gtggctaagtacatgccggt (SEQ ID NO:329)

The entry vectors were recombined with the destination vector pET-DEST42 (Invitrogen) using the LR recombinase enzyme (Invitrogen). These destination vectors were then put into electrocompetent DH10B or BL21 cells.

The alginate lyase clones were then made by digesting (using enzymes Nde I and Bam HI) the PCR products that were generated using *Vibrio splendidus* 12B01 genomic DNA as a template and amplified with the following primer pairs:

Table 10

24214 ndeF	GGAATTC CAT atgacaagaatatgacgactaac (SEQ ID NO:330) for forward primer for V12B01_24214
24214 bamR	CG GGATCC ttattattcccctgcctgcagt (SEQ ID NO:331) for reverse primer for V12B01_24214

24219 ndeF	GGAATTC CAT atgagctatcaaccacttttac (SEQ ID NO:332) for forward primer for V12B01_24219
24219 bamR	CG GGATCC ttacagttgagcaaatgatcc (SEQ ID NO:333) for reverse primer for V12B01_24219

The digested PCR products were then ligated into cut pET28 vector. Certain of the cloned genomic regions of *Vibrio splendidus* B01 were tested for the presence of secreted alginate lyases, and the above-described constructs were tested in various combinations for the ability to confer growth on alginate as a sole source of carbon.

The Vs24254 (SEQ ID NO: 32) region of *Vibro splendidus* encodes a functional external alginate lyase. BL21 cells expressing Vs24254 from the pET28 vector were capable of breaking down alginate in the growth medium. When grown on LB + 2% alginate + 0.1 mM Isopropyl β -D-1-thiogalactopyranoside (IPTG), only cells expressing the Vs24254 gene give a positive TBA assay result of pink color. This assay was performed by spinning down an overnight culture grown on the above mentioned media. The media was then mixed in a 1:1 ratio with 0.8% thiobarbituric acid (TBA), heated for 10 min at 99 degrees Celsius, and assayed for pink coloration. Figure 47 shows the results of this assay. The left tube in Figure 47 represents media taken from an overnight culture of cells expressing Vs24254, while the right hand tube shows the TBA reaction using media from cells expressing Vs24259 (negative control). The lack of pink coloration in the negative control indicates that little or no cleavage of the alginate polymer has occurred. Wildtype *E. coli* cells not expressing any recombinant proteins show the same coloration as the negative control Vs24259 (data not shown).

To test the ability of recombinant *E. coli* to grow on alginate as a sole source of carbon, transformed cells were grown for 19 hours at 30 degrees Celsius with mild shaking in a 96-well plate. Each well held 222 μ l of minimal media (see growth conditions for explanation of minimal media) with the 0.66% carbon source in the form of either degraded alginate or glucose (positive control for growth). All cells were either BL21 with no plasmid (BL21 – negative control), one plasmid (Da or 3a), or two plasmids (Dk3a and Da3k). The plasmids are indicated by the lower case letter: “a”

refers to the plasmid backbone pET-DEST42 and “k” refers to the pENTR/D/TOPO backbone. “D” indicates that the plasmid contains the genomic region Vs24214-24249, while “3” indicates that the plasmid contains the genomic region Vs24189-24209. Thus, Da would be pET-DEST42-Vs24214-24249, Da3k would be pET-DEST42-Vs24214-24249 and pENTR/D/TOPO-Vs24189-24209 and so on.

As shown in Figure 56A, the two vector-constructs pET-DEST42-Vs24214-24249 and pENTR/D/TOPO-Vs24189-24209 when combined in *E. coli* confer growth on degraded alginate as the sole carbon source. This same result is observed when these genomic inserts are switched into the opposite vector (pET-DEST42-Vs24189-24209 and pENTR/D/TOPO-Vs24214-24249). Figure 56B shows growth on glucose as a positive control. Thus, the combined genomic regions of Vs24214-24249 and Vs24189-24209 from *Vibro splendidus* were sufficient to confer on *E. coli* the ability to grow on alginate as a sole source of carbon.

Example 11

Production of Ethanol from Alginate

The ability of recombinant *E. coli* to produce ethanol by growing on alginate on a source of carbon was tested. To generate recombinant *E. coli*, DNA sequences encoding pyruvate decarboxylase (*pdc*), and two alcohol dehydrogenase (*adhA* and *adhB*) of *Zymomonas mobilis* were amplified by polymerase chain reaction (PCR). These amplified fragments were gel purified and spliced together by another round of PCR. The final amplified DNA fragment was digested with BamHI and XbaI ligated into pBBR1MCS-2 pre-digested with the same restriction enzymes. The resulting plasmid is referred to as pBBRPdc-AdhA/B.

E. coli was transformed with either pBBRPdc-AdhA/B or pBBRPdc-AdhA/B + 1.5 Fos (fosmid clone containing genomic region between V12B01_24189 and V12B01_24249; these sequences confer on *E. coli* the ability to use alginate as a sole source of carbon, see Examples 1 and 10), grown in m9 media containing alginate, and tested for the production of ethanol. The results are shown in Figure 57, which demonstrates that the strain harboring pBBRPdc-AdhA/B + 1.5 FOS showed

significantly higher ethanol production when growing on alginate. These results indicate that the pBBRPdc-AdhA/B + 1.5 FOS was able to utilize alginate as a source of carbon in the production of ethanol.

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Claims:

1. A method for converting a polysaccharide to a commodity chemical, comprising:

(a) contacting the polysaccharide, wherein the polysaccharide is optionally derived from biomass, with a polysaccharide degrading or depolymerizing metabolic system, wherein the metabolic system is selected from;

(i) enzymatic or chemical catalysis, and

(ii) a microbial system, wherein the microbial system comprises a recombinant microorganism, wherein the recombinant microorganism comprises one or exogenous genes that allow it to grow on the polysaccharide as a sole source of carbon,

thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide; and

(b) contacting the suitable monosaccharide or oligosaccharide with commodity chemical biosynthesis pathway, wherein the commodity chemical biosynthesis pathway comprises an aldehyde or ketone biosynthesis pathway,

thereby converting the polysaccharide to the commodity chemical

2. The method of claim 1, wherein the biomass is selected from marine biomass and vegetable/fruit/plant biomass.

3. The method of claim 2, wherein the marine biomass is selected from kelp, giant kelp, sargasso, seaweed, algae, marine microflora, microalgae, and sea grass.

4. The method of claim 2, wherein the vegetable/fruit/plant biomass comprises plant peel or pomace.

5. The method of claim 2, wherein the vegetable/fruit/plant biomass is selected from citrus, potato, tomato, grape, gooseberry, carrot, mango, sugar-beet, apple, switchgrass, wood, and stover.

6. The method of claim 1, wherein the polysaccharide is selected from alginate, agar, carrageenan, fucoidan, pectin, polygalacturonate, cellulose, hemicellulose, xylan, arabinan, and mannan.

7. The method of claim 1, wherein the suitable monosaccharide or oligosaccharide is selected from 2-keto-3-deoxy D-gluconate (KDG), D-mannitol, guluronate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose.

8. The method of claim 1, wherein the commodity chemical is selected from methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butanediol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl) ethanol, , 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanediol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanedione, indolyethane, indolyethene, 2-(indole-3-)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal,

pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pentene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione, 4-methyl-1-phenyl-3-hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-

pentanedione, 4-methyl-1-(indole-3-)pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 4-methyl-1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl-1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2,3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3,4-hexanedione, 2,5-dimethyl-3-hydroxy-4-hexanone, 5-methyl-1-phenylhexane, 4-methyl-1-phenylhexane, 5-methyl-1-phenyl-1-hexene, 5-methyl-1-phenyl-2-hexene, 5-methyl-1-phenyl-3-hexene, 4-methyl-1-phenyl-1-hexene, 4-methyl-1-phenyl-2-hexene, 4-methyl-1-phenyl-3-hexene, 5-methyl-1-phenyl-2-hexanol, 5-methyl-1-phenyl-3-hexanol, 4-methyl-1-phenyl-2-hexanol, 4-methyl-1-phenyl-3-hexanol, 5-methyl-1-phenyl-2-hexanone, 5-methyl-1-phenyl-3-hexanone, 4-methyl-1-phenyl-2-hexanone, 4-methyl-1-phenyl-3-hexanone, 5-methyl-1-phenyl-2,3-hexanediol, 4-methyl-1-phenyl-2,3-hexanediol, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-3-hydroxy-2-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 5-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-(4-

hydroxyphenyl)hexane, 5-methyl-1-(4-hydroxyphenyl)-1-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexene, 5-methyl-1-(4-hydroxyphenyl)-3-hexene, 4-methyl-1-(4-hydroxyphenyl)-1-hexene, 4-methyl-1-(4-hydroxyphenyl)-2-hexene, 4-methyl-1-(4-hydroxyphenyl)-3-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexanol, 5-methyl-1-(4-hydroxyphenyl)-3-hexanol, 4-methyl-1-(4-hydroxyphenyl)-2-hexanol, 4-methyl-1-(4-hydroxyphenyl)-3-hexanol, 5-methyl-1-(4-hydroxyphenyl)-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(indole-3-yl)hexane, 5-methyl-1-(indole-3-yl)-1-hexene, 5-methyl-1-(indole-3-yl)-2-hexene, 5-methyl-1-(indole-3-yl)-3-hexene, 4-methyl-1-(indole-3-yl)-1-hexene, 4-methyl-1-(indole-3-yl)-2-hexene, 4-methyl-1-(indole-3-yl)-3-hexene, 5-methyl-1-(indole-3-yl)-2-hexanol, 5-methyl-1-(indole-3-yl)-3-hexanol, 4-methyl-1-(indole-3-yl)-2-hexanol, 4-methyl-1-(indole-3-yl)-3-hexanol, 5-methyl-1-(indole-3-yl)-2-hexanone, 5-methyl-1-(indole-3-yl)-3-hexanone, 4-methyl-1-(indole-3-yl)-2-hexanone, 4-methyl-1-(indole-3-yl)-3-hexanone, 5-methyl-1-(indole-3-yl)-2,3-hexanediol, 4-methyl-1-(indole-3-yl)-2,3-hexanediol, 5-methyl-1-(indole-3-yl)-3-hydroxy-2-hexanone, 5-methyl-1-(indole-3-yl)-2-hydroxy-3-hexanone, 4-methyl-1-(indole-3-yl)-3-hydroxy-2-hexanone, 4-methyl-1-(indole-3-yl)-2-hydroxy-3-hexanone, 5-methyl-1-(indole-3-yl)-2,3-hexanedione, 4-methyl-1-(indole-3-yl)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-

heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2,5-dimethyl-2-heptene, 2,5-dimethyl-3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2,6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone, 2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol,

nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedione, 1-undecene, 1-undecanol, undecanal, undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decdecene, 1-dodecanol, ddodecanal, dodecanoate, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1, 3-propanediol, 4-hydroxybutanal, 1, 4-butanediol, 3-hydroxy-2-butanone, 2, 3-butanediol, 1,5-pentane diol, homocitrate, homoisocitrate, b-hydroxy adipate, glutarate, glutaraldehyde, glutaraldehyde, 2-hydroxy-1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2,3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino pentaldehyde, 1,10-diaminodecane, 1,10-diamino-5-decene,

1,10-diamino-5-hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-phenylbutane, 1-(4-hydroxyphenyl)-4-phenyl-1-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butanol, 1-(4-hydroxyphenyl)-4-phenyl-2-butanone, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydroxyphenyl)-4-phenyl-3-hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl)-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetaldehyde, 1,4-di(indole-3)-butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, and phosphate.

9. A method for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising:

(a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a microbial system for a time sufficient to convert the polysaccharide to a suitable monosaccharide or oligosaccharide, wherein the microbial system comprises,

(i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase and/or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain;

(ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and

(iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase,

thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide.

10. A method for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising:

(a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a chemical or enzymatic catalysis pathway for a time sufficient to convert the polysaccharide to a first monosaccharide or oligosaccharide; and

(b) contacting the first monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert the first monosaccharide or oligosaccharide to the suitable monosaccharide or oligosaccharide, wherein the microbial system comprises,

(i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase

(ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and

(ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase,

thereby converting the polysaccharide to the suitable monosaccharide or oligosaccharide.

11. The method of claim 9 or claim 10, wherein the lyase is selected from an alginate lyase, a pectate lyase, a polymannuronate lyase, a polygluronate lyase, a polygalacturonate lyase and a rhamnogalacturonate lyase.

12. The method of claim 9 or claim 10, wherein the hydrolase is selected from an alginate hydrolase, a rhamnogalacturonate hydrolase, a polymannuronate hydrolase, a pectin hydrolase, and a polygalacturonate hydrolase.

13. The method of claim 9 or claim 10, wherein the transporter is selected from an ABC transporter, a symporter, and an outer membrane porin.

14. The method of claim 13, wherein the ABC transporter is selected from Atu3021, Atu3022, Atu3023, Atu3024, algM1, algM2, AlgQ1, AlgQ2, AlgS, OG2516_05558, OG2516_05563, OG2516_05568, OG2516_05573, TogM, TogN, TogA, TogB, and functional variants thereof.

15. The method of claim 13, wherein the symporter is selected from V12B01_24239 (SEQ ID NO:26), V12B01_24194 (SEQ ID NO:8), and TogT, and functional variants thereof.

16. The method of claim 13, wherein the outermembrane porin comprises a porin selected from V12B01_24269, KdgM, and KdgN, and functional variants thereof.

17. A recombinant microorganism that is capable of growing on a polysaccharide as a sole source of carbon, wherein the polysaccharide is selected from alginate, pectin, tri-galacturonate, di-galacturonate, cellulose, and hemi-cellulose.

18. The recombinant microorganism of claim 17, wherein the polysaccharide is alginate.

19. The recombinant microorganism of claim 17, wherein the polysaccharide is pectin.

20. The recombinant microorganism of claim 17, wherein the polysaccharide is tri-galacturonate.

21. A recombinant microorganism, comprising (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase.

22. The recombinant microorganism of any one of claims 17-21, wherein the microorganism is capable of growing on a polysaccharide as a sole source of carbon.

23. The recombinant microorganism of claim 22, wherein the polysaccharide is selected from alginate, pectin, and tri-galacturonate.

24. A method for converting a suitable monosaccharide or oligosaccharide to a first commodity chemical comprising,

(a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises a recombinant microorganism, wherein the microorganism comprises a commodity chemical biosynthesis pathway,

thereby converting the suitable monosaccharide or oligosaccharide to the first commodity chemical.

25. The method of claim 24, wherein the commodity chemical pathway comprises one or more genes encoding an aldehyde or ketone biosynthesis pathway.

26. The method of claim 25, wherein the aldehyde or ketone biosynthesis pathway is selected from one or more of an acetaldehyde, a propionaldehyde, a butyraldehyde, an isobutyraldehyde, a 2-methyl-butyraldehyde, a 3-methyl-butyraldehyde, a 2-phenyl acetaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, a 2-Indole-3-acetaldehyde, a glutaraldehyde, a 5-amino-pentaldehyde, a succinate semialdehyde, and a succinate 4-hydroxyphenyl acetaldehyde biosynthesis pathway.

27. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises an acetaldehyde biosynthesis pathway and a biosynthesis pathway selected from a propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetaldehyde biosynthesis pathway.

28. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises a propionaldehyde biosynthesis pathway and a biosynthesis

pathway selected from a butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, and phenylacetoaldehyde biosynthesis pathway.

29. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises a butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from an isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

30. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises an isobutyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

31. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises a 2-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

32. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises a 3-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

33. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises a 2-phenyl acetoaldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-(4-hydroxyphenyl) acetaldehyde and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

34. The method of claim 26, wherein the aldehyde or ketone biosynthesis pathway comprises a 2-(4-hydroxyphenyl) acetaldehyde biosynthesis pathway and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

35. The method of claim 24, wherein the first commodity chemical is further enzymatically and/or chemically reduced and dehydrated to a second commodity chemical.

36. A method for converting a suitable monosaccharide or oligosaccharide to a commodity chemical comprising,

(a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises;

(i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and

(ii) one or more genes encoding and expressing an aldehyde reductase,

thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical.

37. The method of claim 36, wherein the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC).

38. The method of claim 37, wherein the IPDC comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 312

39. The method of claim 36, wherein the aldehyde reductase enzyme is a phenylacetaldehyde reductase (PAR).

40. The method of claim 39, wherein the PAR comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 313.

41. The method of claim 36, wherein the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol.

42. A recombinant microorganism, comprising (i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and (ii) one or more genes encoding and expressing an aldehyde reductase.

43. The recombinant microorganism of claim 42, wherein the aldehyde biosynthesis pathway further comprises one or more genes encoding and expressing an enzyme selected from a CoA-linked aldehyde dehydrogenase, an aldehyde dehydrogenase, and an alcohol dehydrogenase.

44. The recombinant microorganism of claim 42, wherein the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC).

45. The recombinant microorganism of claim 42, wherein the aldehyde reductase enzyme is a phenylacetaldehyde reductase (PAR).

46. The recombinant microorganism of claim 42, wherein the microorganism is capable of converting a suitable monosaccharide or oligosaccharide to a commodity chemical.

47. The recombinant microorganism of claim 46, wherein the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol.

48. The recombinant microorganism of claims 17-23 or 42-47, wherein the microorganism comprises reduced ethanol production capability compared to a wild-type microorganism.

49. The recombinant microorganism of claim 48, wherein the microorganism comprises a reduction or inhibition in the conversion of acetyl-coA to ethanol.

50. The recombinant microorganism of claim 48, wherein the recombinant microorganism comprises a reduction of an ethanol dehydrogenase, thereby providing a reduced ethanol production capability.

51. The recombinant microorganism of claim 50, wherein the ethanol dehydrogenase is an adhE, homolog or variant thereof.

52. The recombinant microorganism of claim 50, wherein the microorganism comprises a deletion or knockout of an adhE, homolog or variant thereof.

53. The recombinant microorganism of claims 17-23 or 42-47, wherein the recombinant microorganism comprises one or more deletions or knockouts in a gene encoding an enzyme selected from an enzyme that catalyzes the conversion of acetyl-coA to ethanol, an enzyme that catalyzes the conversion of pyruvate to lactate, an enzyme that catalyzes the conversion of fumarate to succinate, an enzyme that catalyzes the conversion of acetyl-coA and phosphate to coA and acetyl phosphate, an enzyme

that catalyzes the conversion of acetyl-coA and formate to coA and pyruvate, and an enzyme that catalyzes the conversion of alpha-keto acid to branched chain amino acids.

54. The microbial system or recombinant microorganism of any one of claims 1-53, wherein the recombinant microorganism or microbial system comprises a microorganism selected from *Acetobacter aceti*, *Achromobacter*, *Acidiphilium*, *Acinetobacter*, *Actinomadura*, *Actinoplanes*, *Aeropyrum pernix*, *Agrobacterium*, *Alcaligenes*, *Ananas comosus* (M), *Arthrobacter*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus melleus*, *Aspergillus pulverulentus*, *Aspergillus saitoi*, *Aspergillus sojae*, *Aspergillus usamii*, *Bacillus alcalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bifidobacterium*, *Brevibacillus brevis*, *Burkholderia cepacia*, *Candida cylindracea*, *Candida rugosa*, *Carica papaya* (L), *Cellulosimicrobium*, *Cephalosporium*, *Chaetomium erraticum*, *Chaetomium gracile*, *Clostridium*, *Clostridium butyricum*, *Clostridium acetobutylicum*, *Clostridium thermocellum*, *Corynebacterium (glutamicum)*, *Corynebacterium efficiens*, *Escherichia coli*, *Enterococcus*, *Erwinia chrysanthemi*, *Gliconobacter*, *Gluconacetobacter*, *Haloarcula*, *Humicola insolens*, *Humicola insolens*, *Kitasatospora setae*, *Klebsiella*, *Klebsiella oxytoca*, *Kluyveromyces*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Kocuria*, *Lactolactis*, *Lactobacillus*, *Lactobacillus fermentum*, *Lactobacillus sake*, *Lactococcus*, *Lactococcus lactis*, *Leuconostoc*, *Methylocystis*, *Methanolobus siciliae*, *Methanogenium organophilum*, *Methanobacterium bryantii*, *Microbacterium imperiale*, *Micrococcus lysodeikticus*, *Microbacterium*, *Mucor javanicus*, *Mycobacterium*, *Myrothecium*, *Nitrobacter*, *Nitrosomonas*, *Nocardia*, *Papaya carica*, *Pediococcus*, *Pediococcus halophilus*, *Penicillium*, *Penicillium camemberti*, *Penicillium citrinum*, *Penicillium emersonii*, *Penicillium roqueforti*, *Penicillium lilactinum*, *Penicillium multicolor*, *Paracoccus pantotrophus*, *Propionibacterium*, *Pseudomonas*, *Pseudomonas fluorescens*, *Pseudomonas denitrificans*, *Pyrococcus*, *Pyrococcus furiosus*, *Pyrococcus horikoshii*, *Rhizobium*, *Rhizomucor miehei*, *Rhizomucor pusillus* Lindt, *Rhizopus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Rhizopus*

niveus, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Rhodococcus*, *Sccharomyces cerevisiae*, *Sclerotinia libertina*, *Sphingobacterium multivorum*, *Sphingobium*, *Sphingomonas*, *Streptococcus*, *Streptococcus thermophilus* Y-1, *Streptomyces*, *Streptomyces griseus*, *Streptomyces lividans*, *Streptomyces murinus*, *Streptomyces rubiginosus*, *Streptomyces violaceoruber*, *Streptoverticillium mobaraense*, *Tetragenococcus*, *Thermus*, *Thiosphaera pantotropha*, *Trametes*, *Trichoderma*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma viride*, *Trichosporon penicillatum*, *Vibrio alginolyticus*, *Xanthomonas*, yeast, *Zygosaccharomyces rouxii*, *Zymomonas*, and *Zymomonus mobilis*.

55. A commodity chemical produced by the method of any one of claims 1-8 or 24-41.

56. A blended commodity chemical comprising the commodity chemical of claim 55 and a refinery-produced petroleum product.

57. The blended commodity chemical of claim 56, wherein the commodity chemical is selected from a C10-C12 hydrocarbon, 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and indole-3-ethanol.

58. The blended commodity chemical of claim 57, wherein the C10-C12 hydrocarbon is selected from 2,7-dimethyloctane and 2,9-dimethyldecane.

59. The blended commodity chemical of claim 56, wherein the refinery-produced petroleum product is selected from jet fuel and diesel fuel.

60. A method of producing a commodity chemical enriched refinery-produced petroleum product, comprising

(a) blending the refinery-produced petroleum product with the commodity chemical produced by the method of any one of claims 1-8 or 24-41,

thereby producing the commodity chemical enriched refinery-produced petroleum product.

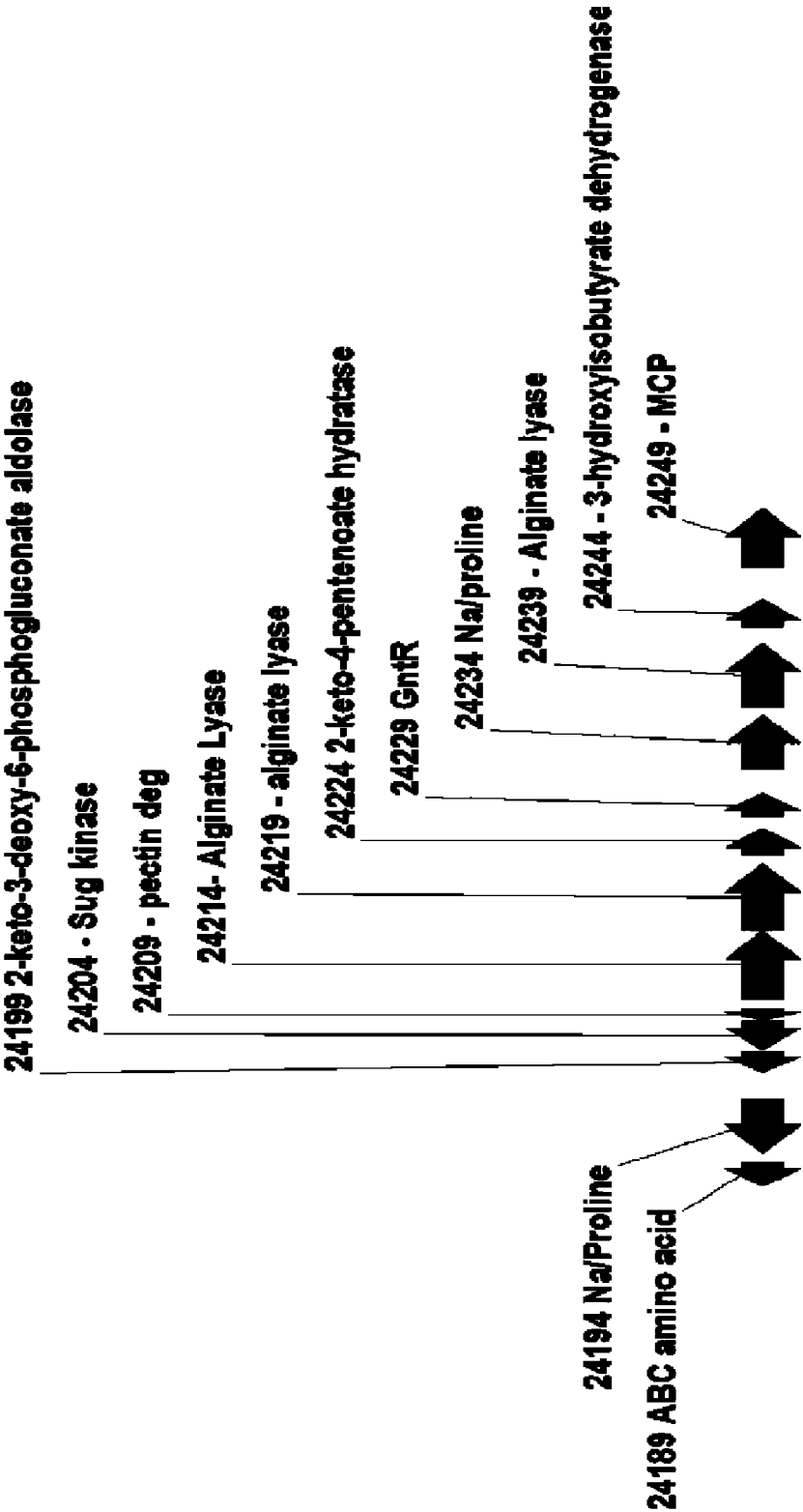


FIG. 1

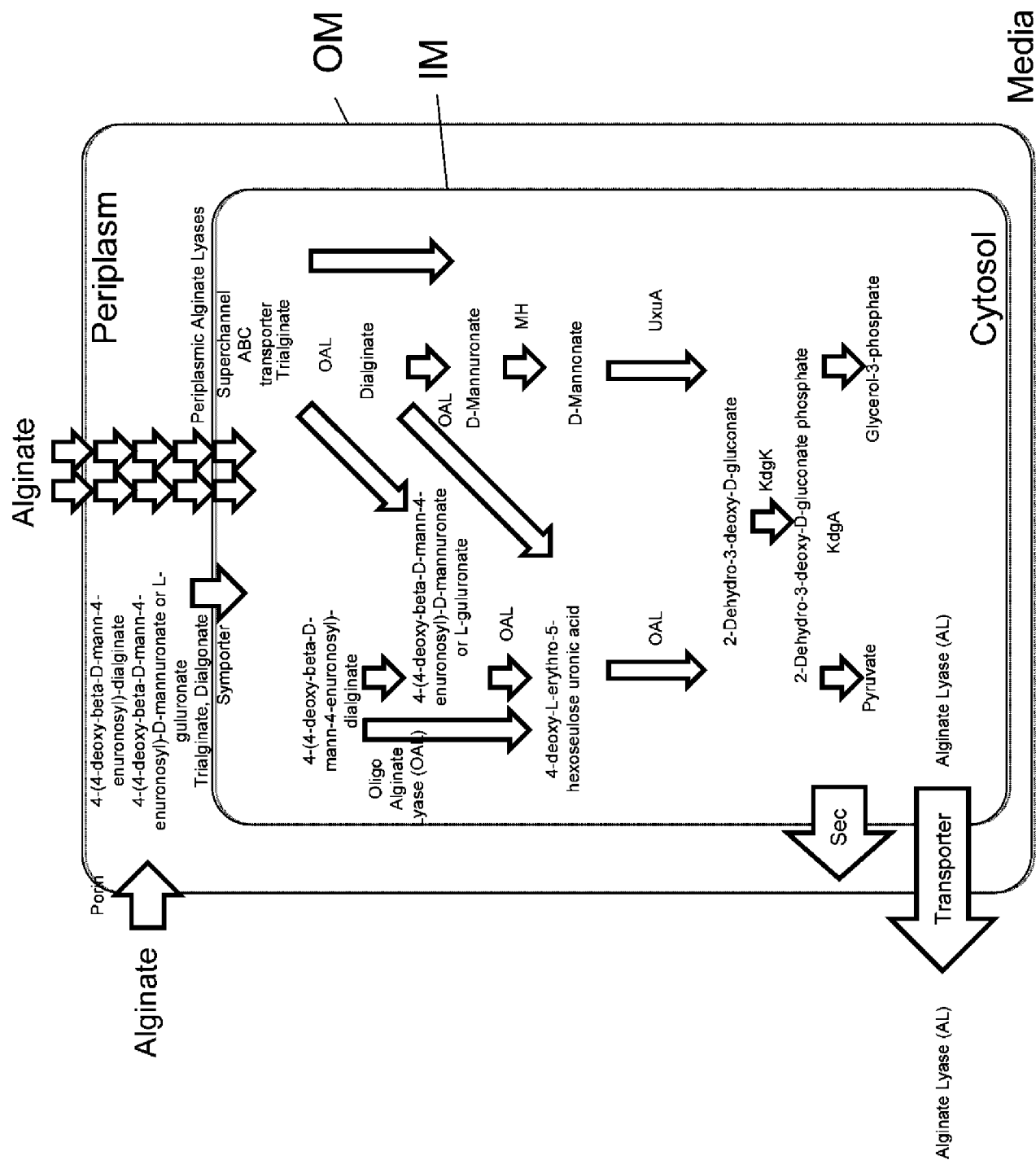


FIG. 2

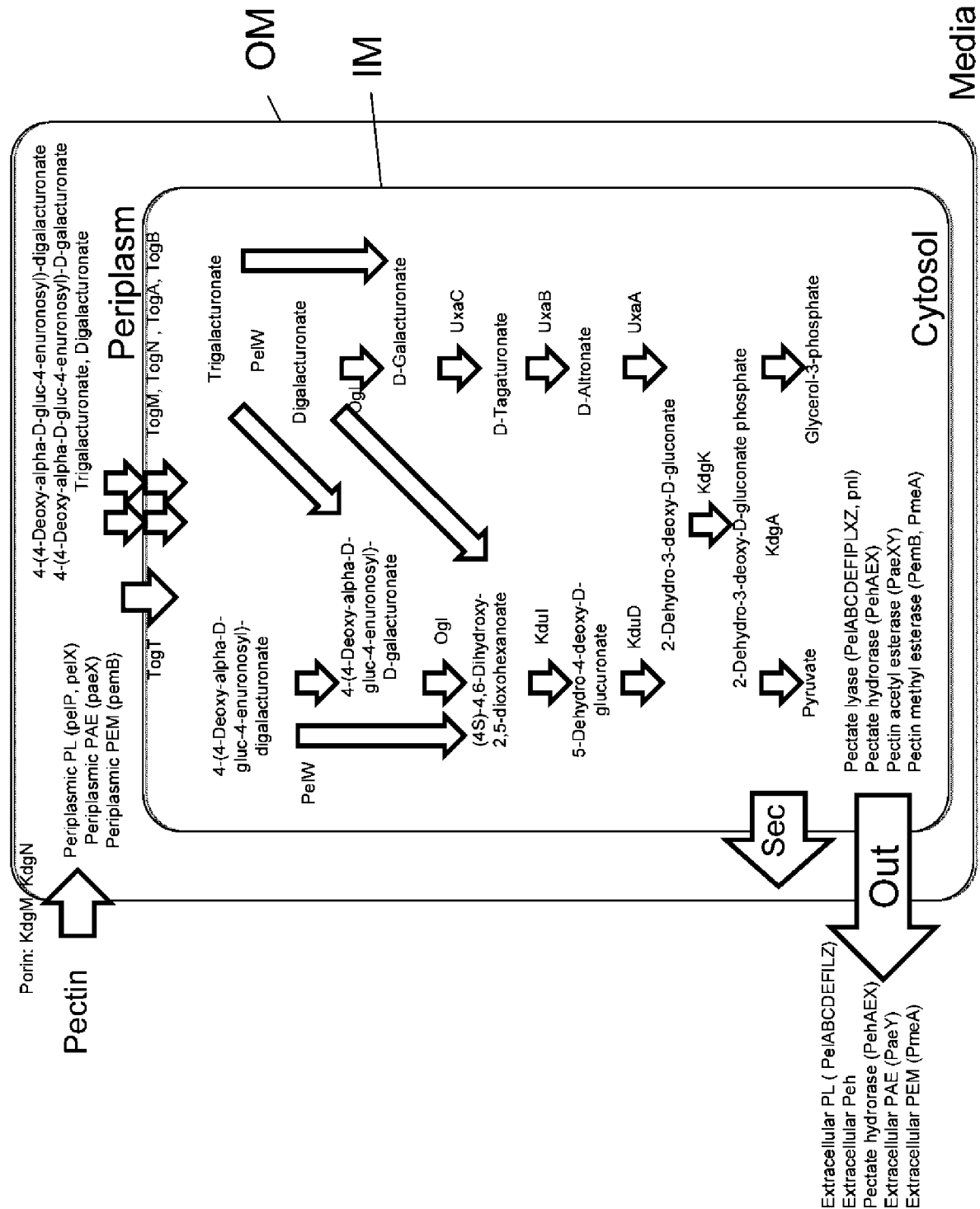
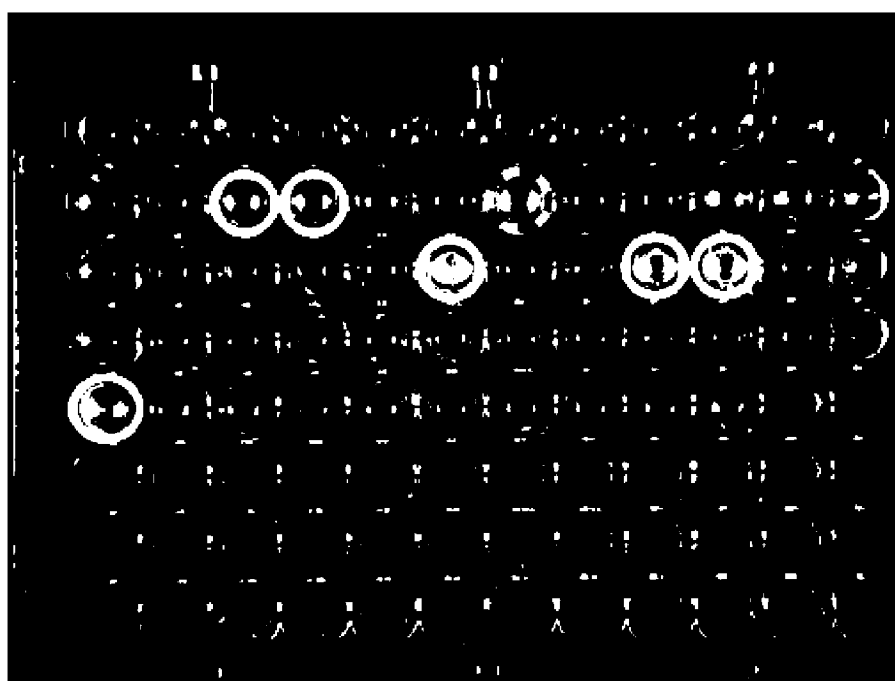


FIG. 3

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E. coli Growing on Alginate*FIG. 4*

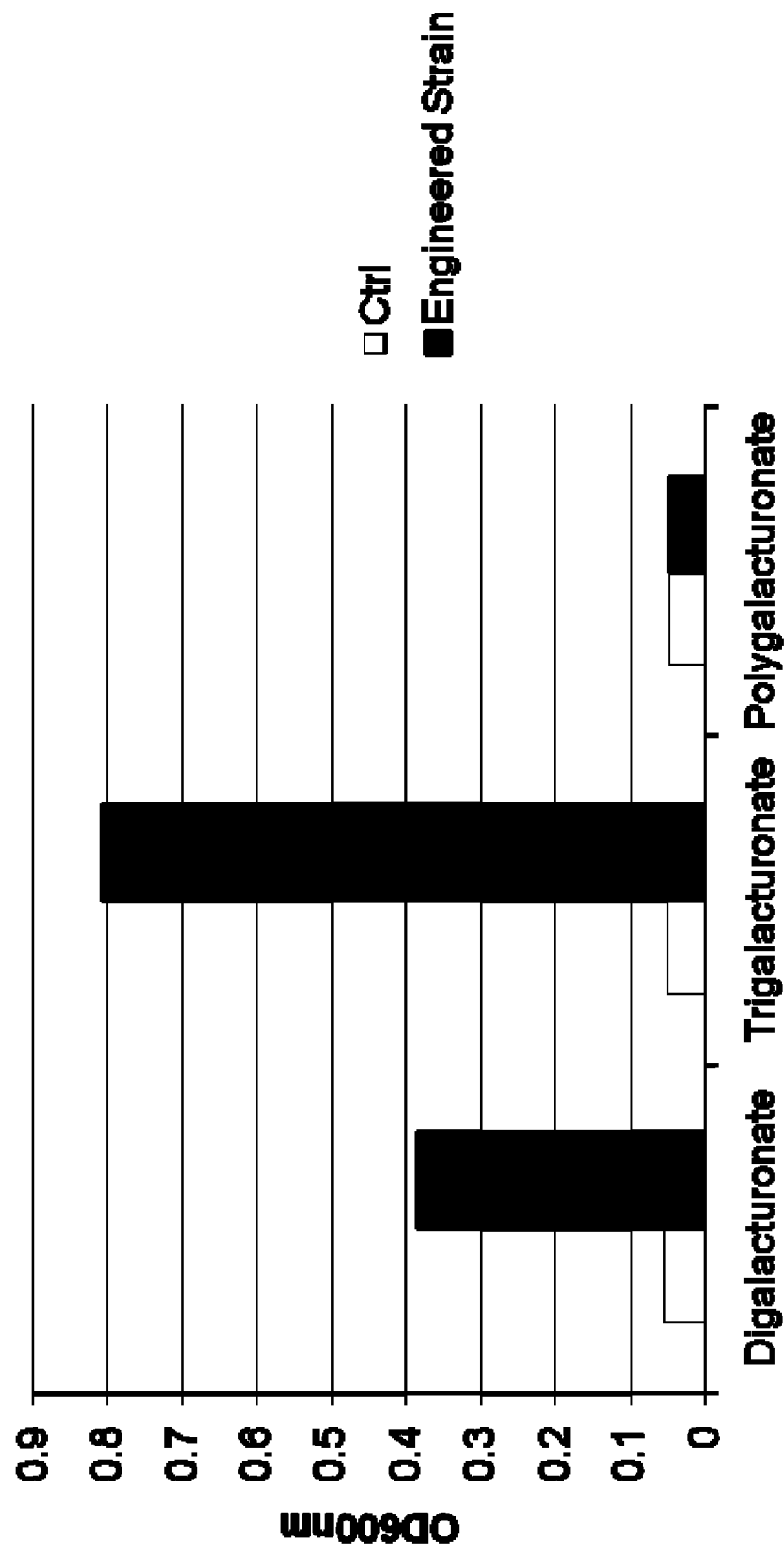


FIG. 5A



FIG. 5B

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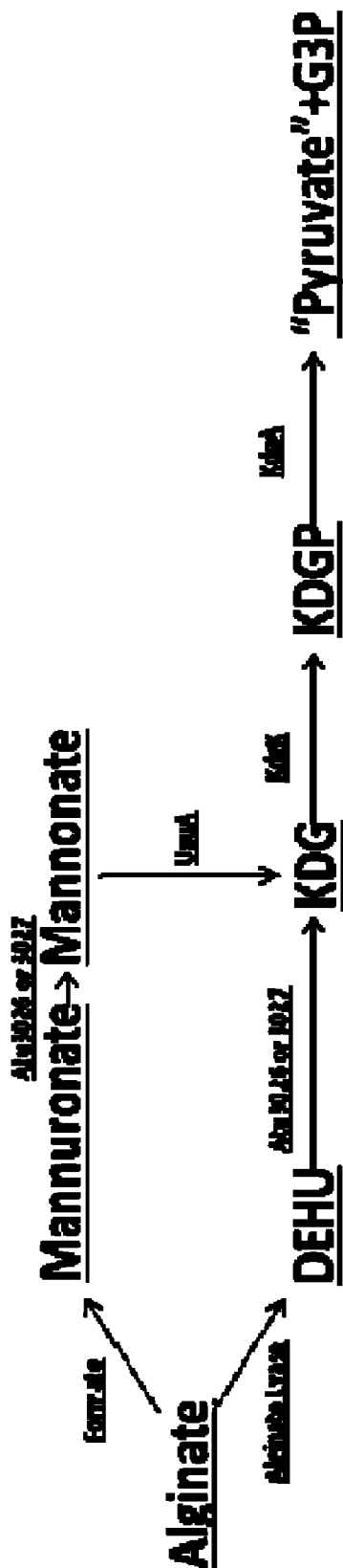


FIG. 6A

Pyruvate formation from alginate (enzymatic degradation route)

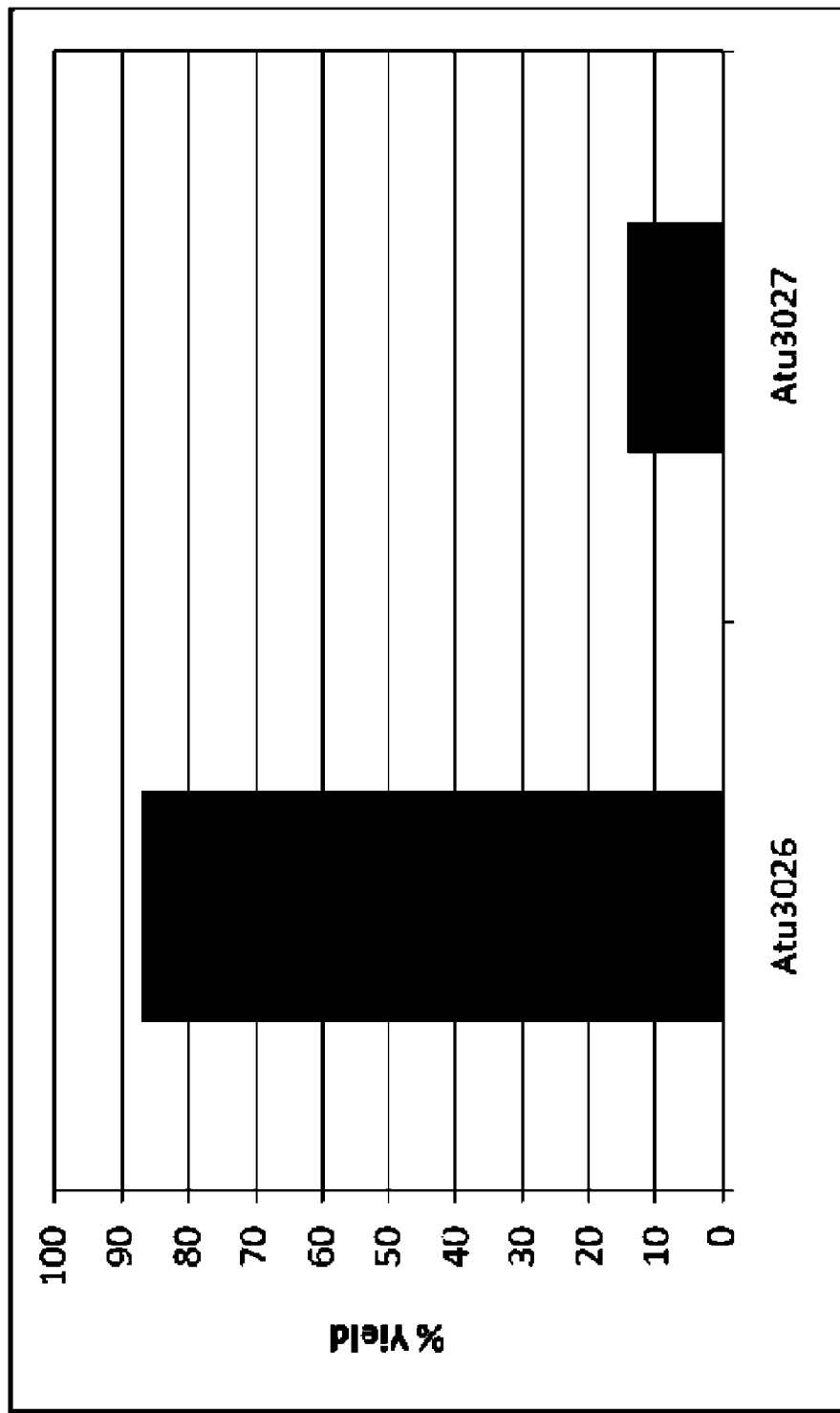


FIG. 6B

Pyruvate formation from alginate (chemical degradation route).

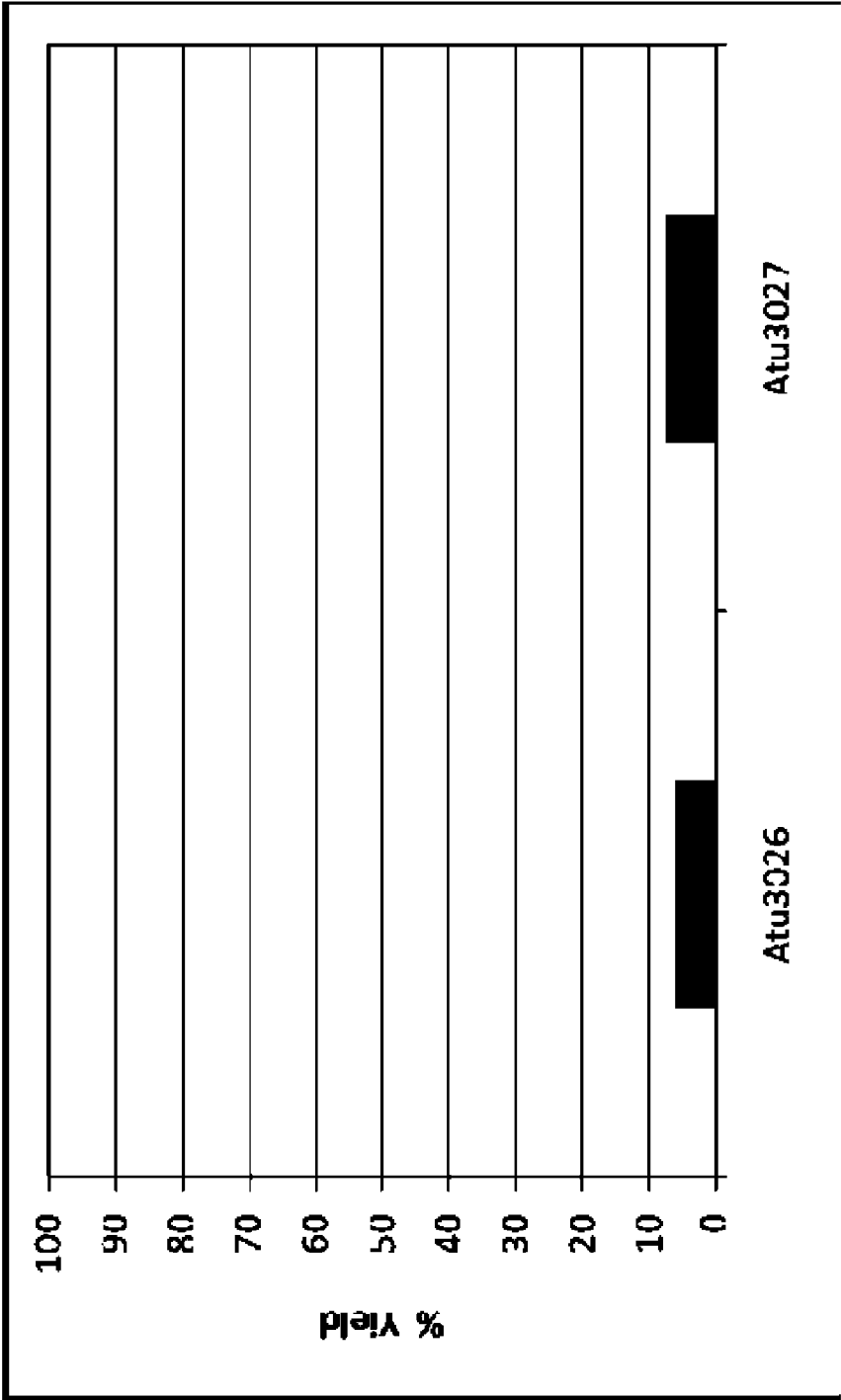


FIG. 6C

DEHU hydrogenase activity monitored by NADPH consumption

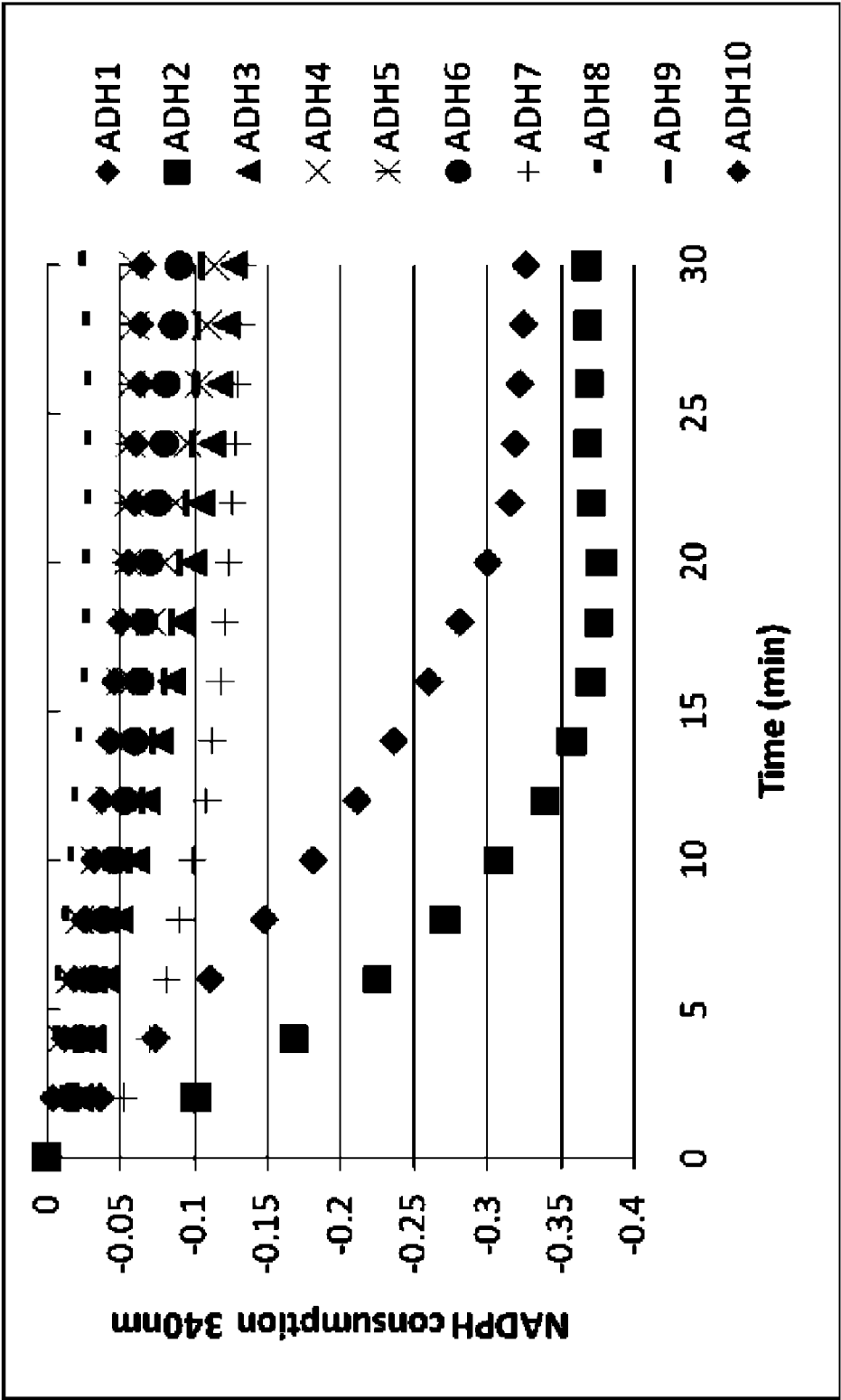


FIG. 7A

Mannuronate hydrogenase activity monitored by NADPH consumption

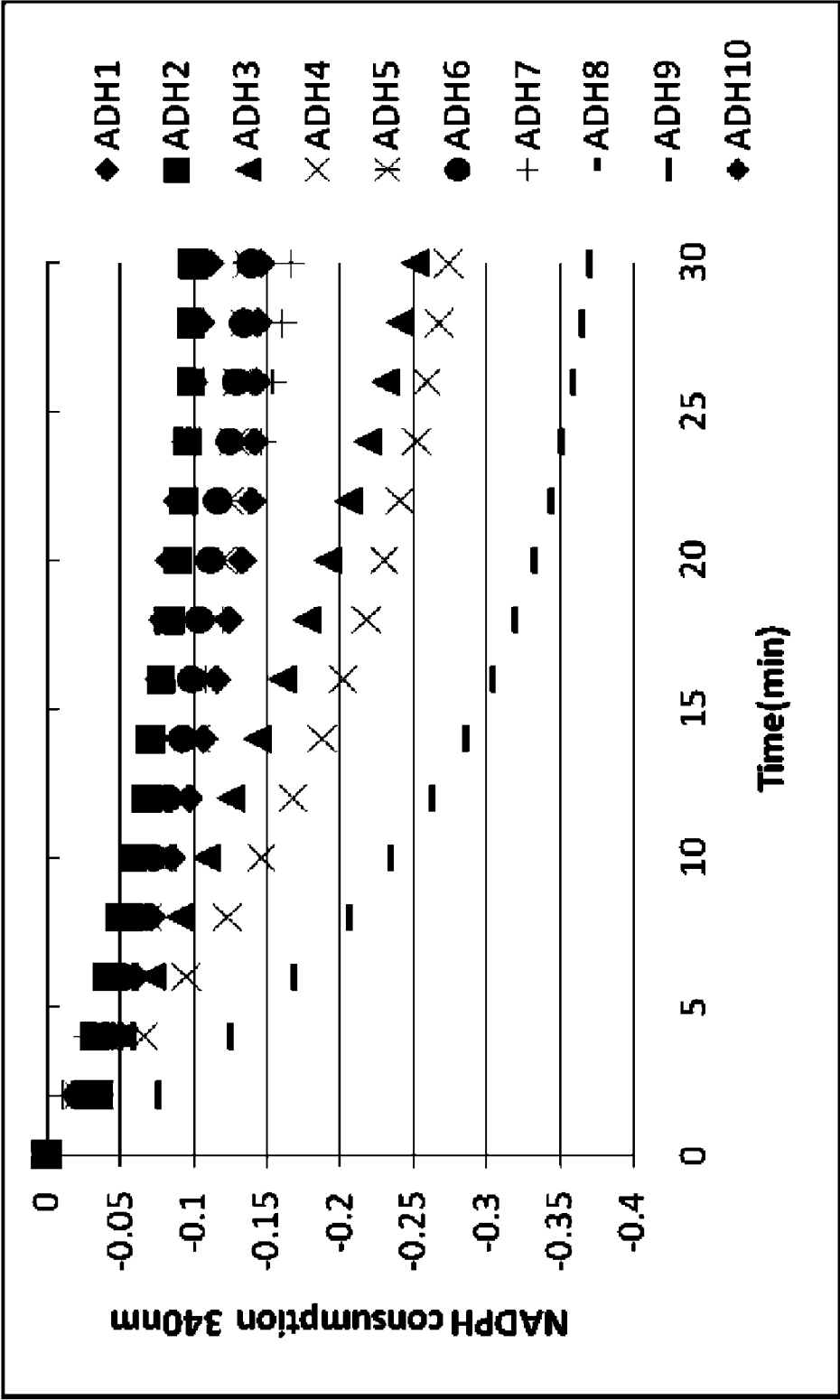


FIG. 7B

GC-MS chromatogram of control

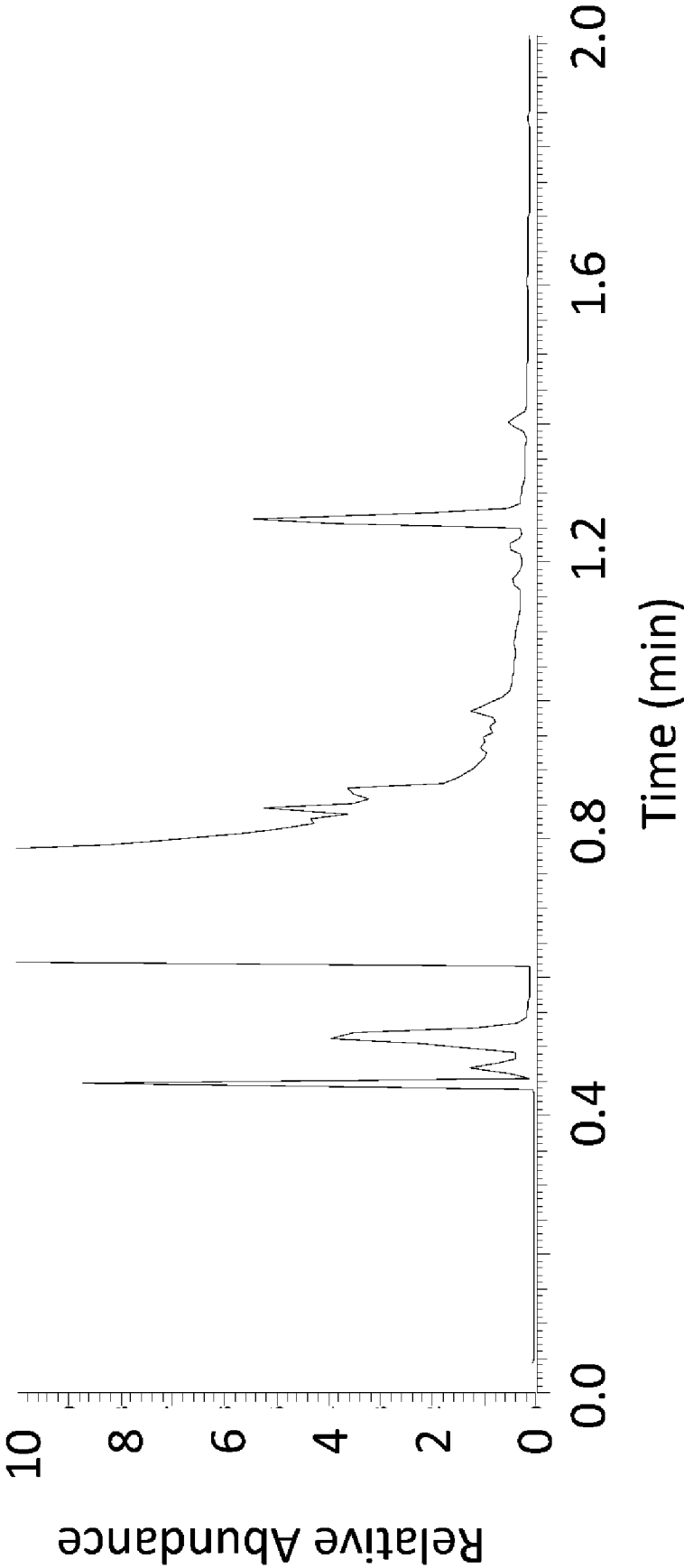


FIG. 8A

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GC-MS chromatogram for isobutyraldehyde, 3-methylpentanol, and 2-methylpentanol production from pBADalsS-ilvCD-leuABCD2

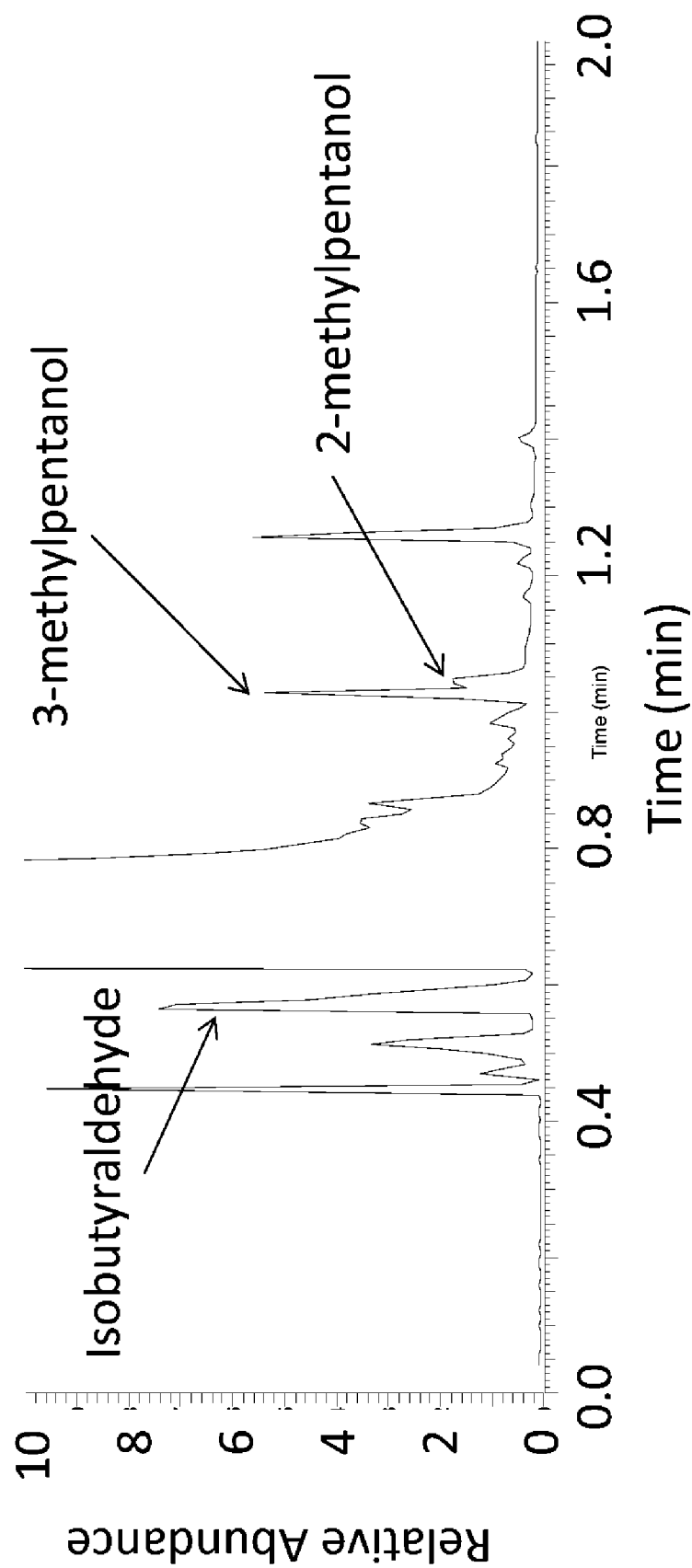


FIG. 8B

GC-MS chromatogram of control

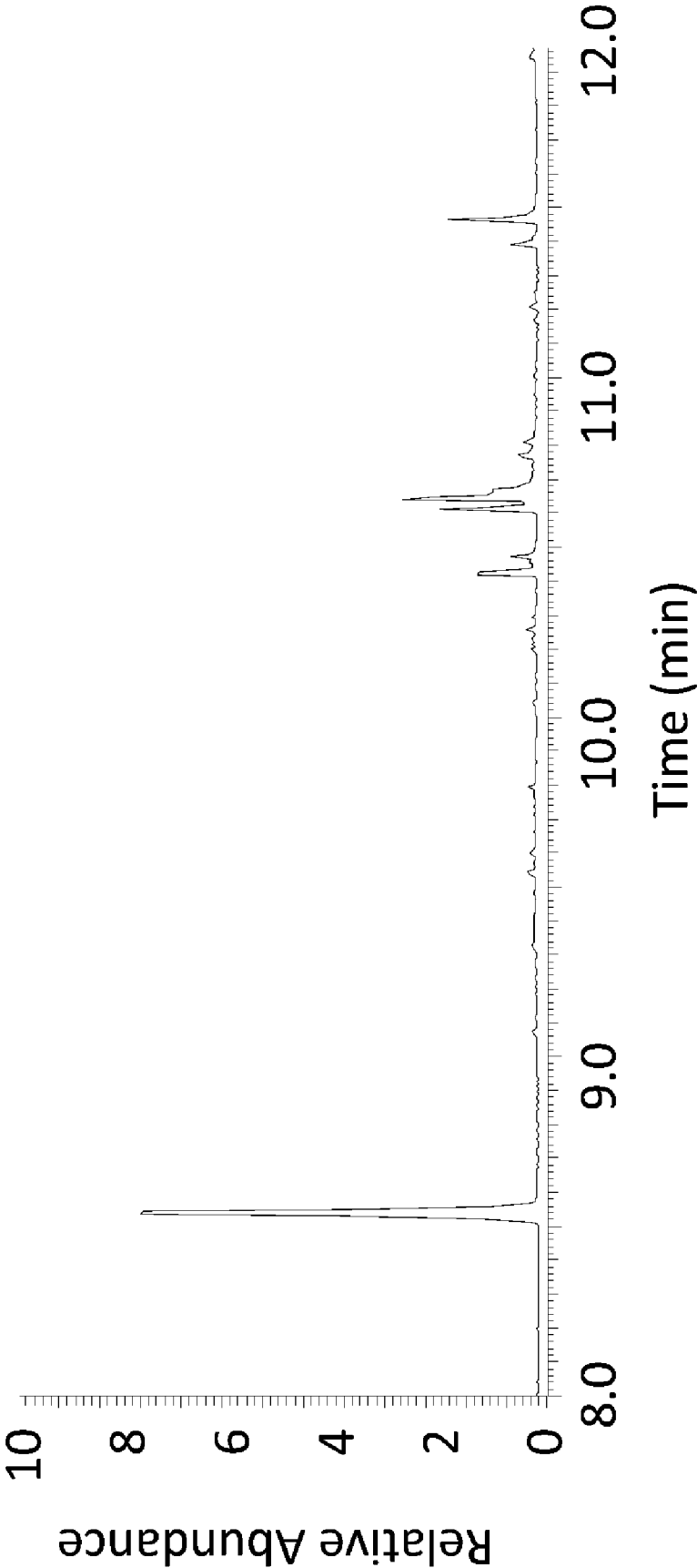


FIG. 9A

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GC-MS chromatogram for 4-hydroxy-2-phenylethanol and indole-3-ethanol
production from pBADtyrA-aroLAC-aroG-*tk*tA-aroBDE and pTrcBALK

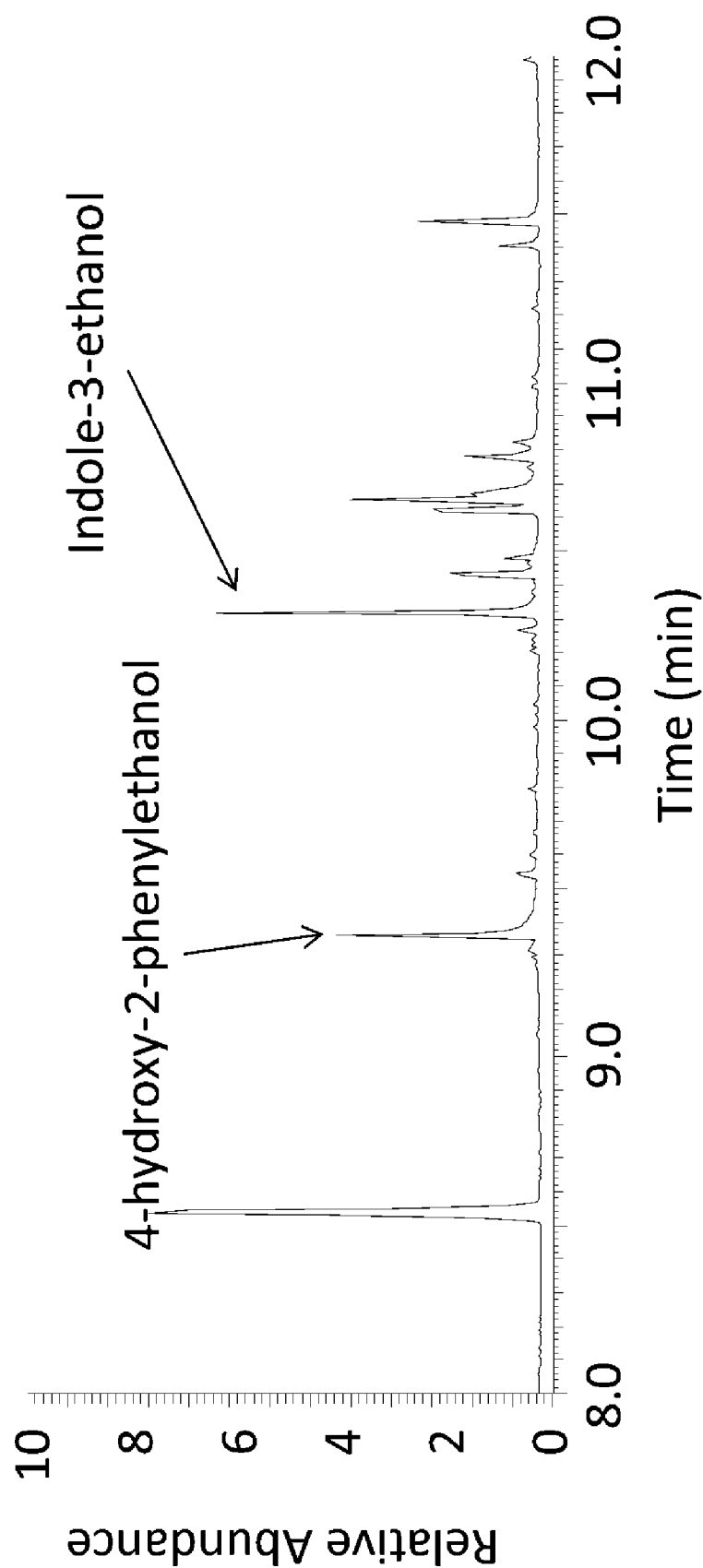


FIG. 9B

Mass spectrometry of isobutanol produced in vivo

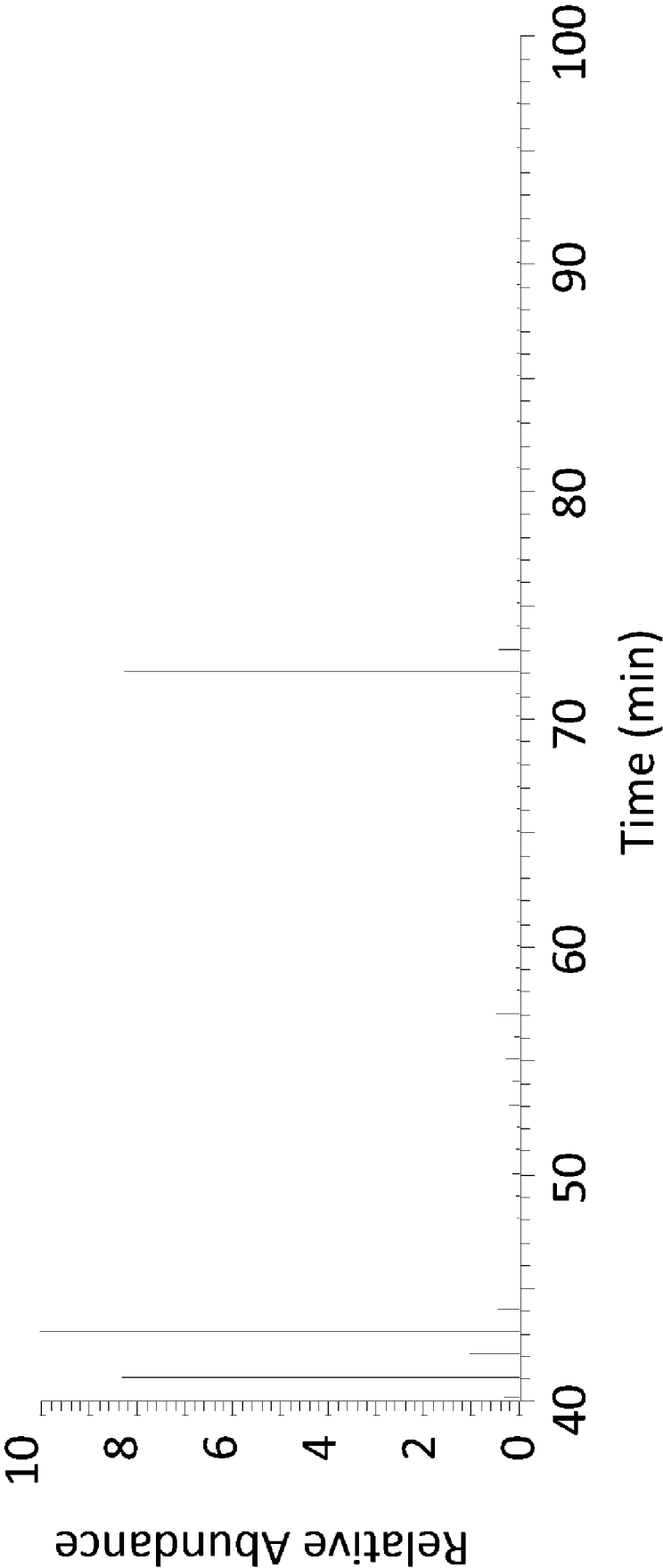


FIG. 10A

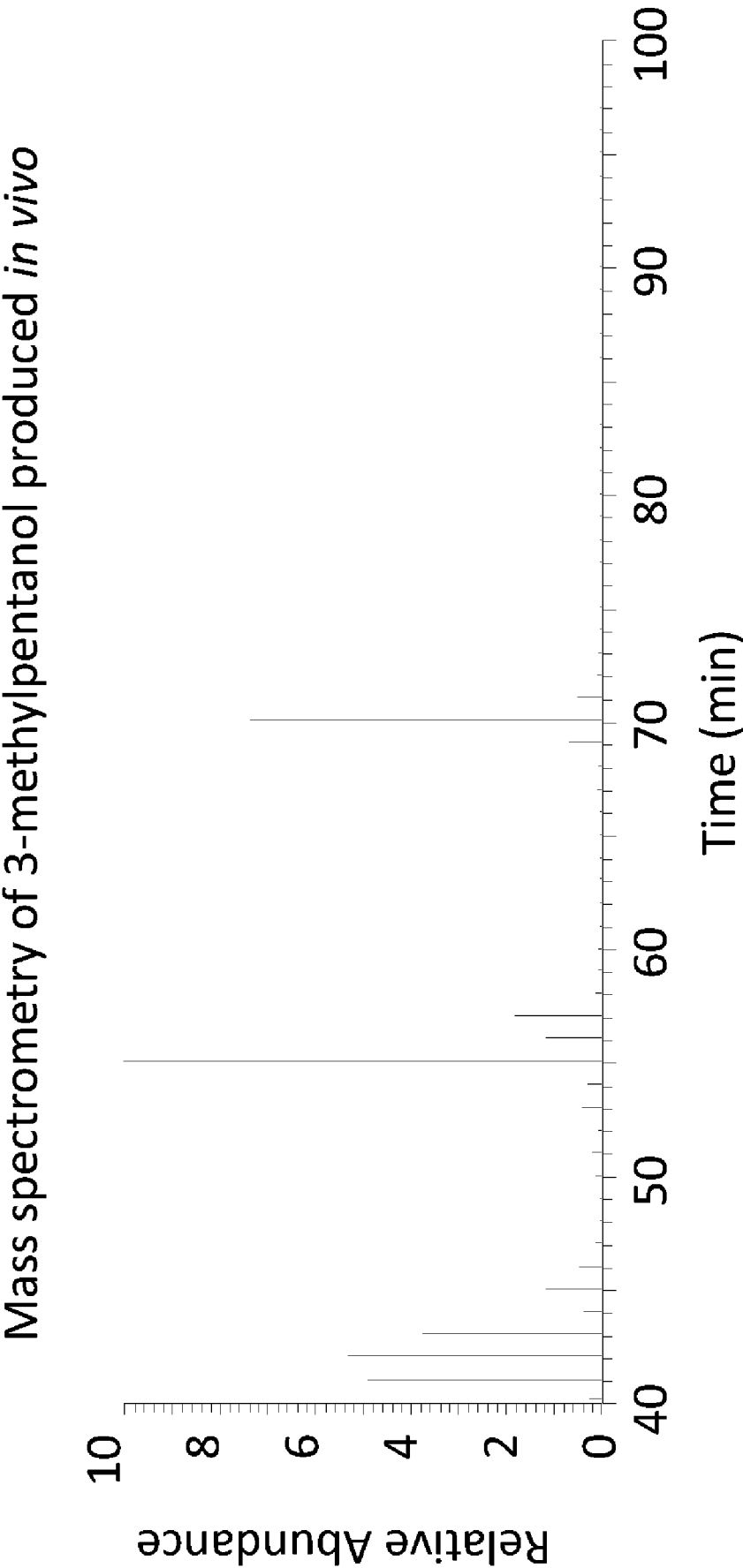


FIG. 10B

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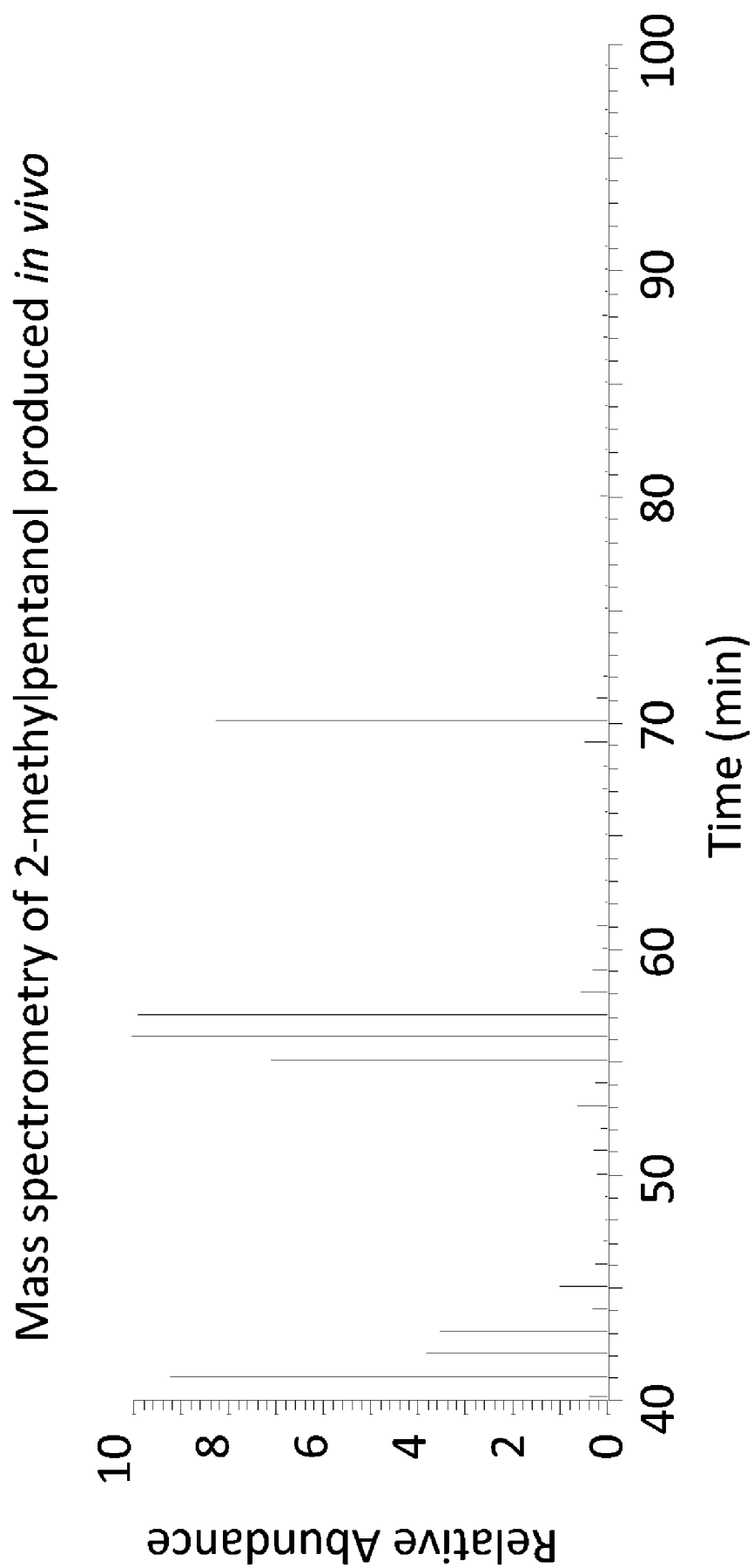


FIG. 10C

Mass spectrometry of 2-phenylethanol produced *in vivo*

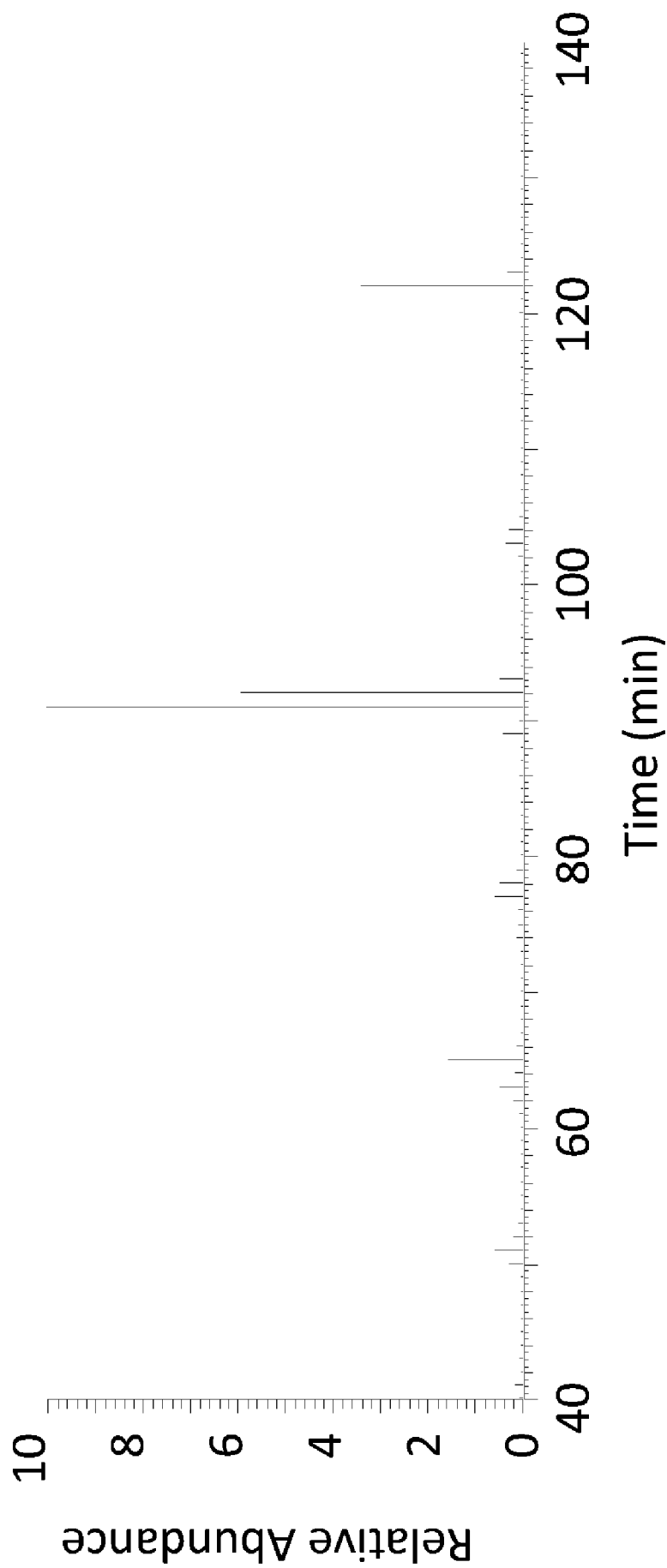


FIG. 11A

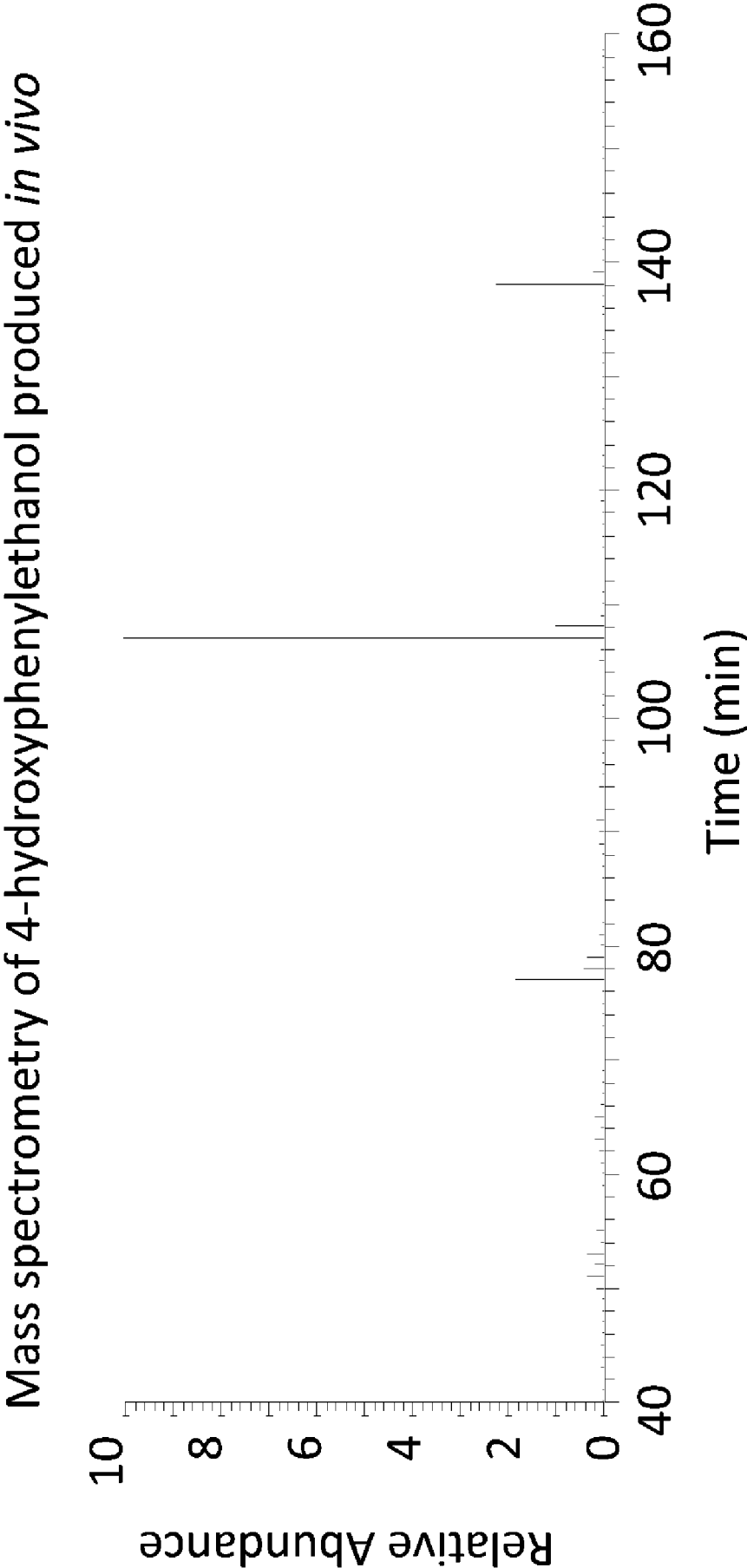


FIG. 11B

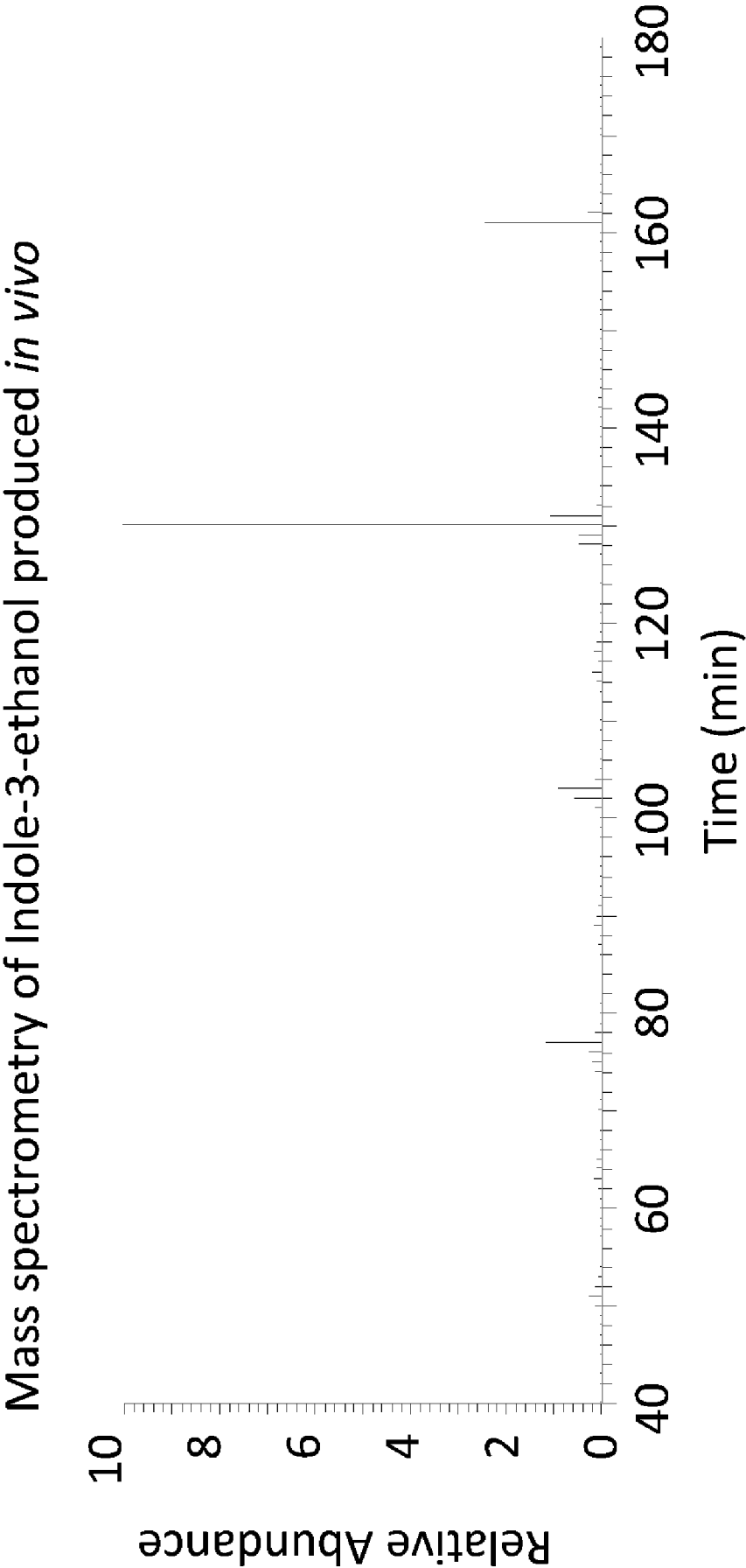


FIG. 11C

Reduction of butyrolin by *ddh1*, *ddh2*, and *ddh3* monitored by NADH consumption.

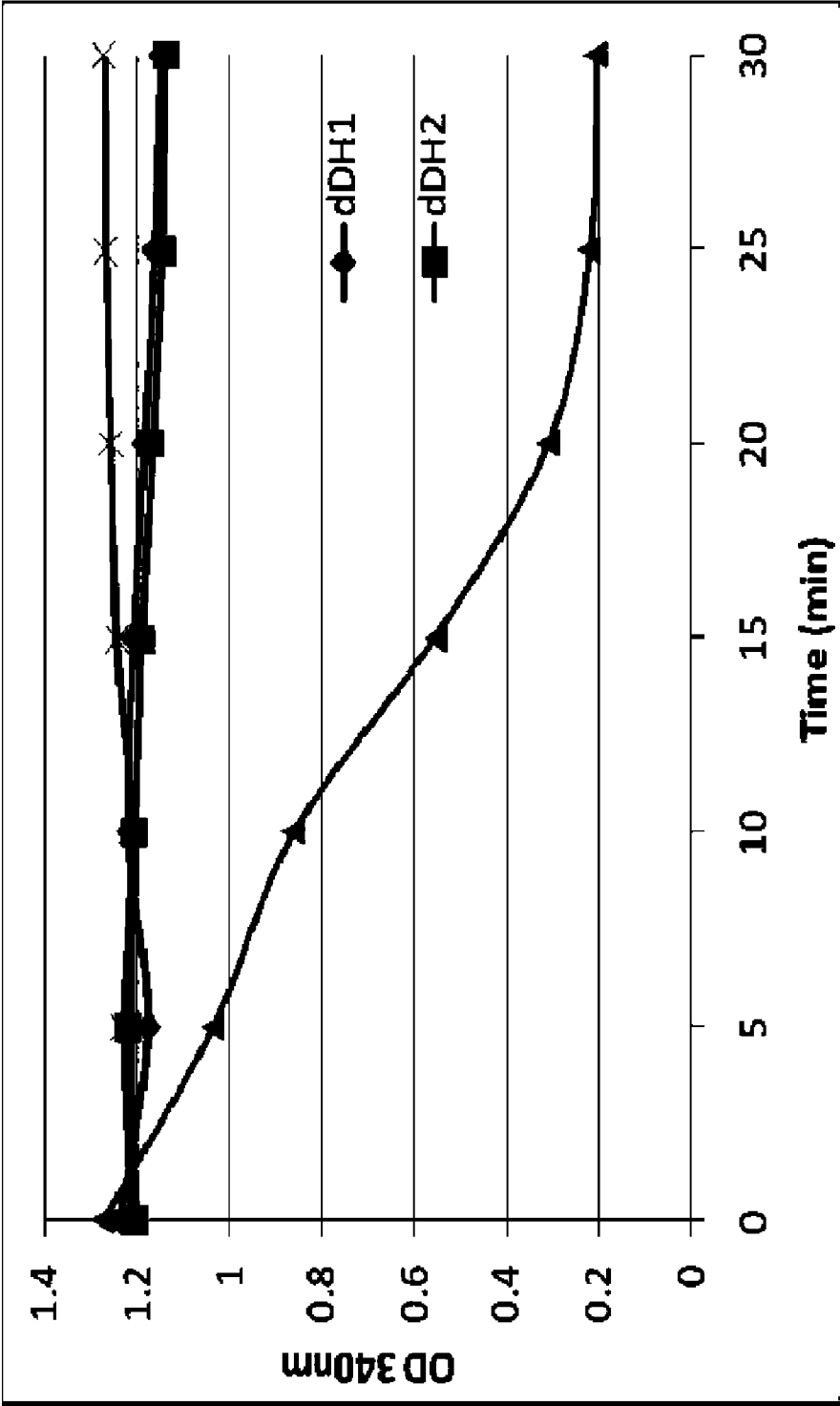


FIG. 12A

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Oxidation activity of *ddh3* towards 1,2-cyclopentanediol and 1,2-cyclohexanediol as measured by NADH production.

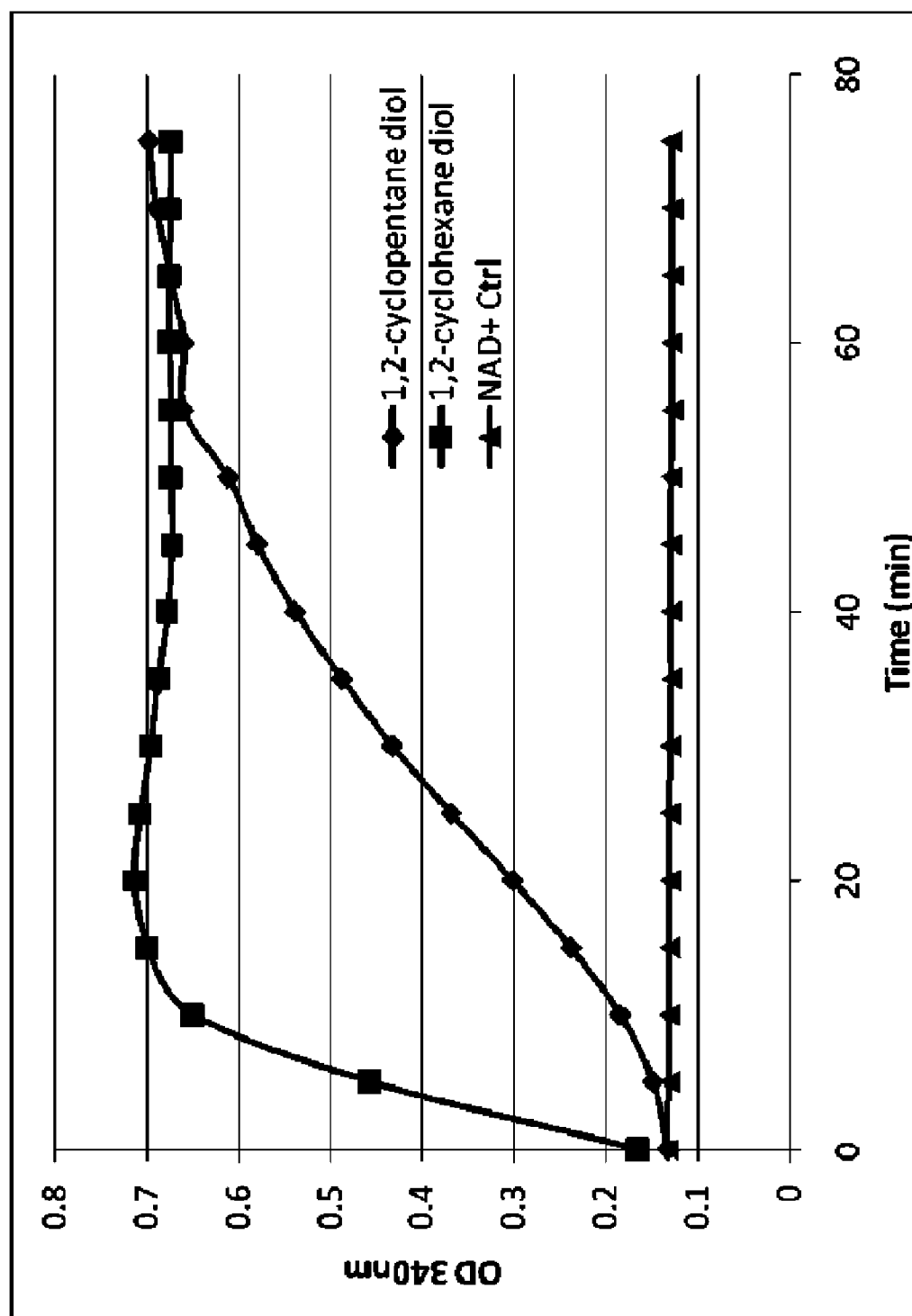


FIG. 12B

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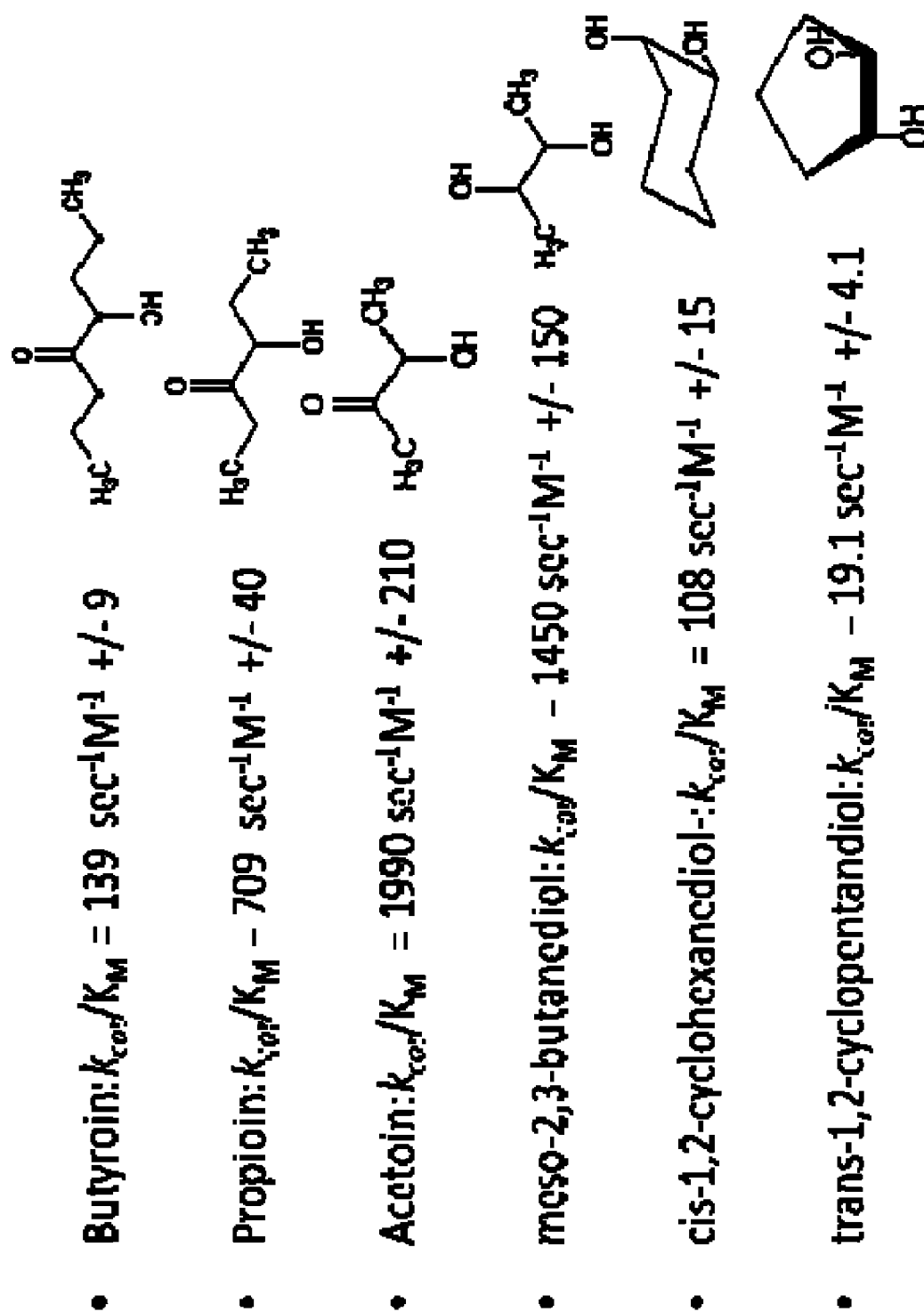


FIG. 13

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Nucleotide sequence of diol dehydrogenase DDH1 isolated from *Lactobacillus brevis* ATCC 367

ATGGCATCAAATGGAAAAGTAGCAATGGTTACCGGTGGCGGACAAGGAATTGGTGAAGC
CATCTCGAAACGGTTAGCTAACGACGGCTTTGCTGTGGCAATTGCTGATTTGAACTTGG
ACAATGCCAACAAGGTCGTTTCTGATATTGAAGCTGCTGGTGGCAAGGCCATTGCGGTC
AAGACCGATGTCTCTGATCGTGATAGCGTGTTTGGCTGCGGTTAATGAAGCGGCCGACAA
GCTGGGCGGGCTTTGACGTTATCGTTAATAACGCCGGCCTTGGCCCAACCACGCCAATTG
ACACCATCACCCAAGAACAGTTTGATACGGTTTATCACGTTAACGTGGGTGGGGTTCTT
TGGGGCATTCAAGCAGCCCATGCGAAGTTCAAGGAATTGGGTCATGGTGGGAAGATCAT
TTCCGCGACGTCTCAAGCCGGGGTTGTTGGTAACCCGAACCTTAGCTCTGTACAGTGGAA
CTAAGTTTGCCATTCGTGGTGTGACCCAAGTTGCGGCGCGTGACTTAGCCGCTGAAGGT
ATCACGGTCAATGCTTATGCACCCGGGATTGTTAAGACACCAATGATGTTTGACATCGC
TCACAAGGTTGGTCAAAATGCTGGTAAAGACGACGAATGGGGGATGCAAACCTTCTCAA
AGGACATCGCTTTATGTCGATTGTCAGAACCAGAAGATGTGGCTAACGGGGTGGCTTTC
TTAGCCGGTCCCGATTCTAACTACATTACGGGTCAAACACTTGAAGTTGATGGTGGGAT
GCAGTTCCACTAA (SEQ ID NO:97)

FIG. 14A

**Polypeptide primary sequence of diol dehydrogenase DDH1 isolated from
Lactobacillus brevis ATCC 367**

MASNGKVAMVTGGGQGIGEAISKRLANDGFAVAIADLNLDNANKVVSDIEAAGGKAIAV
KTDVSDRDSVFAAVNEAADKLGGFDVIVNNAAGLGPTTPIDTITQEQFDTVYHVNVGVL
WGIQAAHAKFKELGHGGKII SATSQAGVVGNNP LALYSGTKFAIRGVTQVAARDLAAEG
ITVNAYAPGIVKTPMMFDIAHKVGQNA GKDD EWGMQTF SKDIALCRLSEPEDVANGVAF
LAGPDSNYITGQTLEVDGGMQFH (SEQ ID NO:98)

FIG. 14B

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Nucleotide sequence of diol dehydrogenase DDH2 isolated from *Pseudomonas putida* KT2440

ATGAATGACCTGAGCCACACCCACATGCGCGCGGCCGTCTGGCATGGCCGCCACGATAT
TCGTGTGGAACAGGTACCTTTGCCGGCCGACCCTGCGCCGGGCTGGGTGCAGATCAAGG
TGGACTGGTGCGGCATCTGCGGCTCCGACCTGCACGAATATGTTGCCGGGCCCGGTGTTT
ATCCCGGTAGAGGCCCGCCACCCGCTGACCGGCATTCAGGGCCAGTGCATCCTCGGCCA
CGAATTCTGCGGCCACATCGCCAAGCTTGGCGAAGGCGTGGAAGGCTATGCCGTAGGCG
ACCCGGTGGCGGCAGACGCGTGCCAGCATTGTGGTACCTGCTATTACTGCACCCATGGC
CTGTACAACATCTGCGAACGCCTGGCGTTACCGGCCCTGATGAACAACGGTGCCTTCGC
CGAGCTGGTCAACGTGCCCCGCCAACCTGCTCTACCGGCTGCCGCAGGGCTTCCCTGCCG
AAGCCGGGGCACTGATCGAGCCGCTGGCGGTGGGTATGCACGCGGTGAAAAAGCCGGC
AGCCTGCTTGGGCAAACCGTTGTAGTGGTTGGGGCCGGCACCATCGGCCTGTGCACCAT
CATGTGCGCCAAGGCTGCAGGTGCGGCACAGGTCATCGCCCTTGAGATGTCCTCTGCGC
GCAAAGCCAAGGCCAAGGAAGCGGGCGCCAACGTGGTGCTGGACCCAGCCAGTGCAT
GCCCTGGCGGAAATCCGCGCACTGACTGCTGGGCTGGGCGCCGATGTGAGTTTTGAGTG
CATCGGCAACAAACATACGGCCAAGCTGGCCATCGACACCATCCGCAAAGCAGGCAAGT
GCGTGCTGGTGGGTATTTTCGAAGAGCCCAGCGAGTTCAACTTCTTCGAGCTGGTGTCC
ACCGAGAAGCAAGTGCTGGGGGCGTTGGCGTACAACGGCGAGTTTGCTGACGTGATTGC
CTTCATTGCTGATGGTCGGCTGGATATTGCCCCGCTGGTAACCGGCCGGATCGGATTGG
AGCAGATTGTCGAGCTGGGCTTCGAGGAACTGGTGAACAACAAAGAGGAGAACGTGAAG
ATCATCGTTTCACCAGGTGTGCGCTGA (SEQ ID NO:99)

FIG. 15A

Polypeptide sequence of diol dehydrogenase DDH2 isolated from *Pseudomonas putida* KT2440

MNDLSHTMRAAVWHGRHDIRVEQVPLPADPAPGWVQIKVDWCGICGSDLHEYVAGPVF
IPVEAPHPLTGIQQQCILGHEFCGHIAKLGEGVEGYAVGDPVAADACQHCGTCTYYCTHG
LYNICERLAFTGLMNNGAFAELVNVPANLLYRLPQGFPAEAGALIEPLAVGMHAVKKAG
SLLGQTVVVVGAGTIGLCTIMCAKAAGAAQVIALEMSSARKAKAKEAGANVVLDPDQCD
ALAEIRALTAGLGADVSEFCIGNKHTAKLAIDTIRKAGKCVLVGIFEEPSEFNFELVS
TEKQVLGALAYNGEFADVIAFIADGRDLRPLVTGRIGLEQIVELGFEELVNNKEENVK
IIVSPGVR (SEQ ID NO:100)

FIG. 15B

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Nucleotide sequence of diol dehydrogenase DDH3 isolated from *Klebsiella pneumoniae* MGH78578

ATGAAAAAAGTCGCACTTGTTACCGGCGCCGGCCAGGGGATTGGTAAAGCTATCGCCCT
TCGTCTGGTGAAGGATGGATTTGCCGTGGCCATTGCCGATTATAACGACGCCACCGCCA
AAGCGGTCGCCTCGGAAATCAACCAGGCCGGCGGACACGCCGTGGCGGTGAAAGTGGAT
GTCTCCGACCGCGATCAGGTATTTGCCGCCGTTGAACAGGCGCGCAAAACGCTGGGCGG
CTTCGACGTCATCGTCAATAACGCCGGTGTGGCACCGTCTACGCCGATCGAGTCCATTA
CCCCGGAGATTGTGACAAAAGTCTACAACATCAACGTCAAAGGGGTGATCTGGGGTATT
CAGGCGGCGGTTCGAGGCCTTTAAGAAAGAGGGGCACGGCGGGAAAATCATCAACGCCTG
TTCCCAGGCCGGCCACGTTCGCAACCCGGAGCTGGCGGTGTATAGCTCCAGTAAATTCG
CGGTACGCGGCTTAACCCAGACCGCCGCTCGCGACCTCGCGCCGCTGGGCATCACGGTC
AACGGCTACTGCCCGGGGATTGTCAAACGCCAATGTGGGCCGAAATTGACCGCCAGGT
GTCCGAAGCCCGCGGTAAACCGCTGGGCTACGGTACCGCCGAGTTCGCCAAACGCATCA
CTCTCGGTCTGTCTGTCCGAGCCGGAAGATGTCGCCGCCTGCGTCTCCTATCTTGCCAGC
CCGATTCTGATTACATGACCGGTCAGTCGTTGCTGATCGACGGCGGGATGGTATTTAA
CTAA (SEQ ID NO:101)

FIG. 16A

Polypeptide sequence of diol dehydrogenase DDH3 isolated from *Klebsiella pneumoniae* MGH78578

MKKVALVTGAGQGIGKAIALRLVKDGFVAIAIDYNDATAKAVASEINQAGGHAVAVKVD
VSDRDQVFAAVEQARKTLGGFDVIVNNAGVAPSTPIESITPEIVDKVYNINVKGVIWGI
QAAVEAFKKEGHGGKIINACSQAGHVGNPVELAVYSSSKFAVRGLTQTAARDLAPLGITV
NGYCPGIVKTPMWAEIDRQVSEAAGKPLGYGTAEFAKRITLGRLSEPEDVAACVSYLAS
PDSYMTGQSLIDGGMVFN (SEQ ID NO:102)

FIG. 16B

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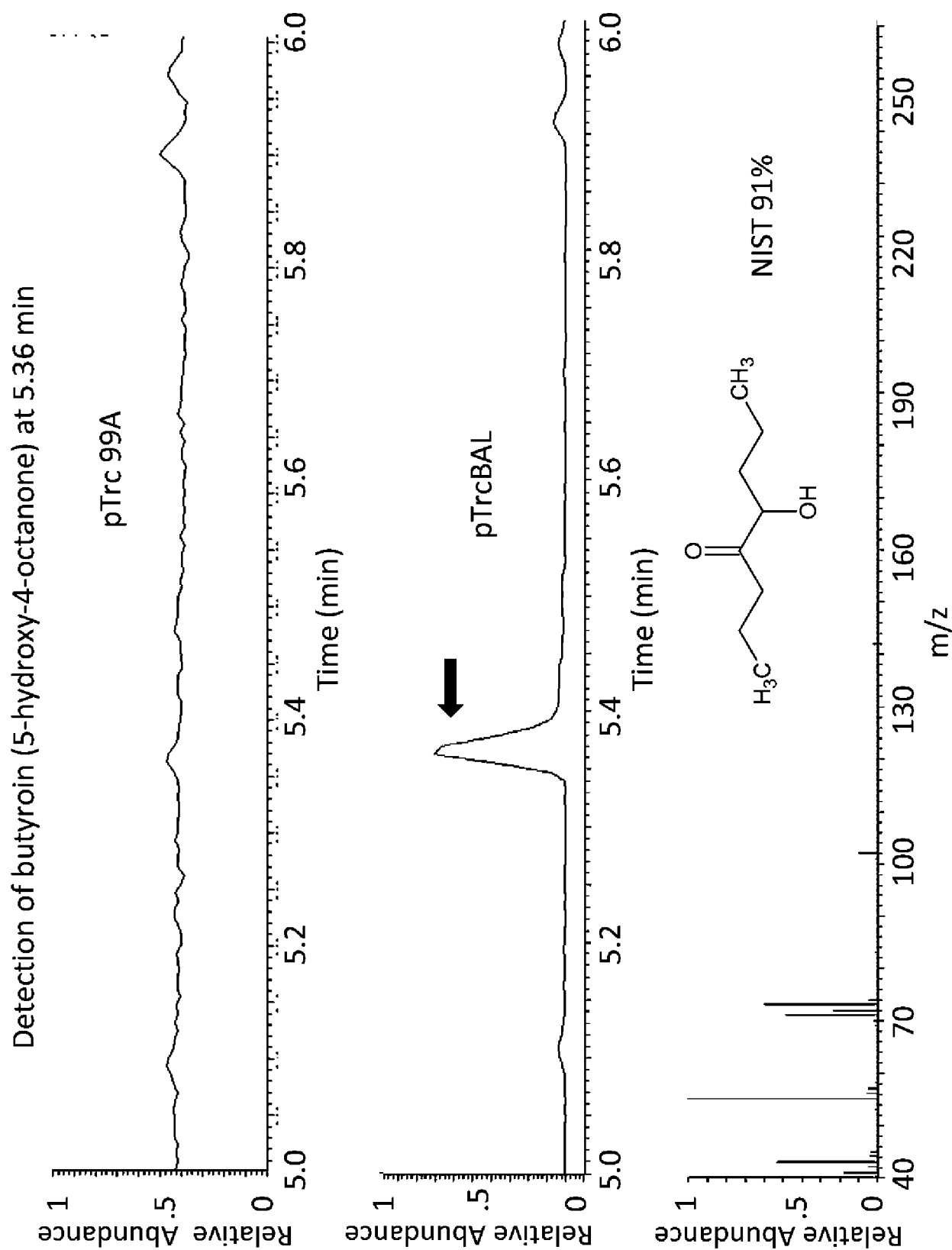


FIG. 17A

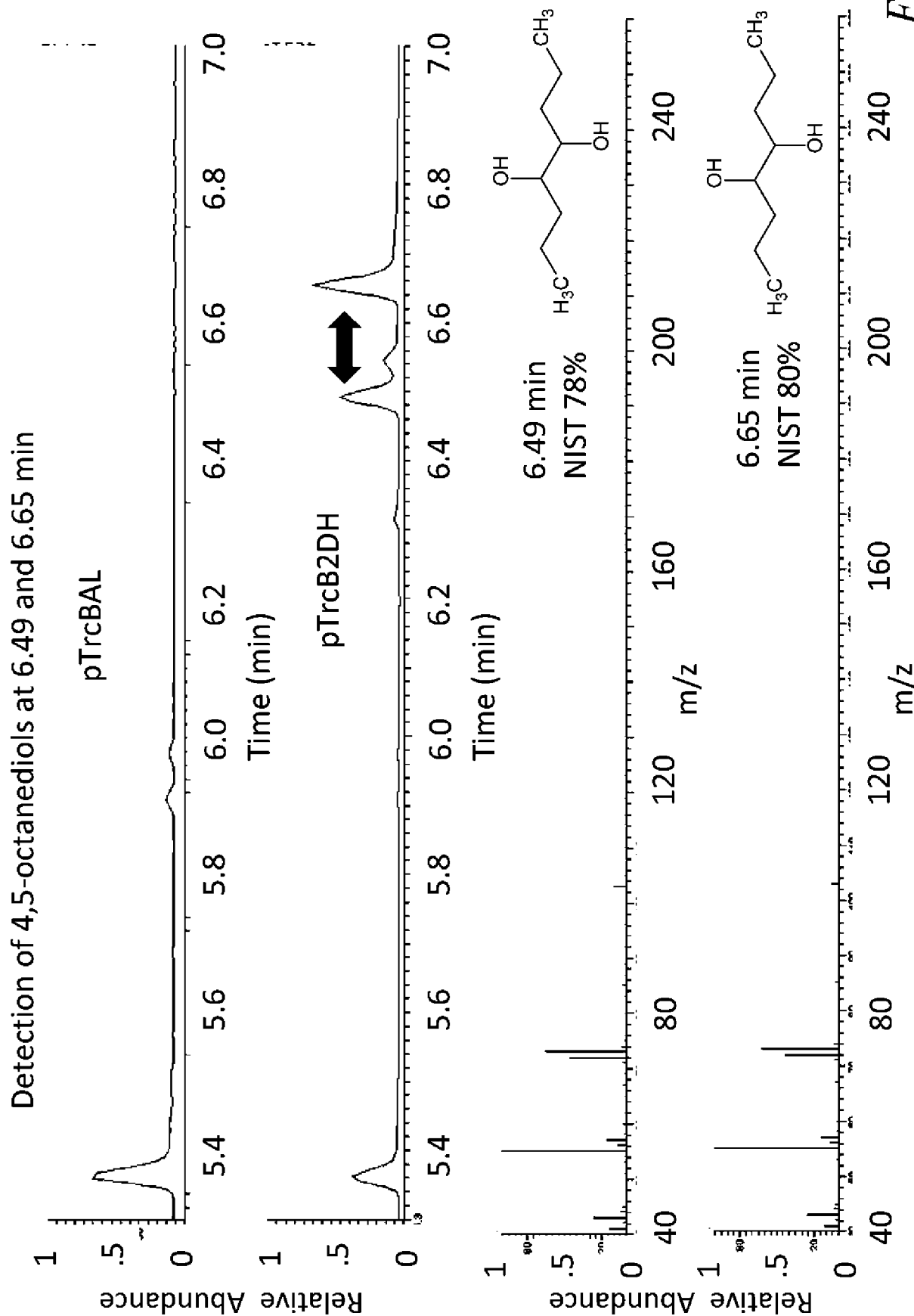
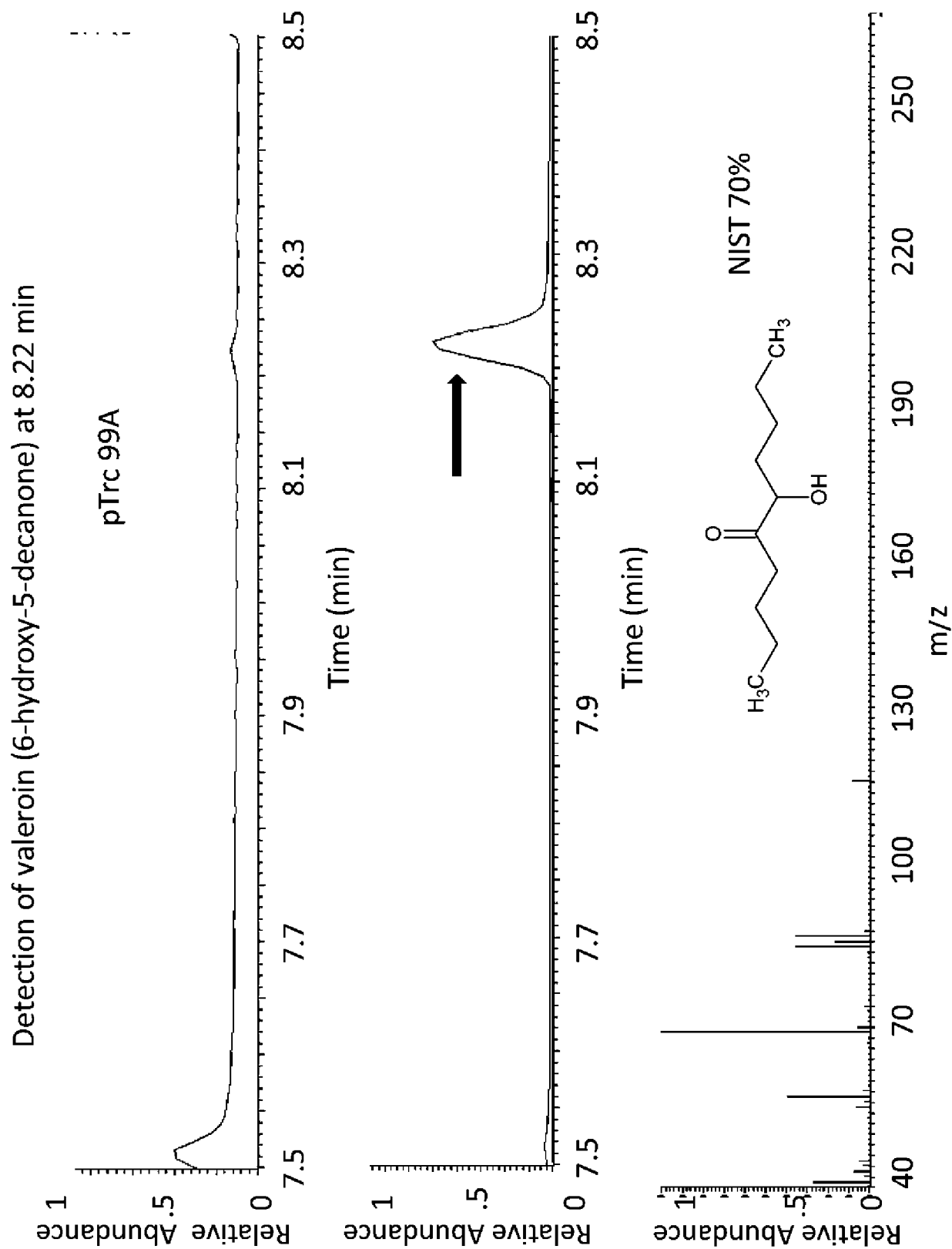


FIG. 17B

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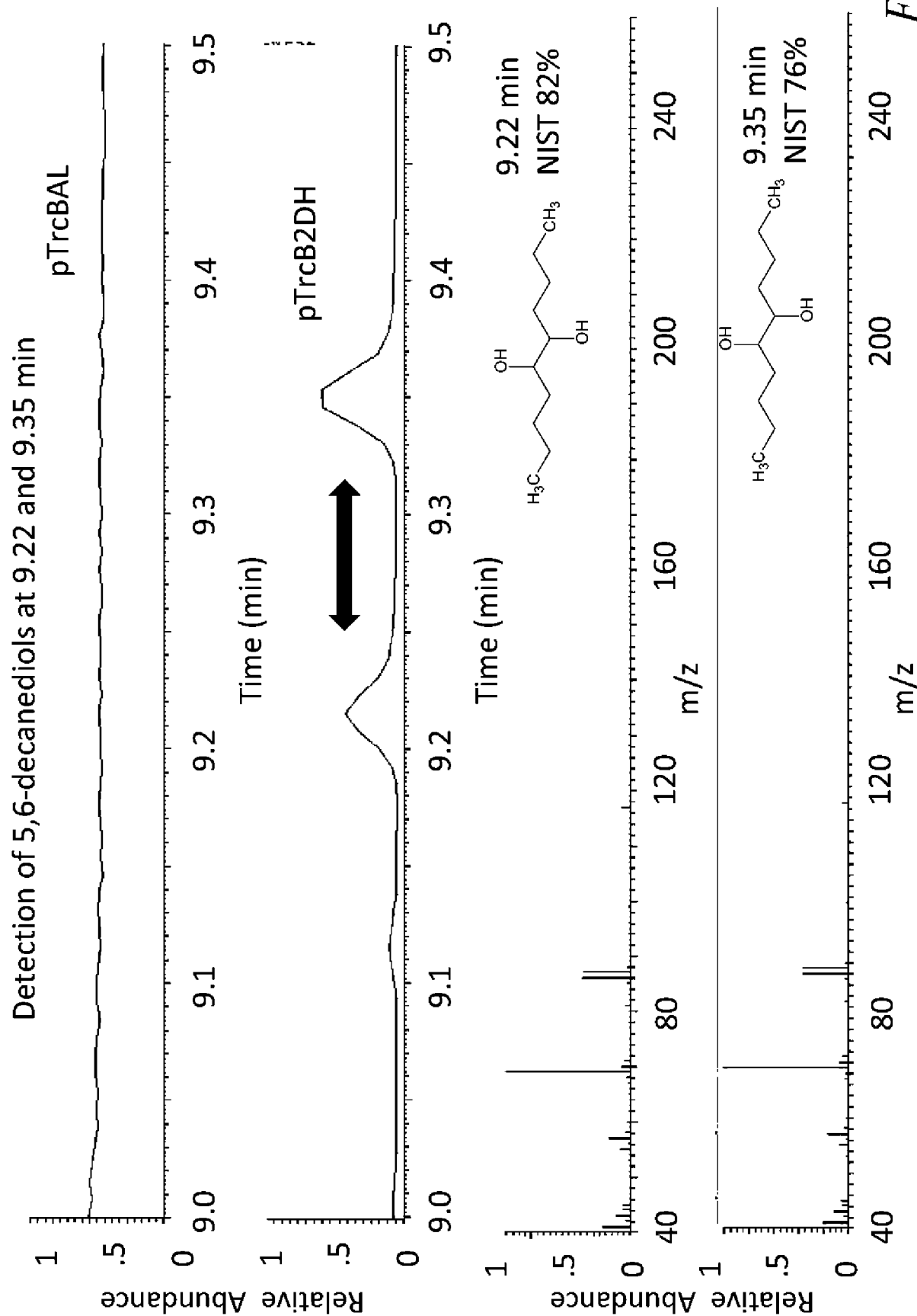
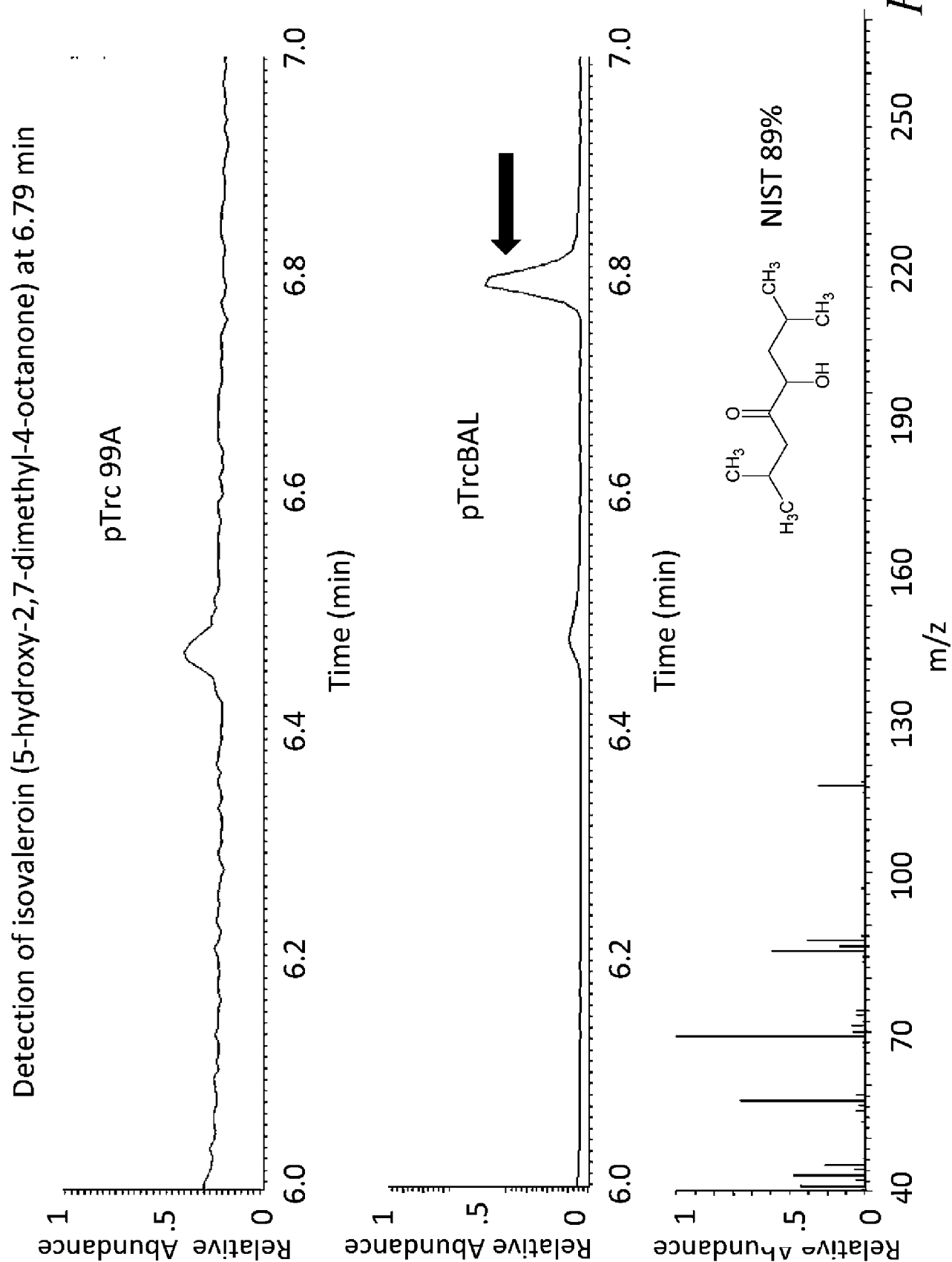


FIG. 18B

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Detection of 2,7-dimethyl-4,5-octanediols at 7.95 and 8.15 min

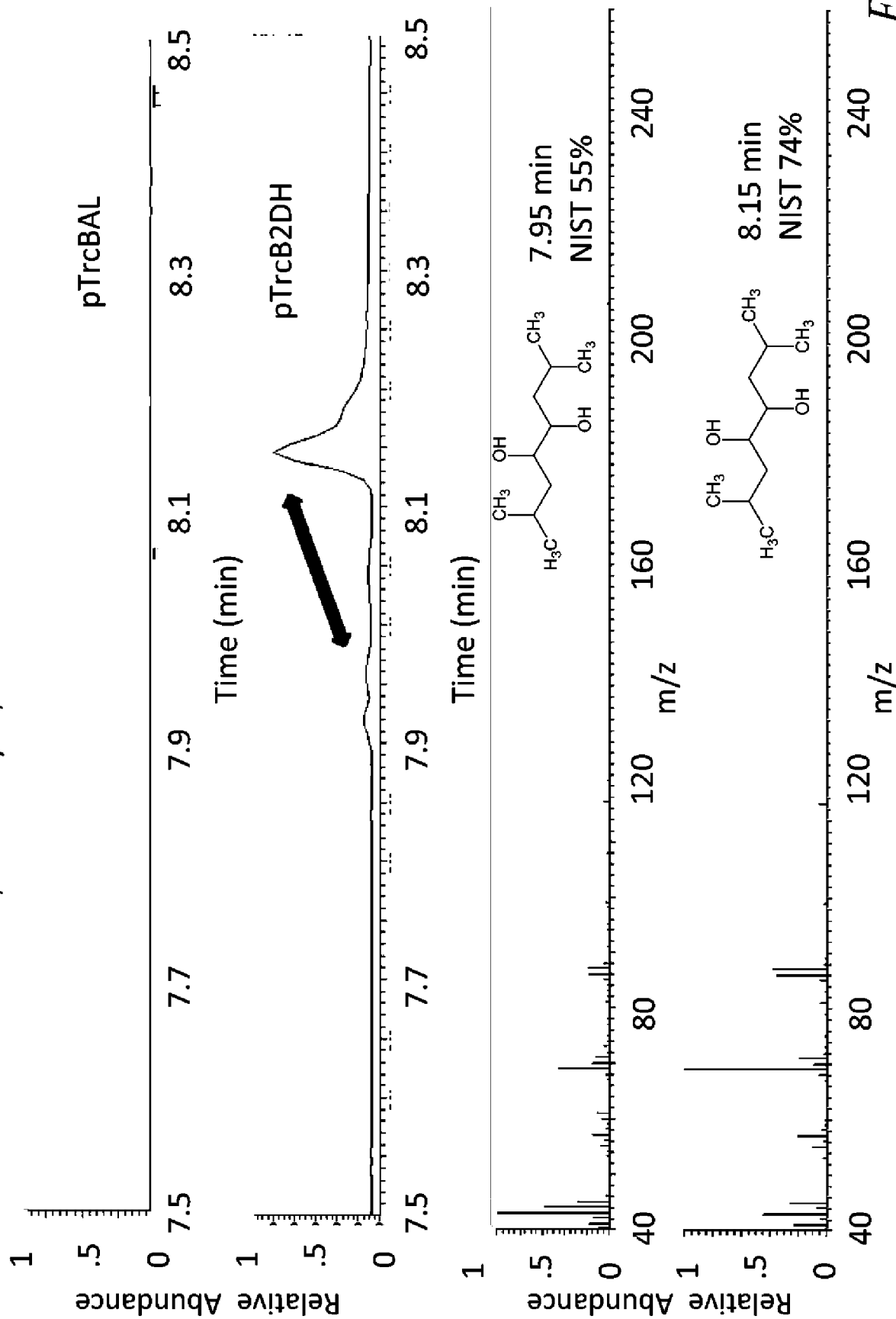
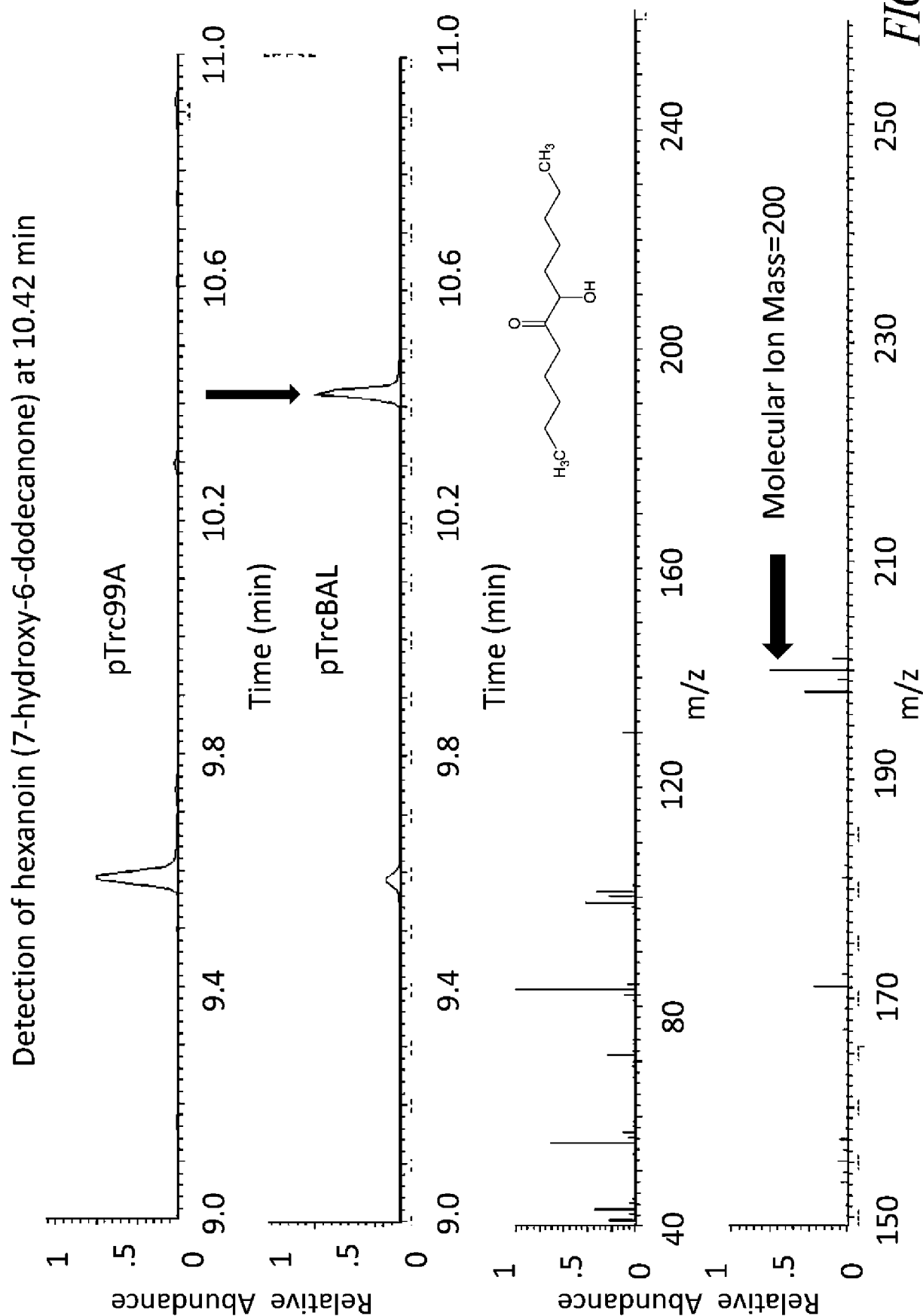
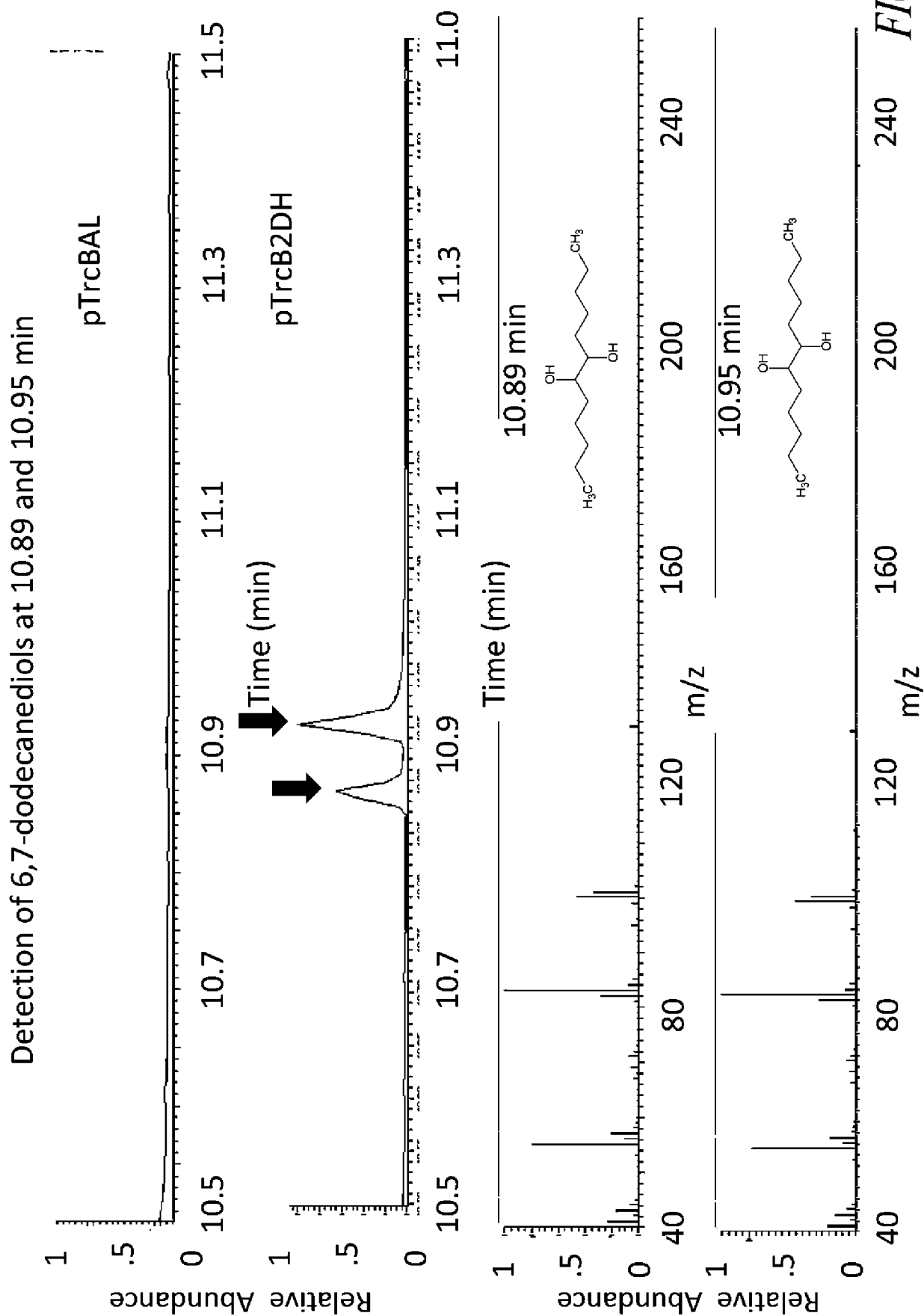


FIG. 19B

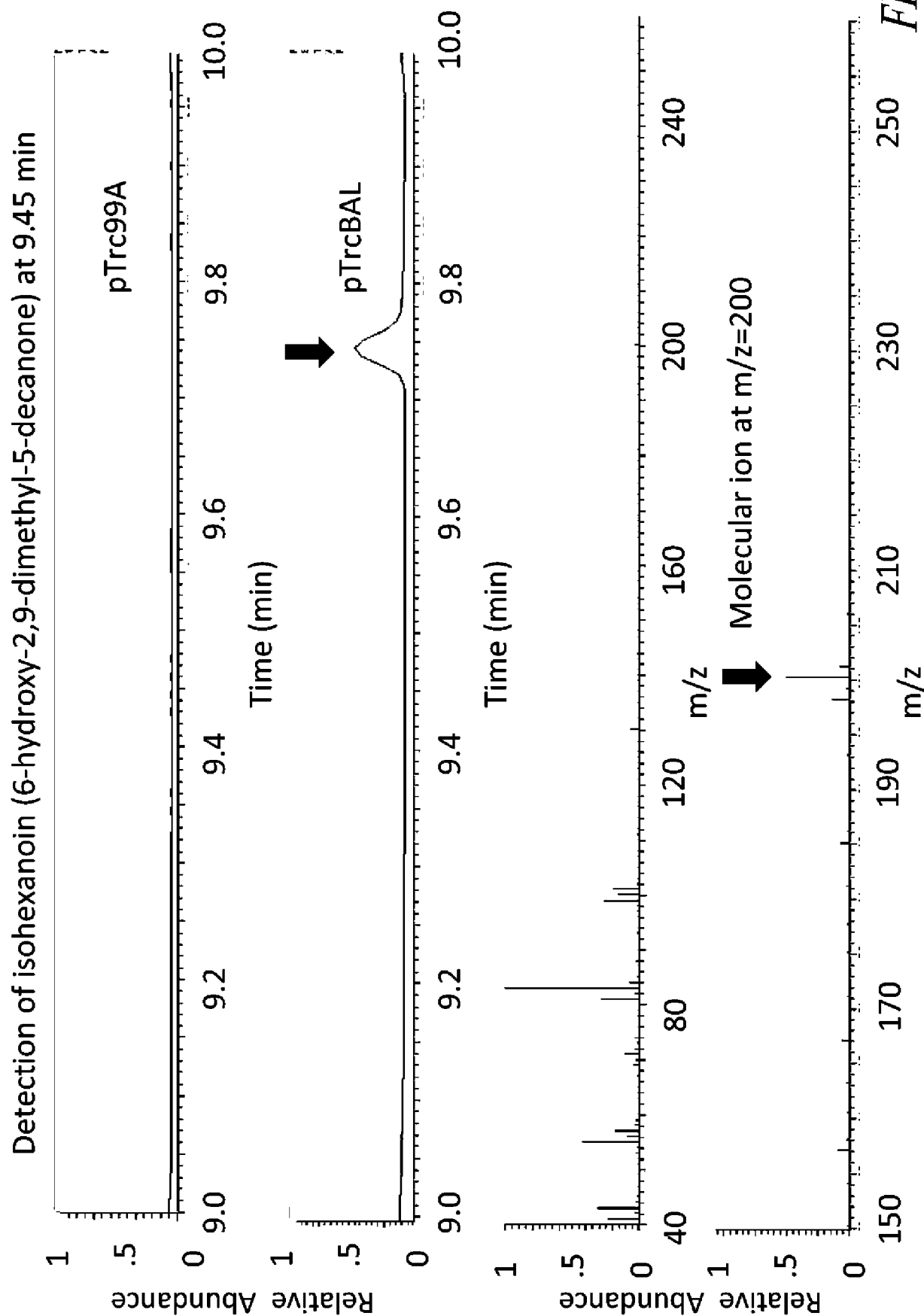
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Detection of 2,9-dimethyl-5,6-decanediols at 10.38 and 10.44 min

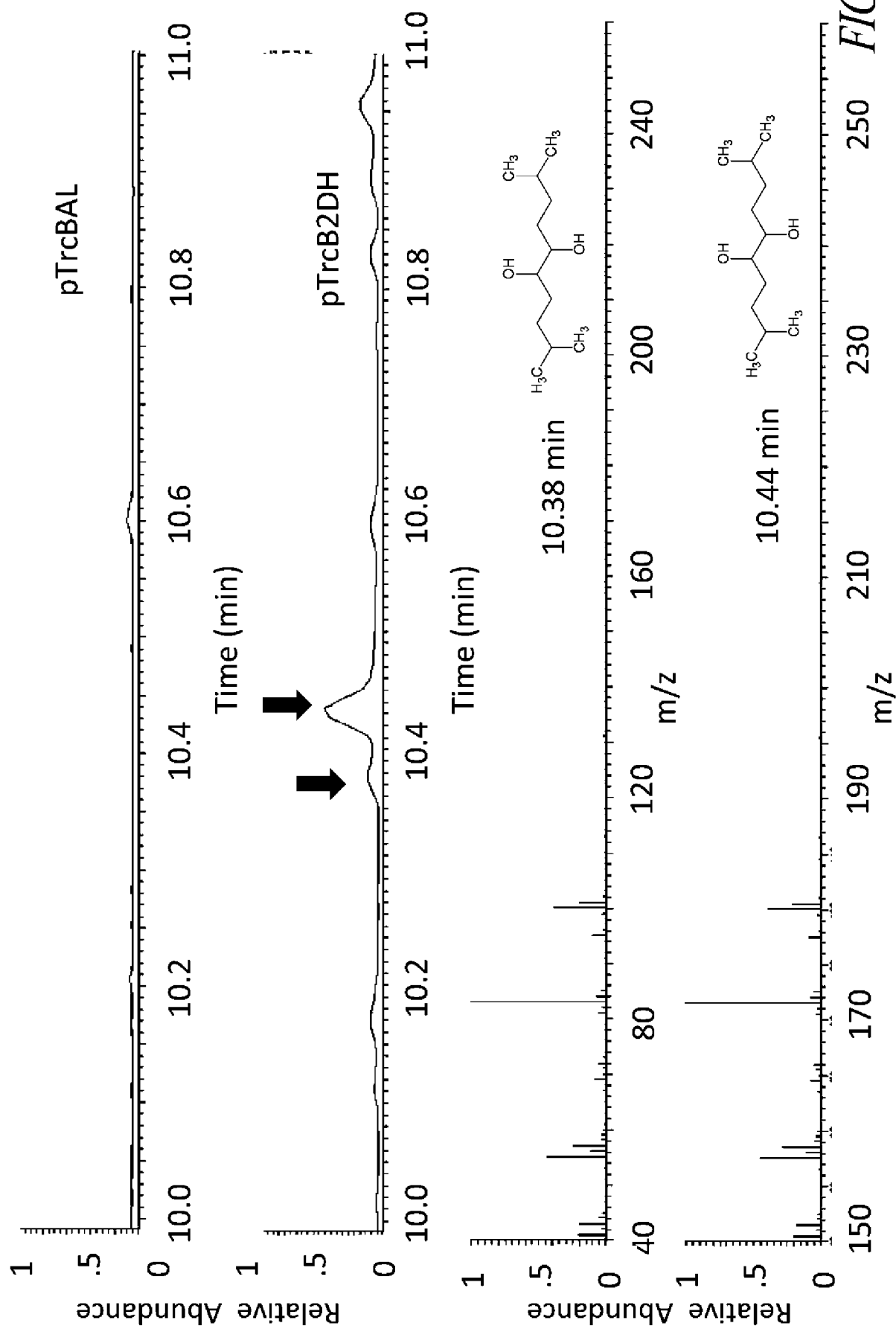
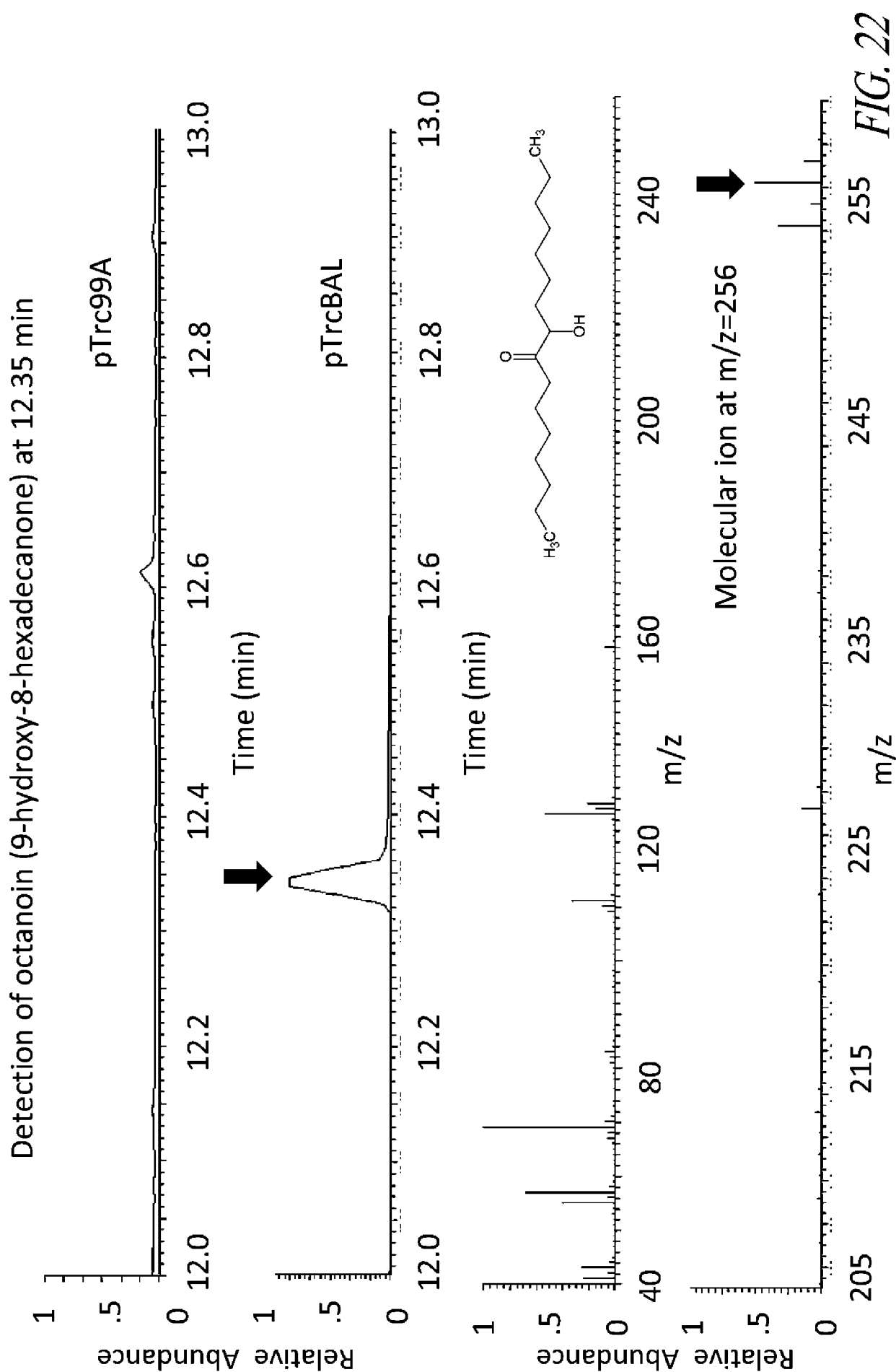
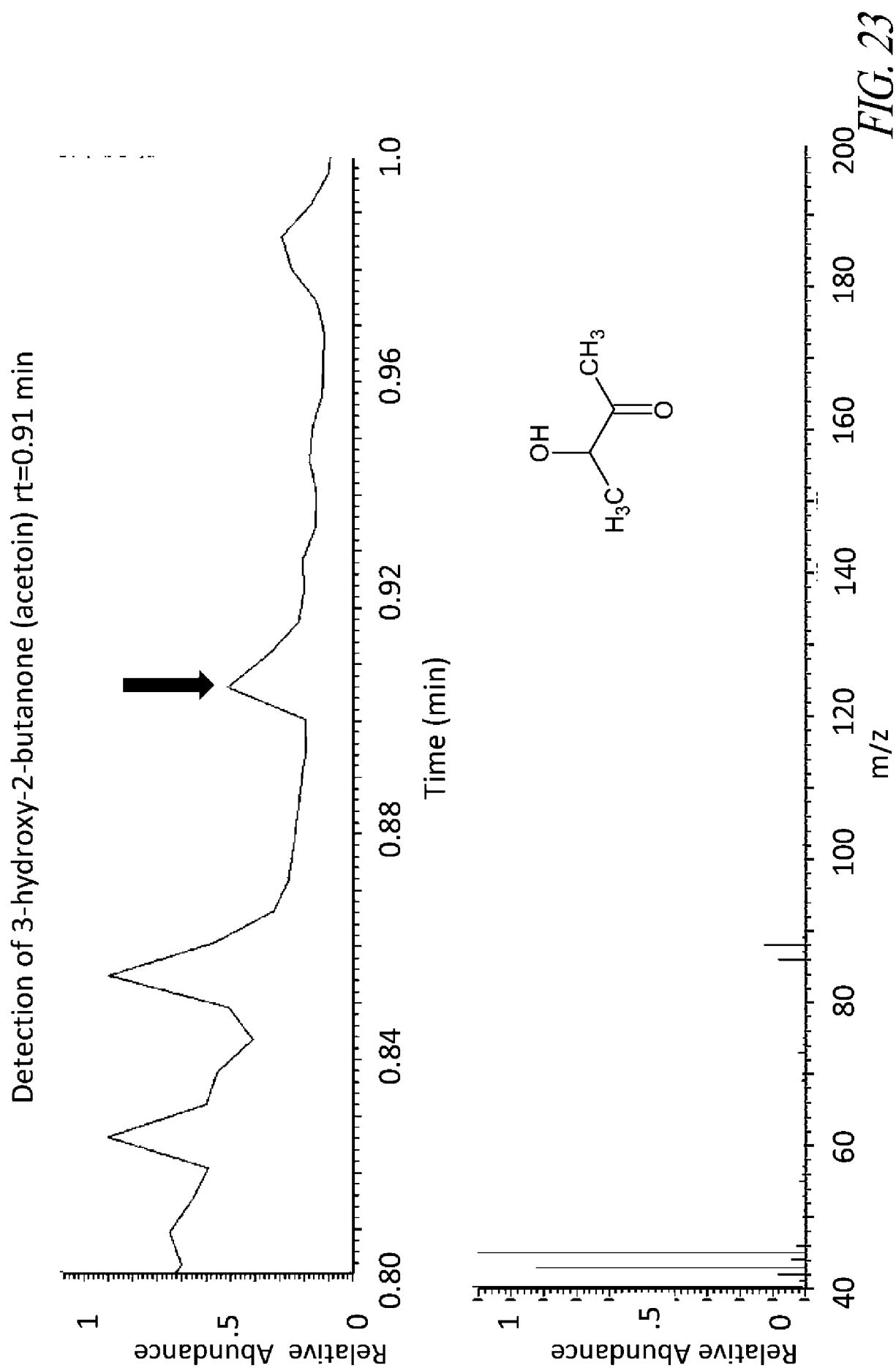


FIG. 21B

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Detection 4-hydroxy-3-hexanone (propioin) rt=2.62 min

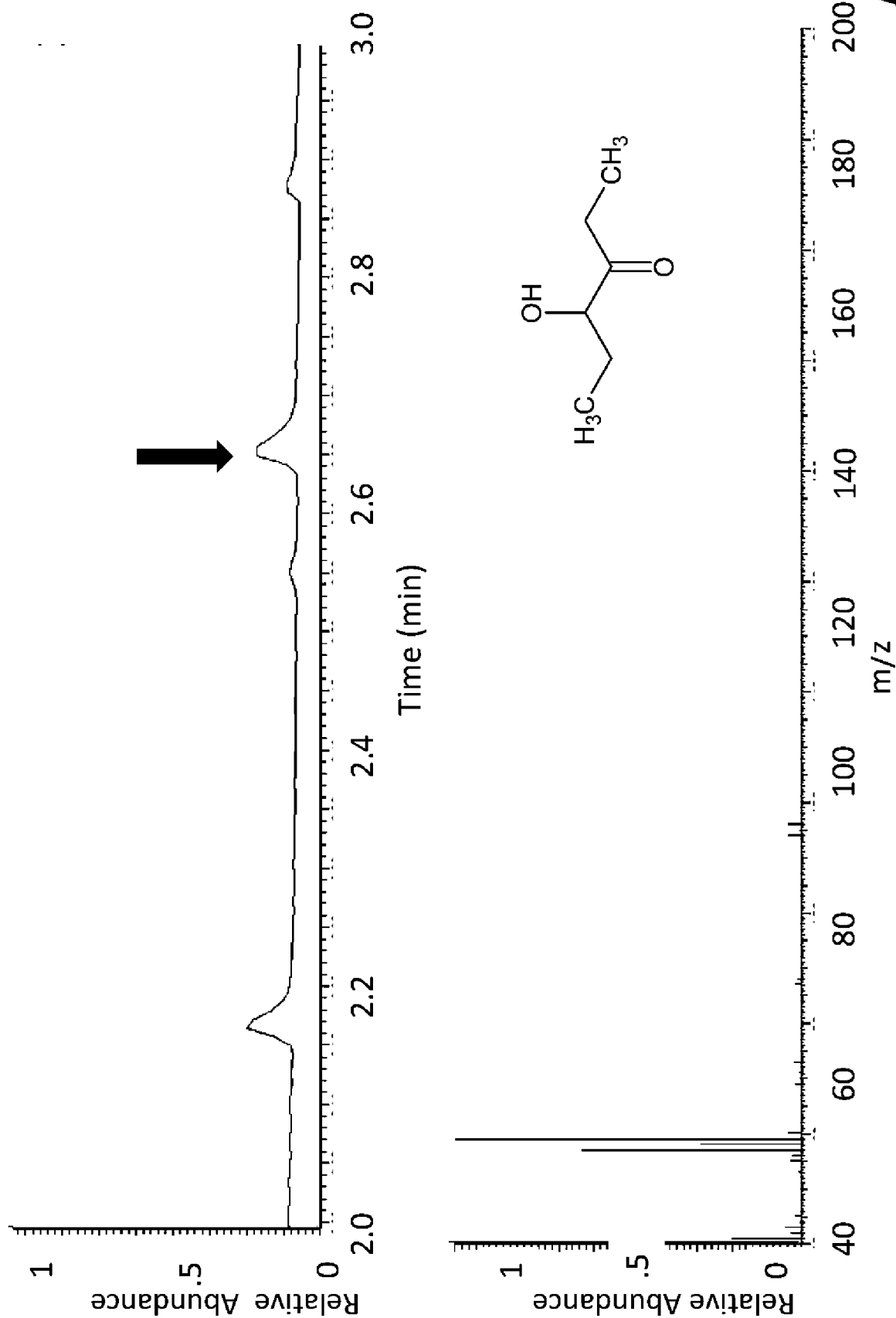


FIG. 24A

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Detection 3,4-hexanediol rt=3.79 min

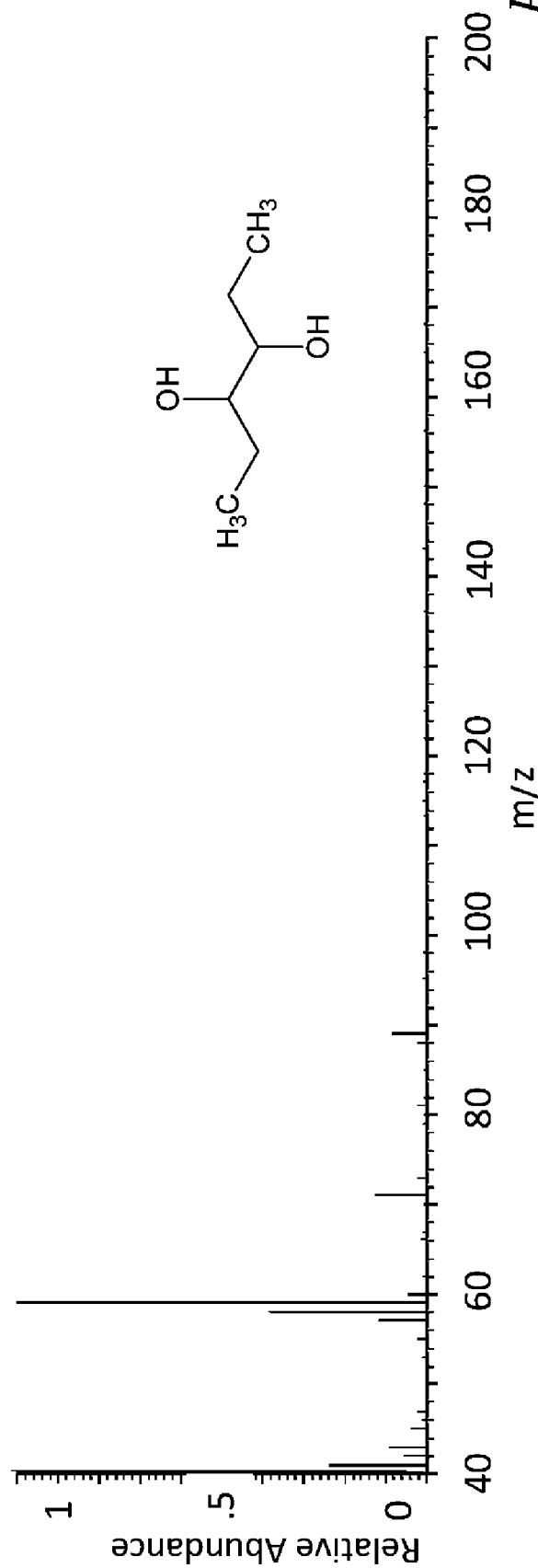
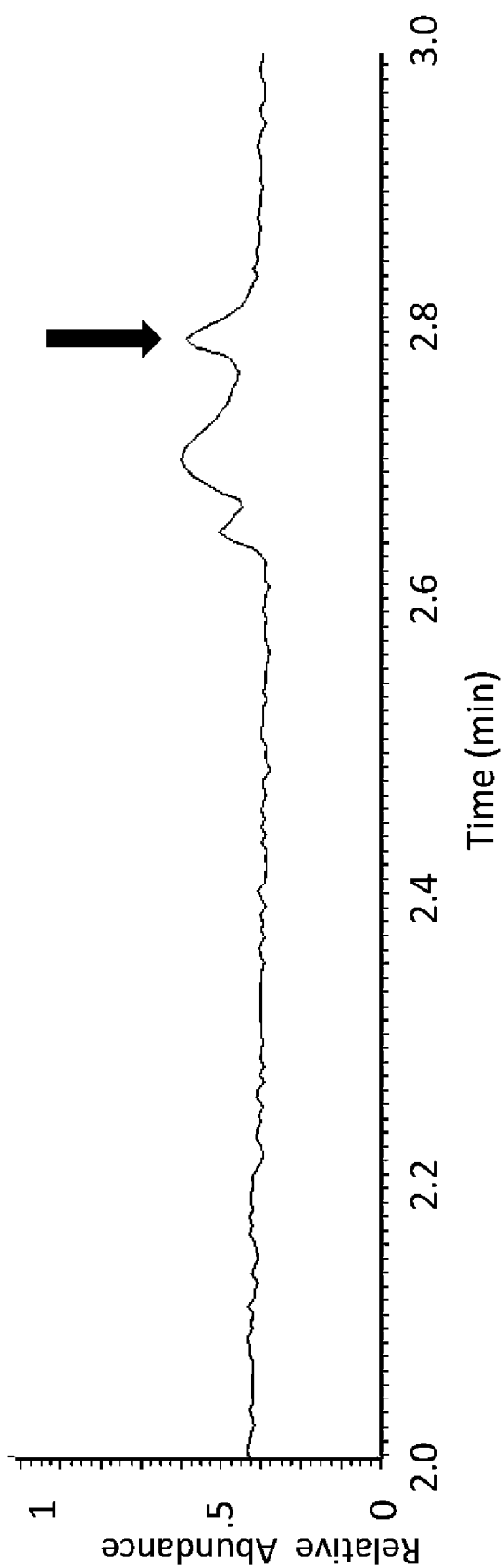


FIG. 24B

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Detection 1,4-diphenyl-3-hydroxy-2-butanone rt=13.66 min

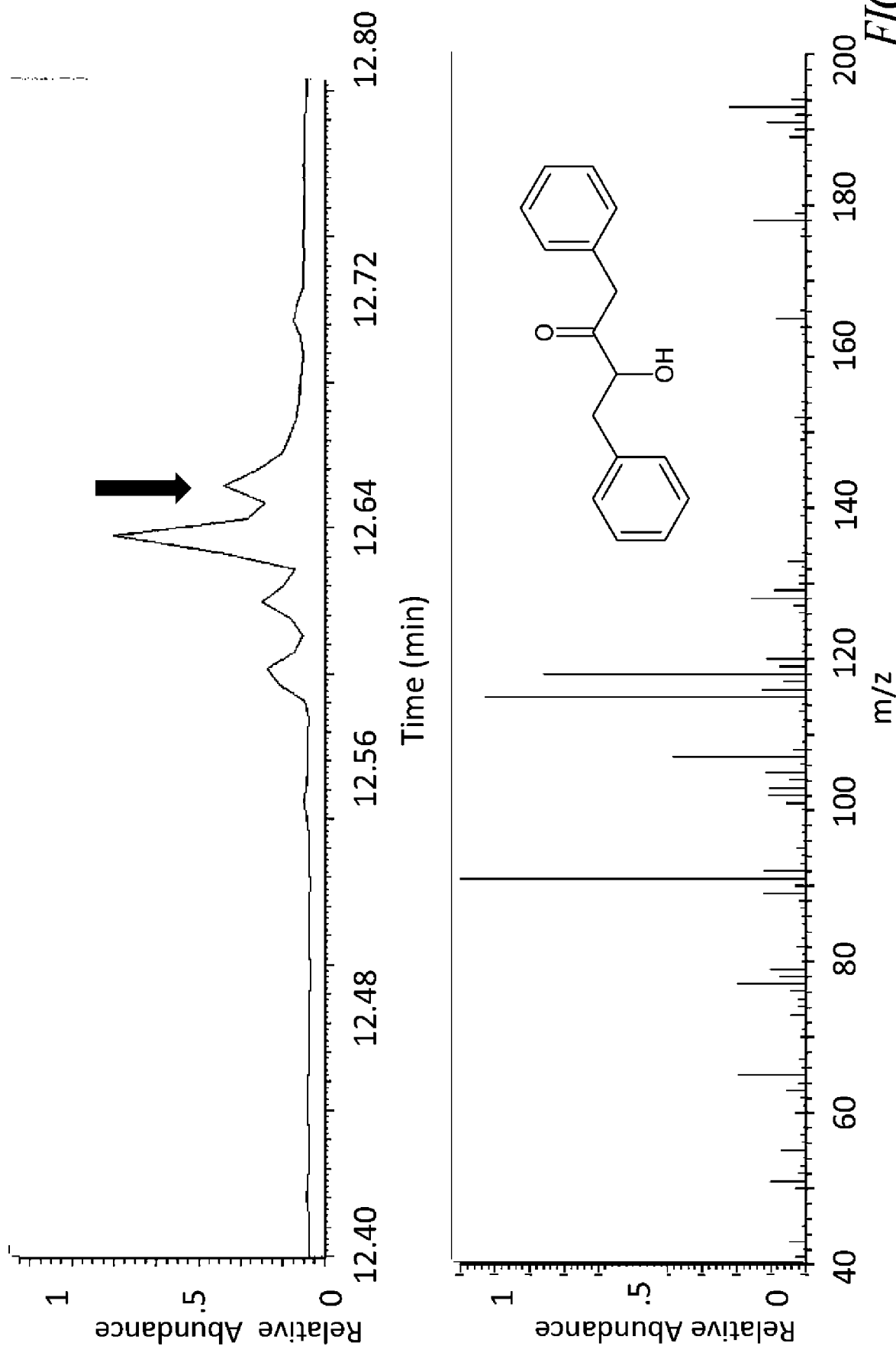


FIG. 25

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GCMS data confirming the presence of 4,5-octanediols in the sample extraction

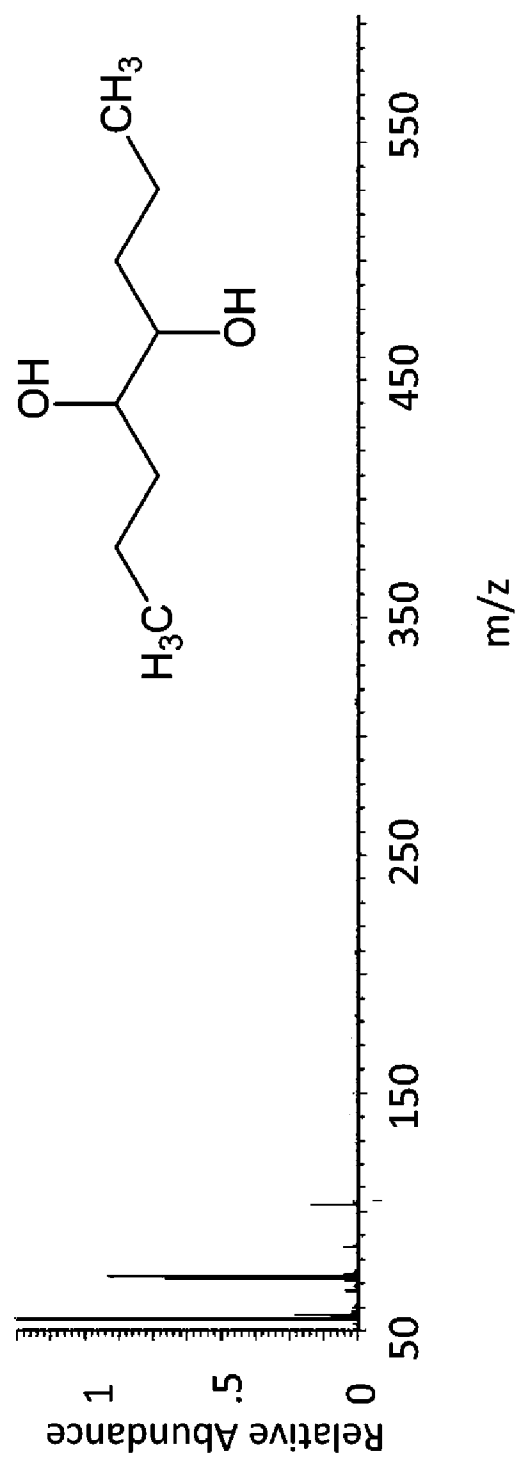
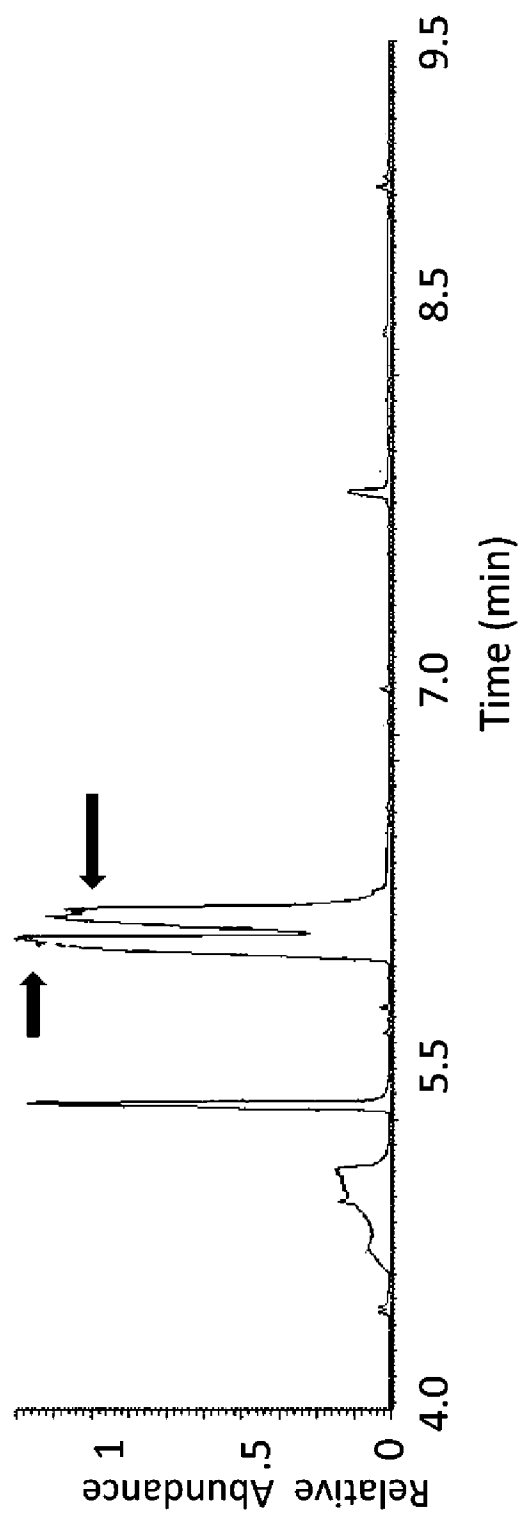


FIG. 26A

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GCMS data confirming the presence of 4-octanone in the sample extraction

Sample Solution - Peak at 4.55 identified as 4-octanone

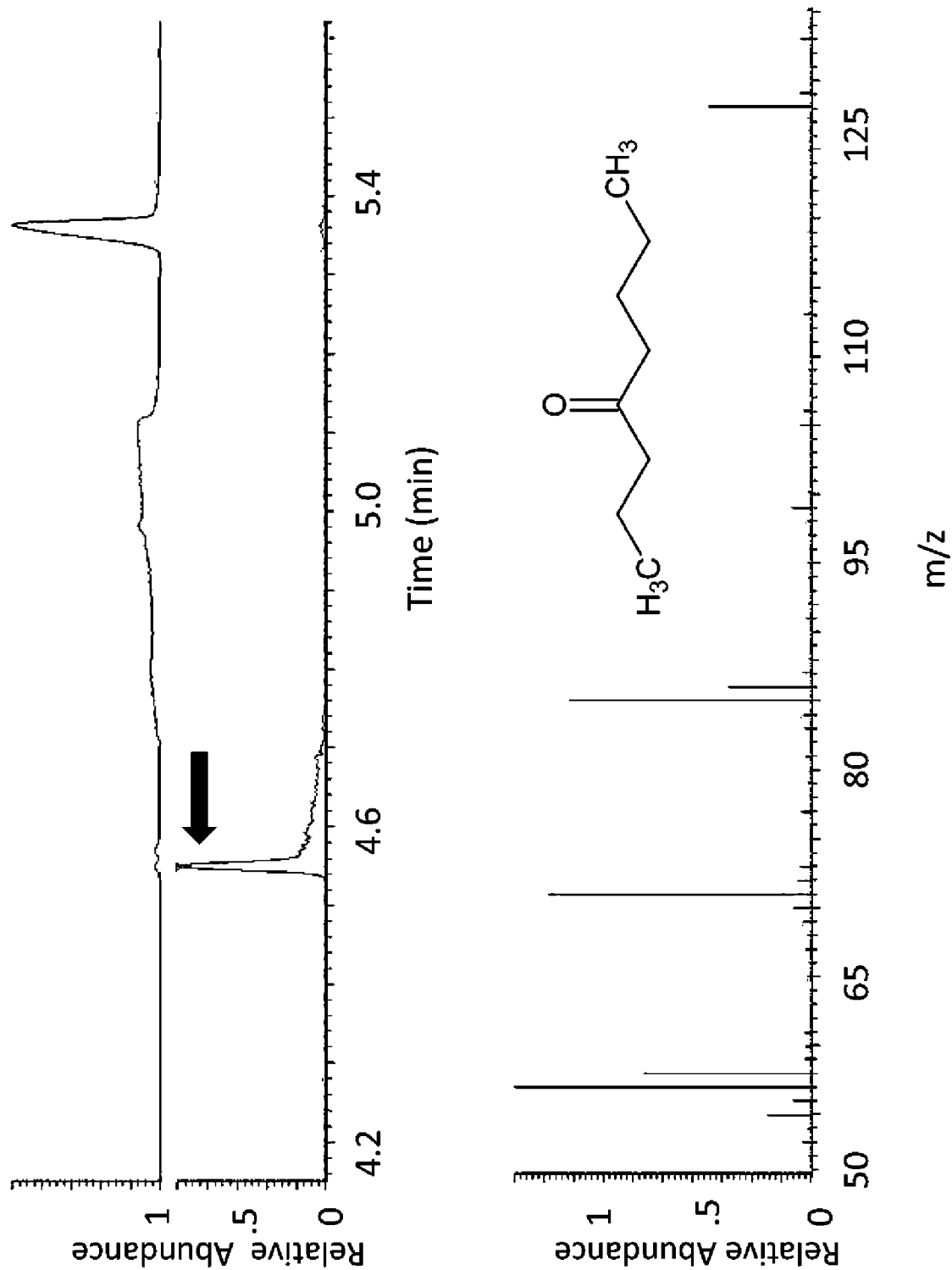


FIG. 26B

Comparison between the sample extraction gas chromatograph (top) and the 4-octanone standard gas chromatograph (bottom)

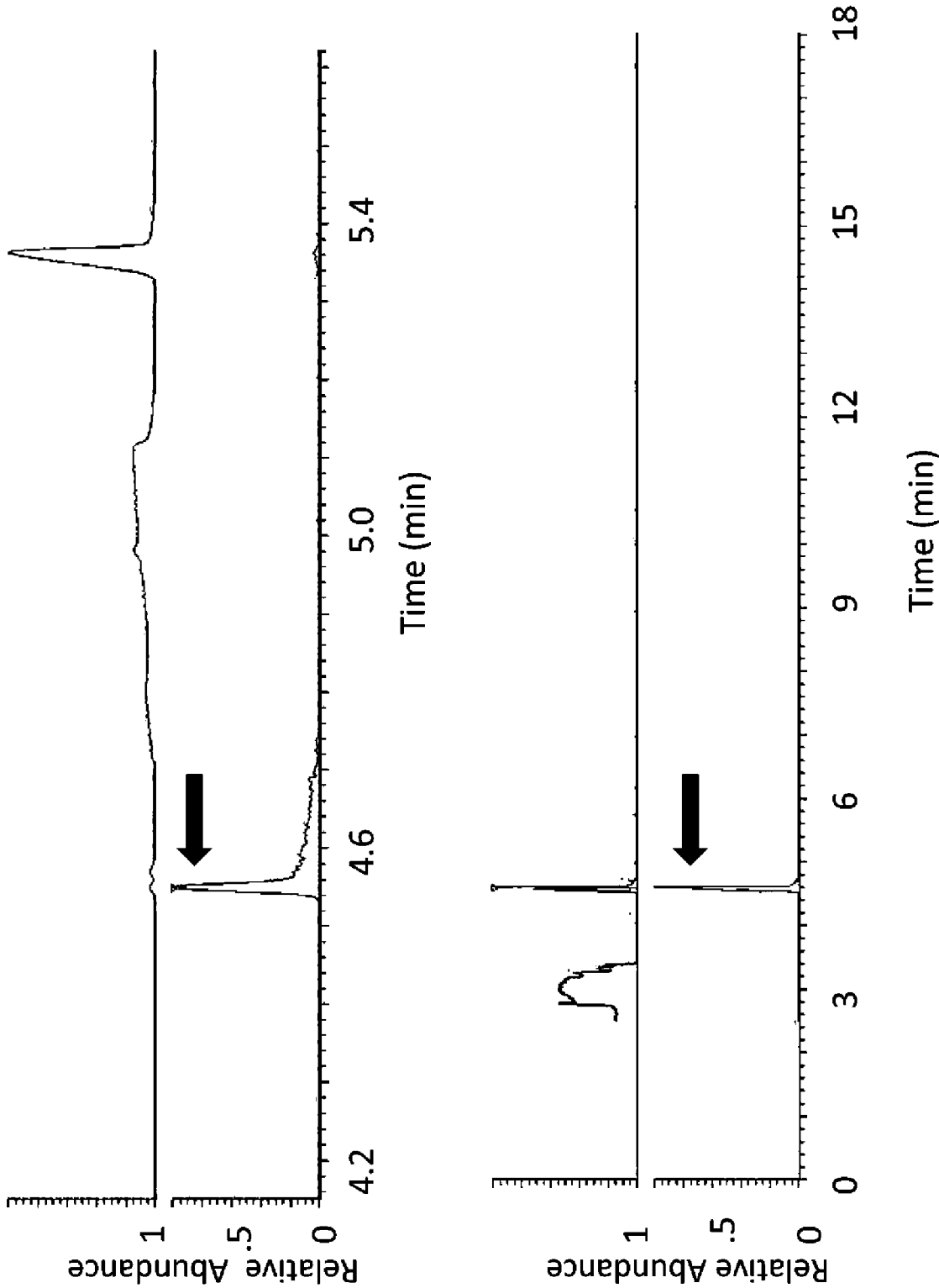


FIG. 27A

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Comparison between the sample mass spectrum (top) and the 4-octanone standard mass spectrum (bottom)

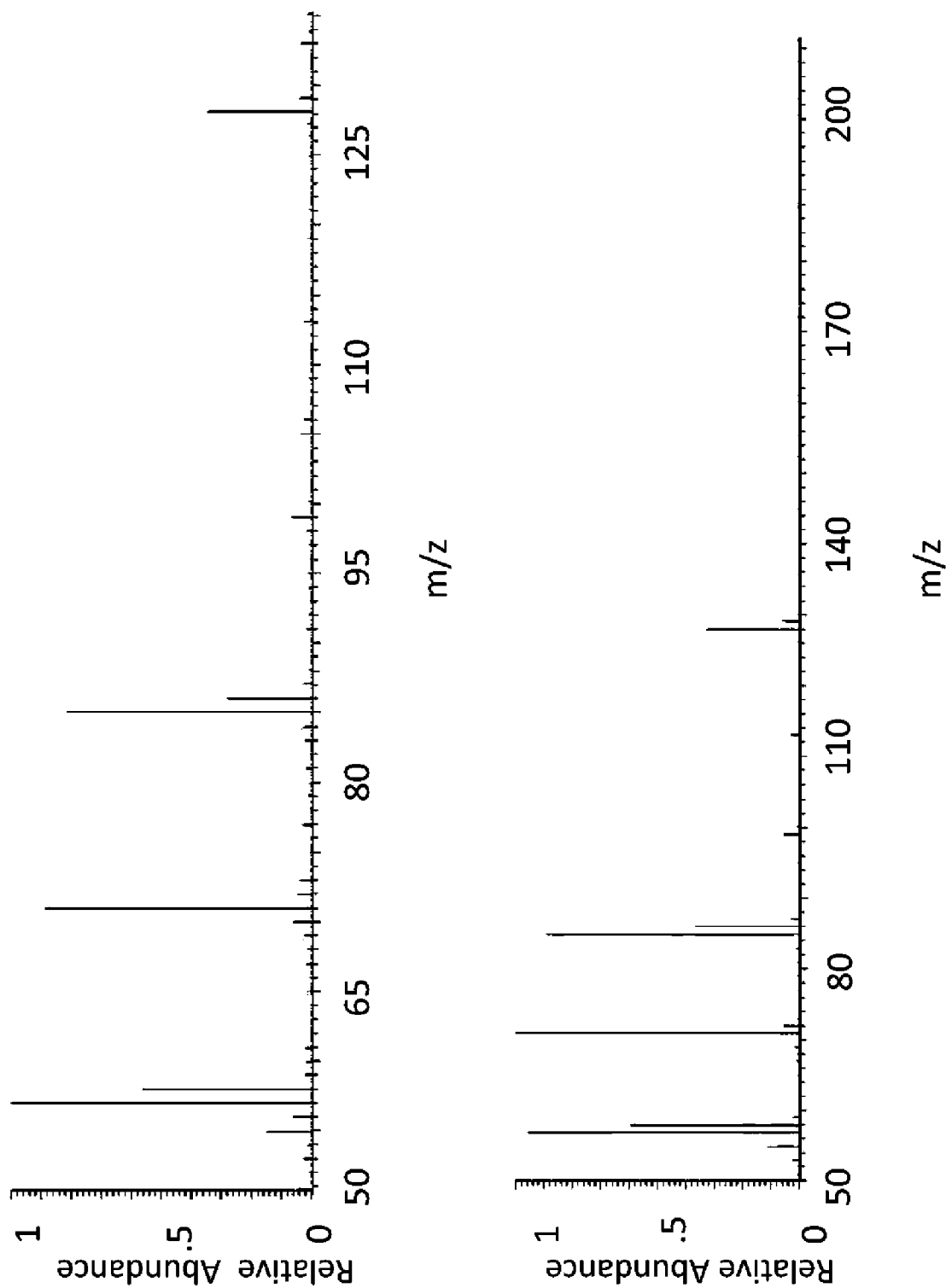


FIG. 27B

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Nucleotide sequence of diol dehydratase large subunit (*pduC*) isolated from *Klebsiella pneumoniae* MGH78578

ATGAGATCGAAAAGATTTGAAGCACTGGCGAAACGCCCTGTGAATCAGGATGGTTTCGTTAAGGA
GTGGATTGAAGAGGGCTTTATCGCGATGGAAAGCCCTAACGATCCCAAACCTTCTATCCGCATCG
TCAACGGCGCGGTGACCGAACTCGACGATAAACCGGTTGAGCAGTTCGACCTGATTGACCACTTT
ATCGCGCGCTACGGCATTAACTCTCGCCCGGGCCGAAGAAGTGATGGCCATGGATTGCGTTAAGCT
CGCCAACATGCTCTGCGACCCGAACGTTAAACGCAGCGACATCGTGCCGCTCACTACCGCGATGA
CCCCGGCGAAAATCGTGGAAGTGGTGTGCGCATATGAACGTGGTCGAGATGATGATGGCGATGCAA
AAAATGCGCGCCCGCCGACGCGCTCCAGCAGGCGCATGTCACATAATATCAAAGATAATCCGGT
ACAGATTGCCGCCGACGCGCTGAAGGCGCATGGCGCGGCTTTGACGAGCAGGAGACCACCGTCG
CCGTGGCGCGCTACGCGCCGTTCAACGCCATCGCCCTGCTGGTCGGTTCACAGGTTGGCCGCCCC
GGCGTCTCACCCAGTGTTGCTGGAAGAAGCCACCGAGCTGAAACTGGGCATGCTGGGCCACAC
CTGCTATGCCGAAACCATTTCGGTATACGGTACGGAACCGGTGTTTACCGATGGCGATGACACCC
CGTGGTCGAAAGGCTTCCTCGCCTCCTCCTACGCCTCGCGCGGCCTGAAAATGCGCTTTACCTCC
GGTTCGGGCTCGGAGGTGCAGATGGGCTATGCCGAAGGCAAATCGATGCTTTATCTCGAAGCGCG
CTGCATCTACATCACCAAAGCCGCGGGGTGCAAGGCCCTGCAGAATGGCTCCGTCAGCTGTATCG
GCGTGCCGTCCGCGCTGCCGTCCGGGATCCGCGCCGTA CTGGCGGAAAACCTGATCTGCTCAGCG
CTGGATCTGGAGTGCGCCTCCAGCAACGATCAAACCTTTACCCACTCGGATATGCGGCGTACCGC
GCGTCTGCTGATGCAGTTCCTGCCAGGTACCGACTTTATCTCCTCCGGTTACTCGGCGGTGCCGA
ACTACGACAACATGTTTCGCCGTTCCAACGAAGATGCCGAAGACTTCGATGACTACAACGTGATC
CAGCGCGACCTGAAGGTGATGGCGGCCTGCGGCCGGTGCGTGAAGAGGACGTGATCGCCATTGCG
CAACAAAGCCGCCGCGCGCTGCAGGCGGTATTTGCCGGCATGGGTTTGCCGCCATTATACGGATG
AAGAAGTAGAAGCCGCCACCTACGCCCACGGTTCAAAAAGATATGCCGTGAGCGCAATATCGTCGAG
GACATCAAGTTTGCTCAGGAGATCATCAACAAGAACCGCAACGGCCTGGAGGTGGTGAAAGCCCT
GGCGAAAGGCGGCTTCCCCGATGTGCGCCAGGACATGCTCAATATTCAGAAAGCCAAGCTCACCG
GCGACTACCTGCATACCTCCGCCATCATTTGTTGGCGAGGGCCAGGTGCTCTCGGCCGTGAATGAC
GTGAACGATTATGCCGGTCCGGCAACAGGCTACCGCCTGCAAGCGAGCGCTGGGAAGAGATTAA
AAATATCCCGGGCGCGCTCGATCCCAATGAACCTTGCTAA (SEQ ID NO:103)

FIG. 28A

Polypeptide sequence of diol dehydratase large subunit was isolated from *Klebsiella pneumoniae* MGH78578 (*pduC*)

MRSKRFEALAKRPVNQDGFVKIEWIEEGFIAMESPNDPKPSIRIVNGAVTELDKPEQFDLIDHF
IARYGINLARAEEVMAMDSVKLANMLCDPNVKRSDIVPLTTAMTPAKIVEVVSHMNVVEMMMAMQ
KMRARRTPSQQAHTVNIKDNPVQIAADAAEGAWRGFDEQETTVAVARYAPFNAIALLVGSQVGRP
GVLTQCSLEEATELKLGLMHTCYAETISVYGTEPVFTDGGDTPWSKGF LASSYASRGLKMRFTS
GSGSEVQMGYAEGKSMLYLEARCIYITKAAGVQGLQNGSVSCIGVPSAVPSGIRAVLAENLICSA
LDLECASSNDQTFTHSDMRRTARLLMQFLPGTDFISSGYSAPVNYDNMFAGSNEDAEDFDDYNVI
QRDLKVDGGLRPVREEDVIAIRNKAARALQAVFAGMGLPITDEEVEAATYAHGSKDMPERNIVE
DIKFAQEIINKNRNGLEVVKALAKGGFPDVAQDMLNIQAKLTGDYLHTSAIIVGEGQVLSAVND
VNDYAGPATGYRLQGERWEEIKNIPGALDPNELG (SEQ ID NO:104)

FIG. 28B

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Nucleotide sequence of diol dehydratase medium subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduD*)

ATGGAAATTAACGAAACGCTGCTGCGCCAGATTATCGAAGAGGTGCTGTCCGAGATGAAATCAGG
CGCAGATAAGCCGGTCTCCTTTAGCGCGCCTGCGGCTTCTGTCCCTCTGCCGCGCCGGTCCGCC
TTGCGCCTGTGTCCGGCGACAGCTTCCTGACGGAAATCGGCGAAGCCAAACCCGGCACGCAGCAG
GATGAAGTCATTATTGCCGTGCGGCCAGCGTTTGGTCTGGCGCAAACCGCCAATATCGTCCGGCAT
TCCGCATAAAAAATATTCTGCGCGAAGTGATCGCCGGCATTGAGGAAGAAGGCATCAAAGCCCGGG
TGATCCGCTGCTTTAAGTCTTCTGACGTGCGCTTCGTGGCAGTGGAAGGCAACCGCCTGAGCGGC
TCCGGCATCTCGATCGGTATTTCAGTCGAAAGGCACCACCGTCATCCACCAGCGCGGCCTGCCGCC
GCTTTCCAATCTGGAACCTCTTCCCGCAGGCGCCGCTGCTGACGCTGGAAACCTACCGTCAGATTG
GCAAAAACGCCGCGCGCTACGCCAAACGCGAGTCGCCGCAGCCGGTGCCGACGCTTAACGATCAG
ATGGCTCGTCCCAAATACCAGGCGAAGTCGGCCATTTTGACATTAAAGAGACCAAATACGTGGT
GACGGGCAAAAACCCGCAGGAAGTGCAGTGGCGCTTTAA (SEQ ID NO:105)

FIG. 29A

Polypeptide sequence of diol dehydratase medium subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduD*)

MEINETLLRQIIIEVLSEMKSGADKPVSFSAAPVAVAPVSGDSFLTEIGEAKPGTQQ
DEVI IAVGPAFGLAQ TANIVGIPHKNILREVIAGIEEEGIKARVIRCFKSSDVAFVAVEGNRLSG
SGISIGIQSKGTTVIHQRLPPLSNLELFPQAPLLTLETYRQIGKNAARYAKRESPQPVPTLNDQ
MARPKYQAKSAILHIKETKYVVTGKNPQELRVAL (SEQ ID NO:106)

FIG. 29B

Nucleotide sequence of diol dehydratase small subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduE*)

ATGAATACCGACGCAATTGAATCCATGGTACGCGACGTGCTGAGCCGGATGAACAGCCTACAGGA
CGGGATAACGCCCGCGCCAGCCGCGCCGACAAACGACACCGTTCCGCCAGCCAAAAGTTAGCGACT
ACCGGTTAGCGACCCGCCATCCGGAGTGGGTCAAACCGCTACCAATAAAACGCTCGATGACCTG
ACGCTGGAGAACGTATTAAGCGATCGCGTTACGGCGCAGGACATGCGCATCACTCCGGAAACGCT
GCGTATGCAGGCGGCGATCGCCAGGATGCCGGACGCGATCGGCTGGCGATGAACTTTGAGCGGG
CCGCAGAGCTCACCGCGGTTCGCGACGACCGAATCCTTGAGATCTACAACGCCCTGCGCCATAC
CGTTCCACCCAGGCGGAGCTACTGGCGATCGTGTATGACCTCGAGCATCGCTACCAGGCACGACT
CTGTGCCGCTTTGTTTCGGGAAGCGGCCGGGCTGTACATCGAGCGTAAGAAGCTGAAAGGCGACG
ATTAA (SEQ ID NO:107)

FIG. 29C

Polypeptide sequence of diol dehydratase small subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduE*)

MNTDAIESMVRDVL SRMNSLQDGITPAPAAPTNDTVRQPKVSDYPLATRHPEWVK TATNKTLLDDL
TLENVLSDRVTAQDMRITPETLRMQAAIAQDAGRDR LAMNFERAAELTAVPDDRILEIYNALRPY
RSTQAE LLA IADDLEHRYQARLCAAFVREAAGLYIERKKLKGDD (SEQ ID NO:108)

FIG. 29D

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Oxidation of 4-octanol monitored by NADH production (2ADH 1-10)

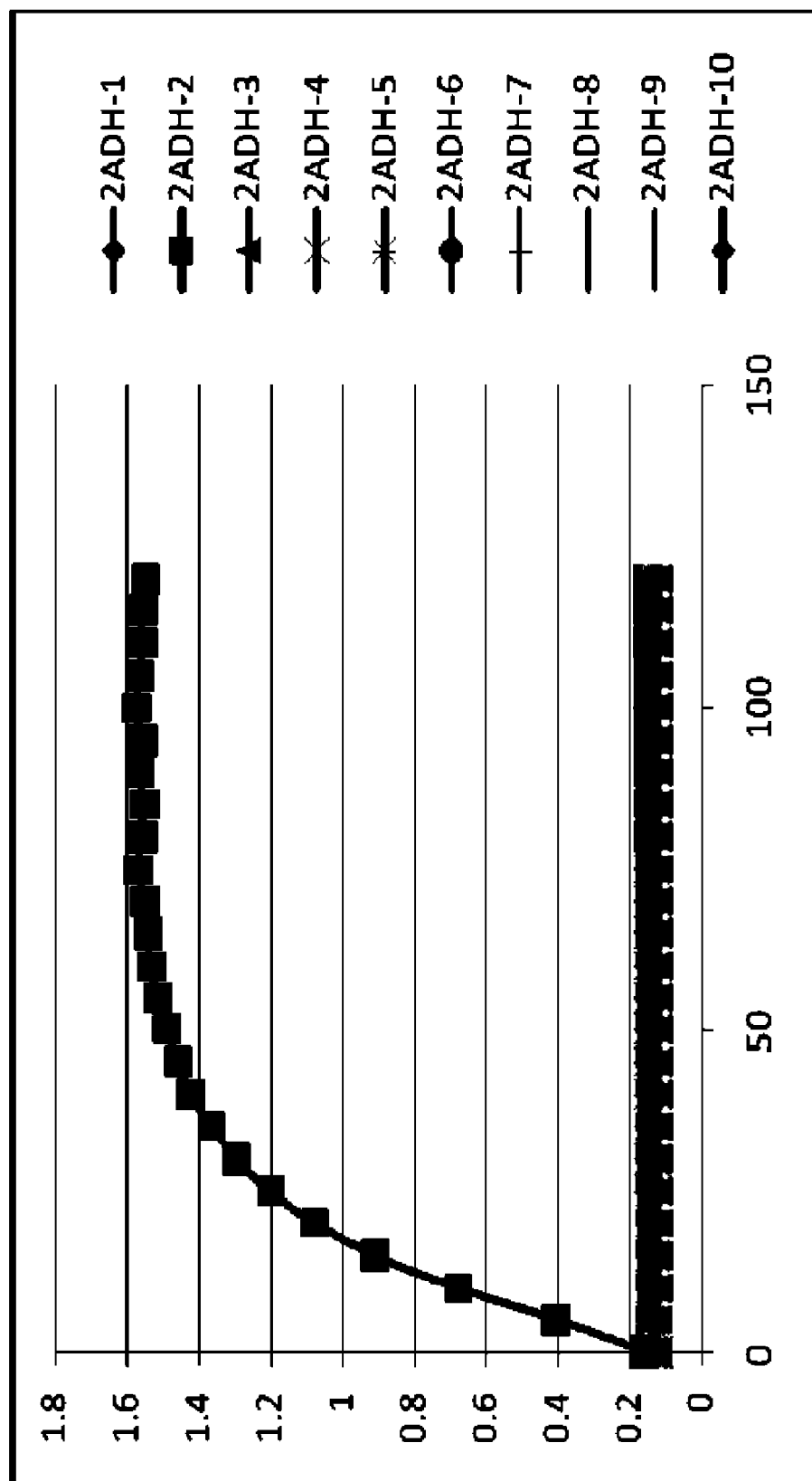


FIG. 30A

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Oxidation of 4-octanol monitored by NADPH production (2ADH 1-10)

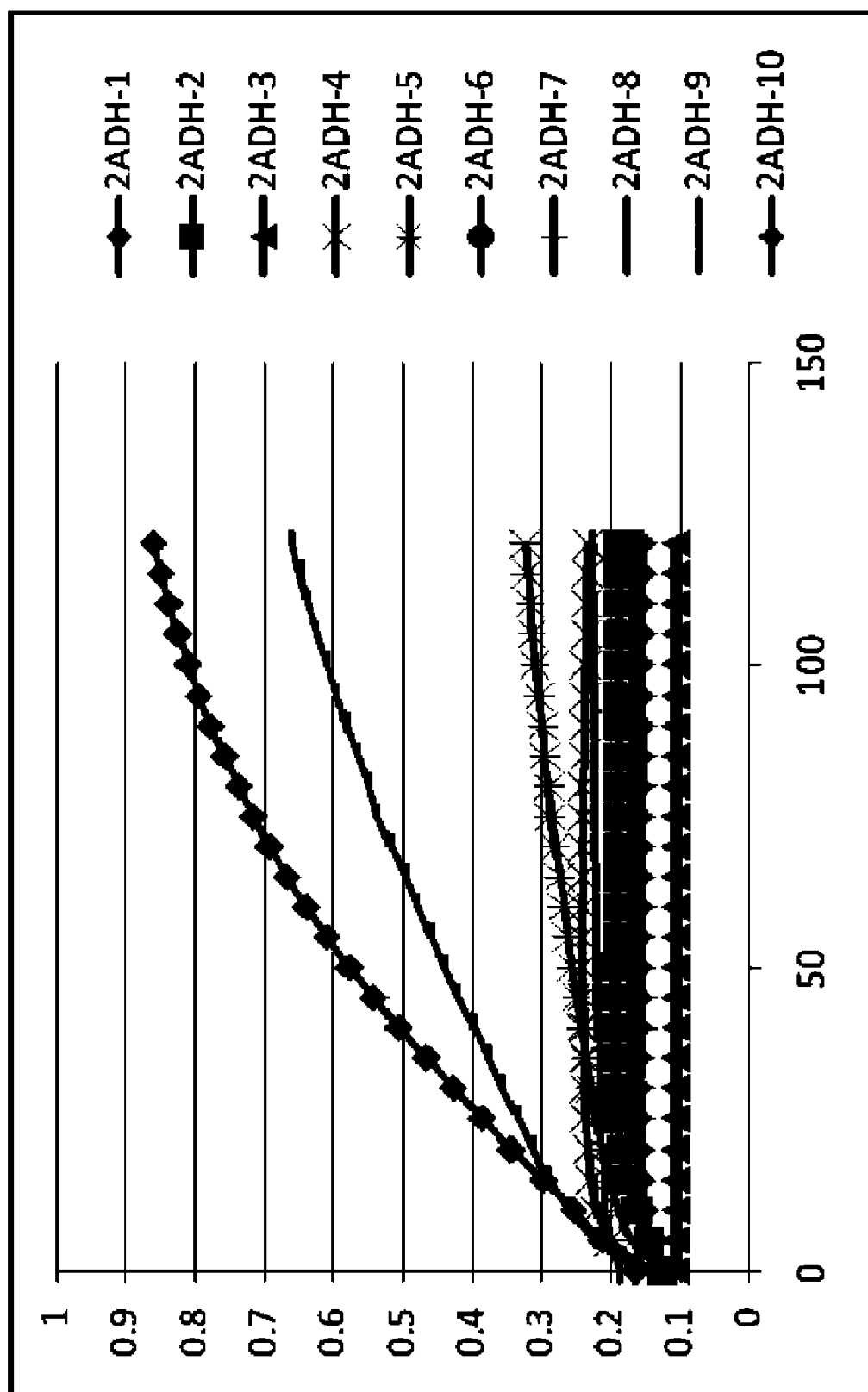


FIG. 30B

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Oxidation of 4-octanol monitored by NADH production (2ADH11-18)

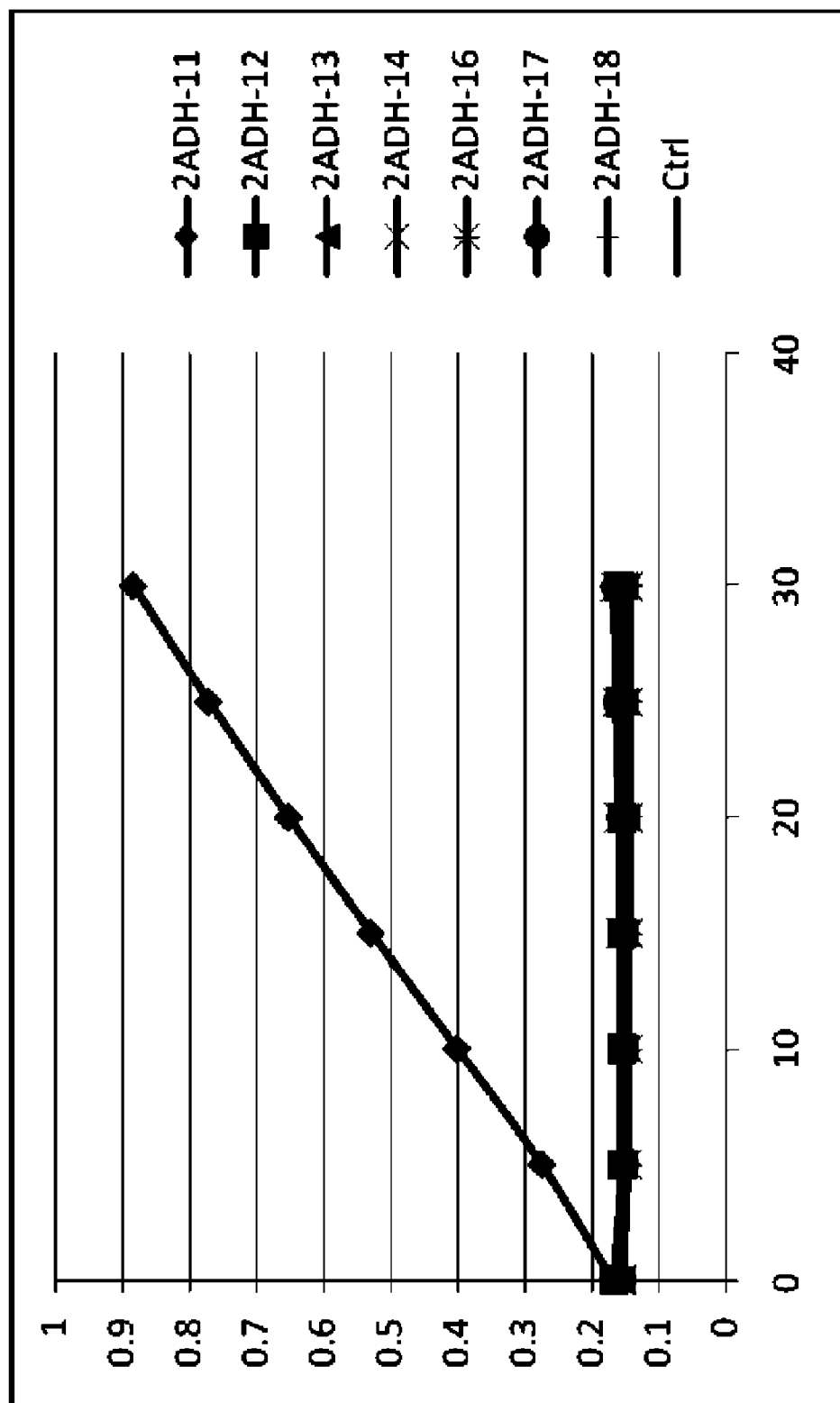


FIG. 31A

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Oxidation of 4-octanol monitored by NADPH production (2ADH 11-18)

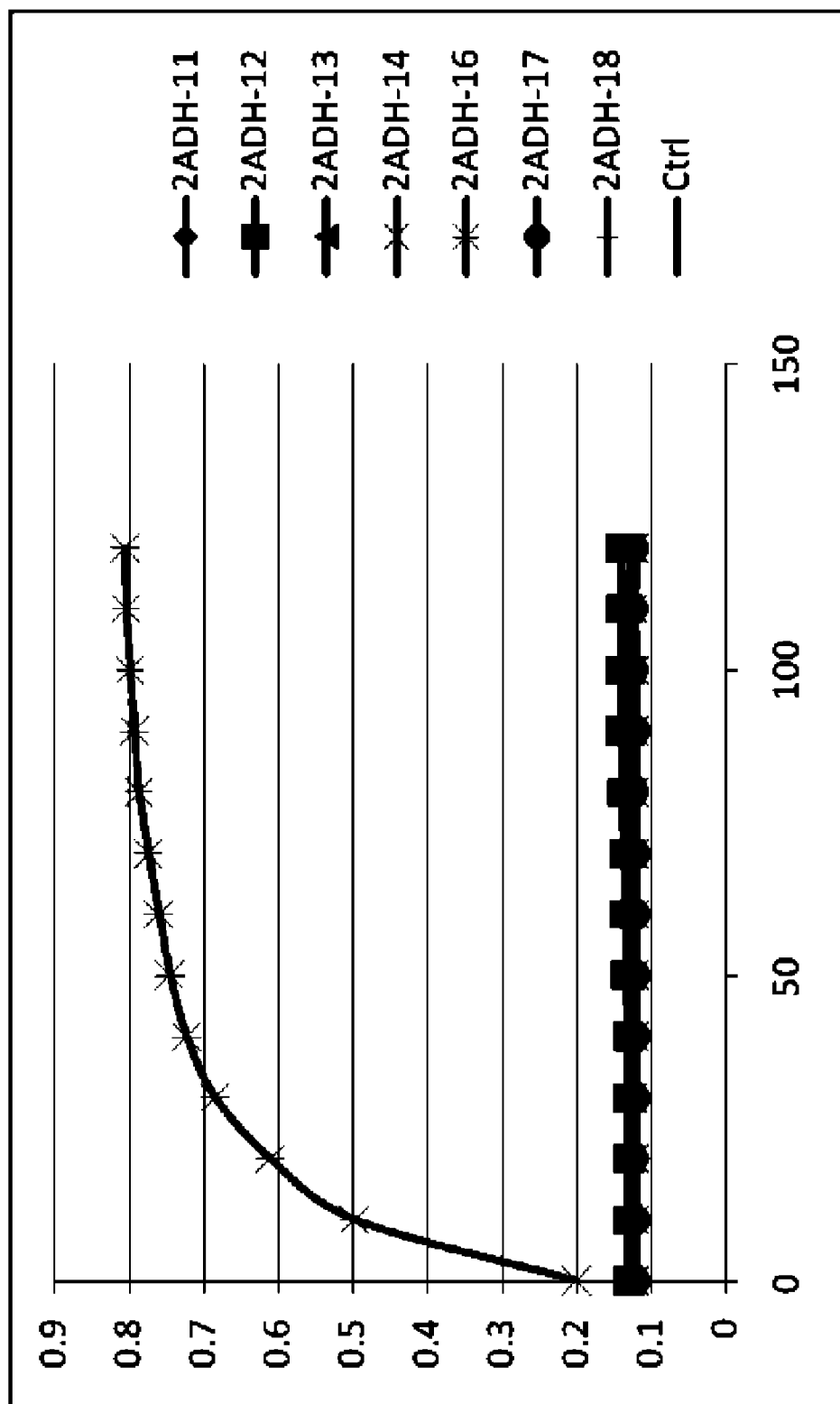


FIG. 31B

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Oxidation of 2,7-dimethyl octanol monitored by NADH production (2ADH 11-18)

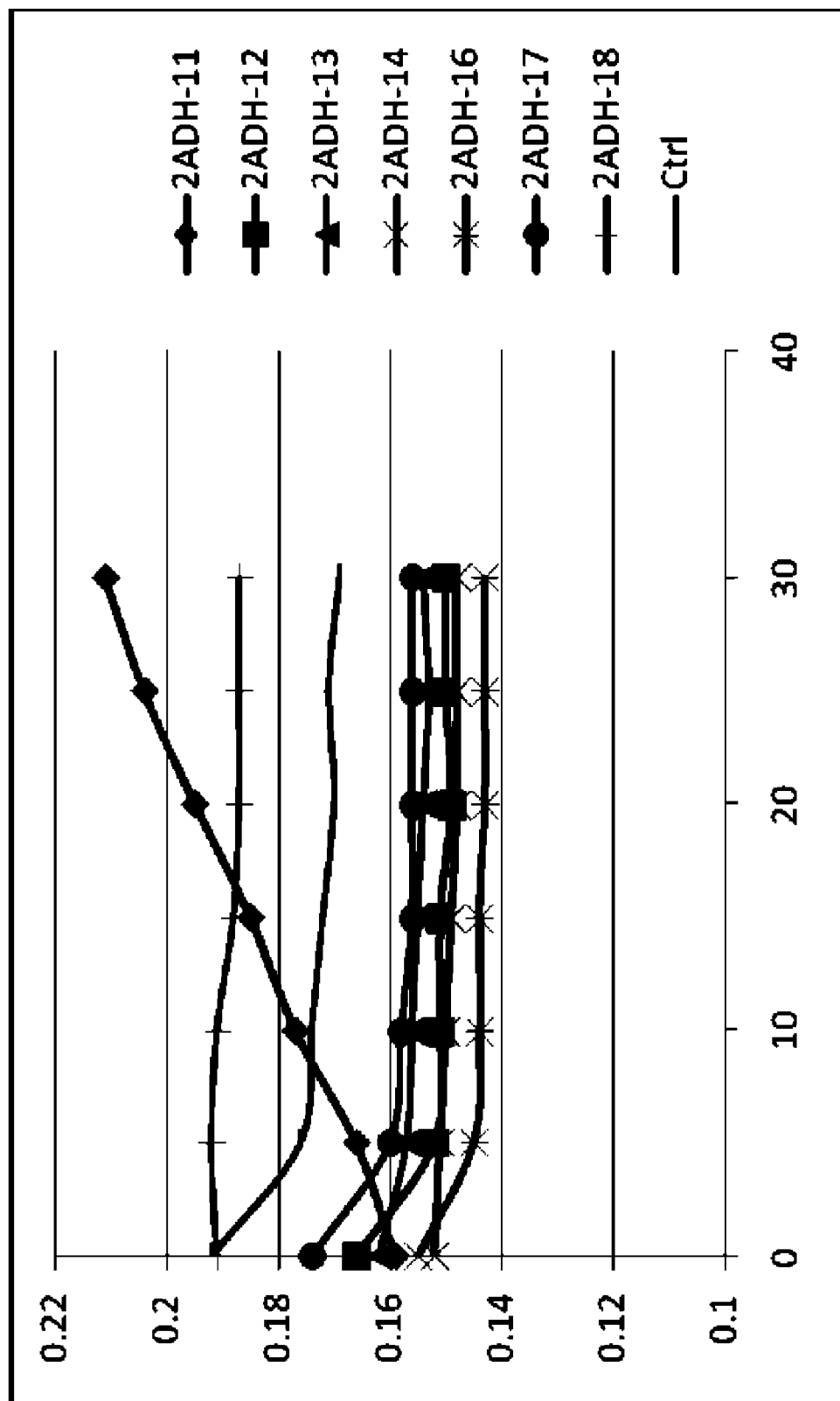


FIG. 32A

Oxidation of 2,7-dimethyl octanol monitored by NADPH production (2ADH 11-18)

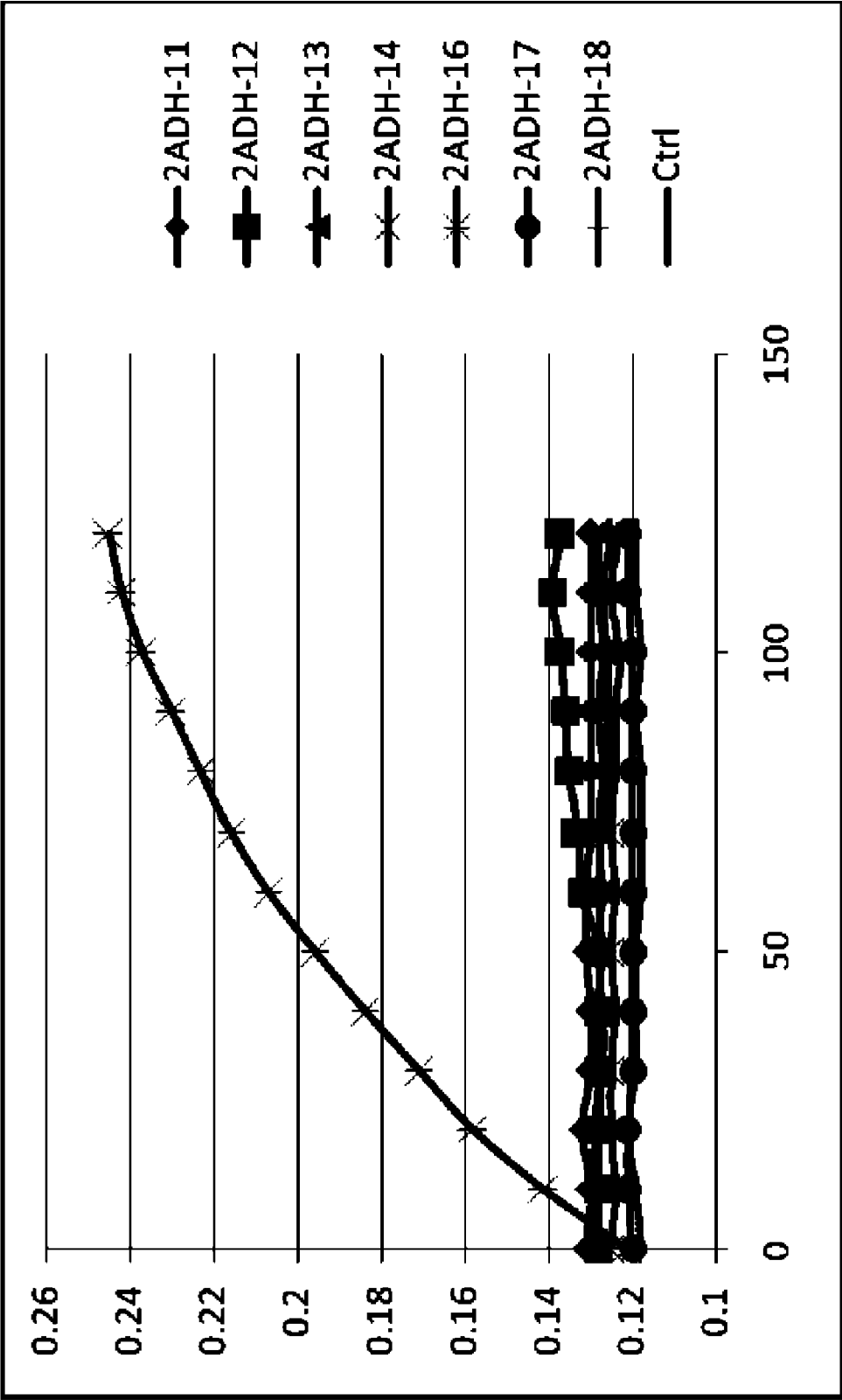


FIG. 32B

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Reduction of 2,7-dimethyl octanol monitored by NADPH consumption

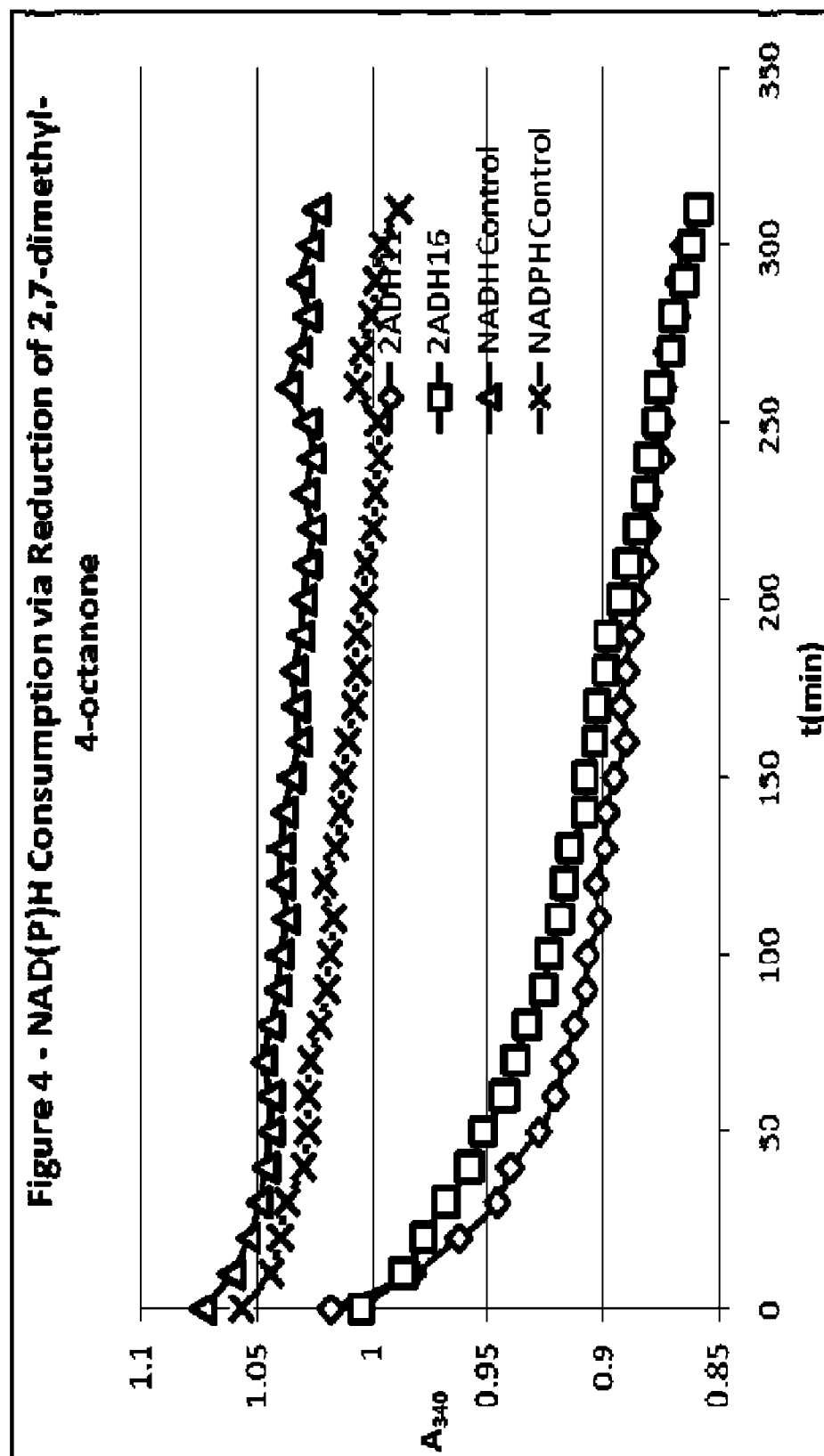


FIG. 33A

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Activity of 2ADH11 and 2ADH16 Towards Various Substrates

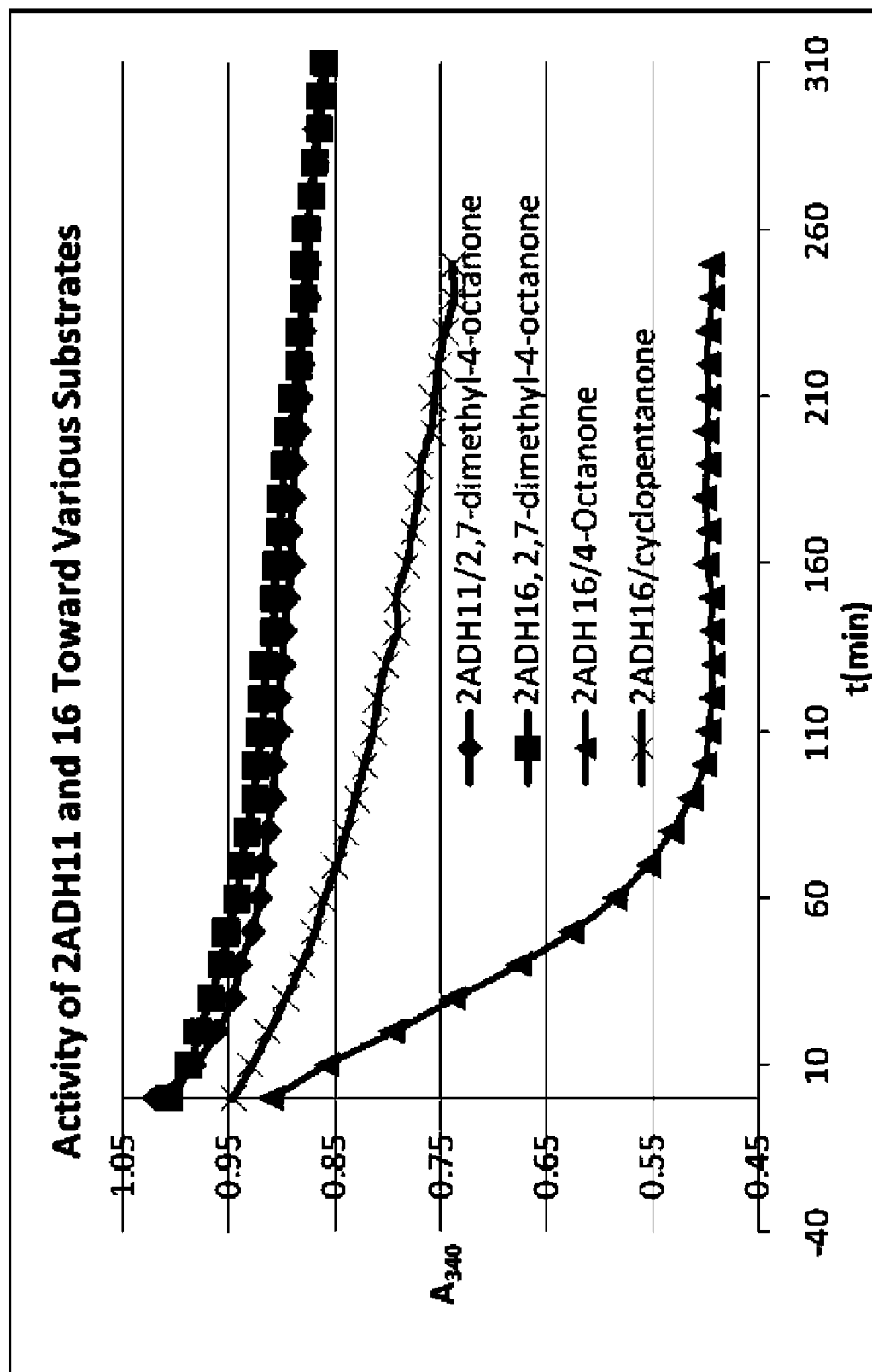


FIG. 33B

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Oxidation of cyclopentanol catalyzed by 2ADH as monitored by NADH or NADPH formation

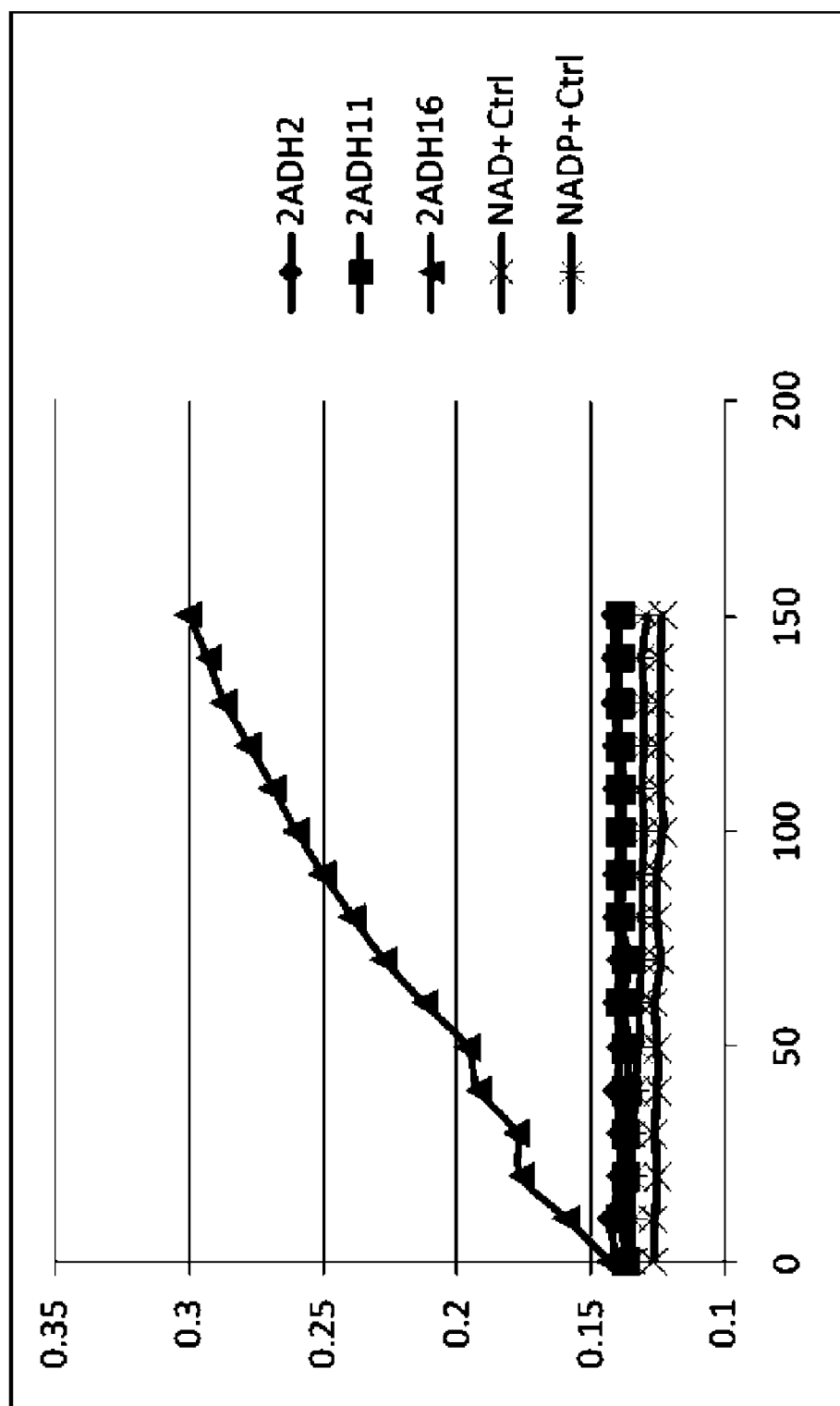


FIG. 34A

Reduction of cyclopentanone catalyzed by 2ADH as monitored by NADPH consumption

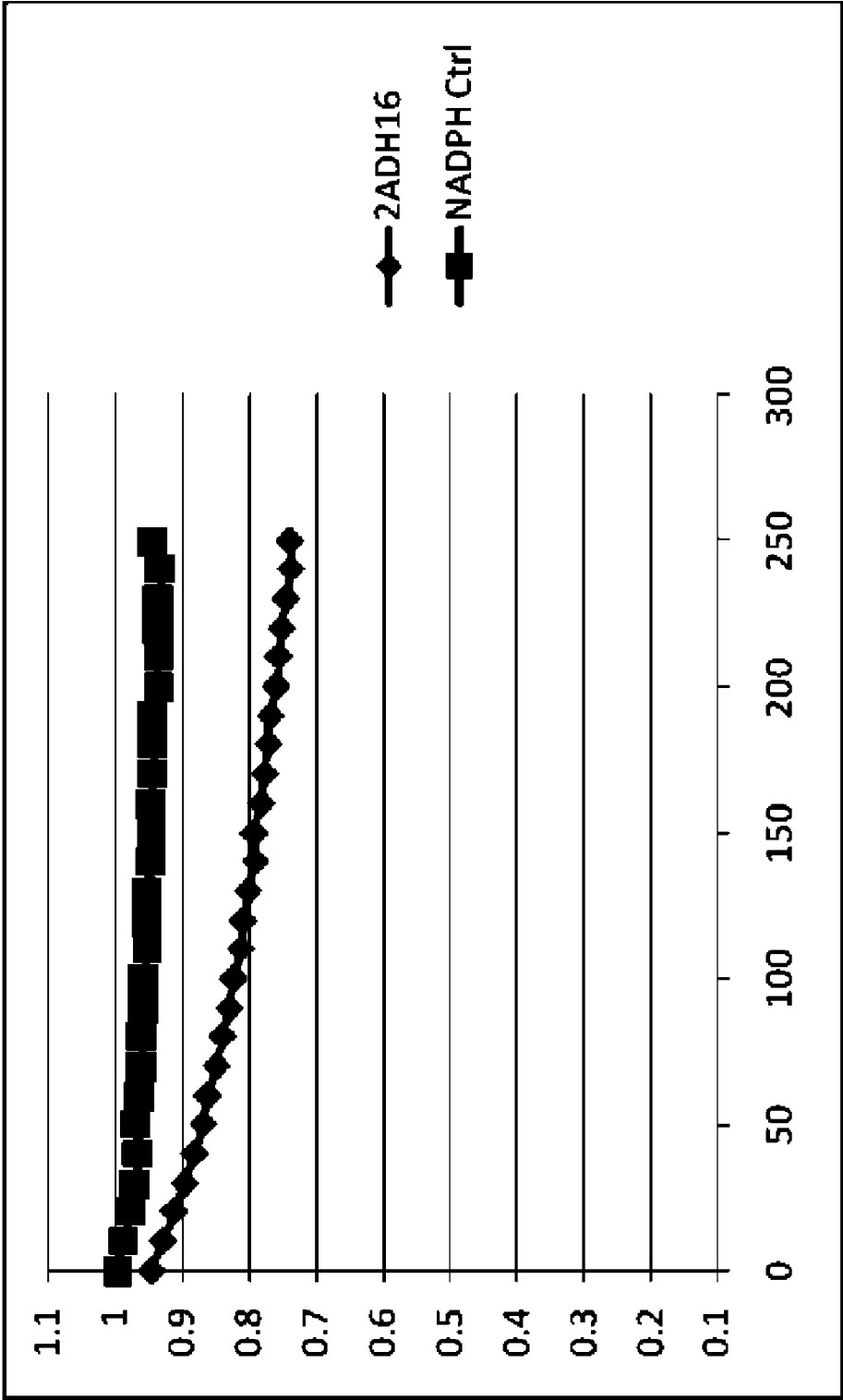


FIG. 34B

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Calculated Rate Constants for Reduction Reactions of 2ADH16

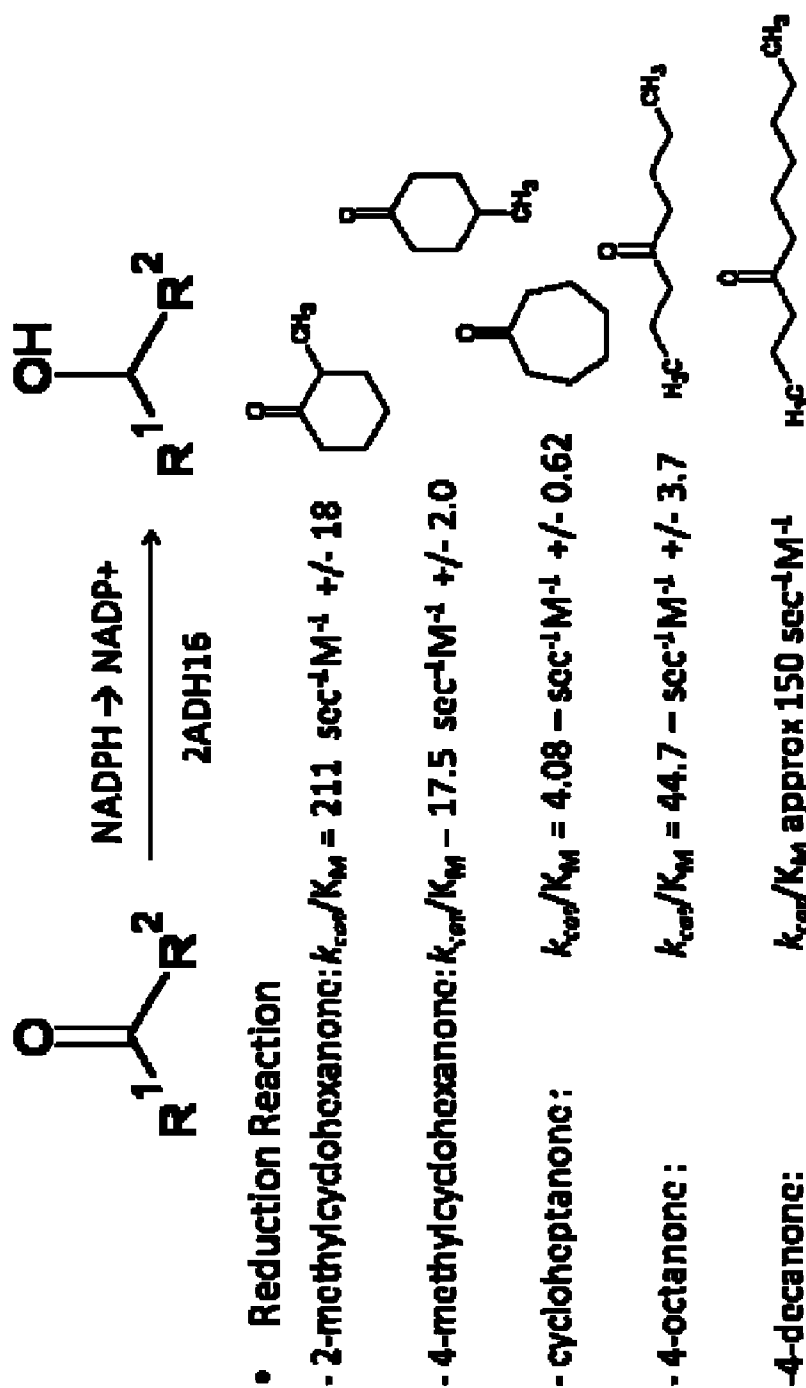
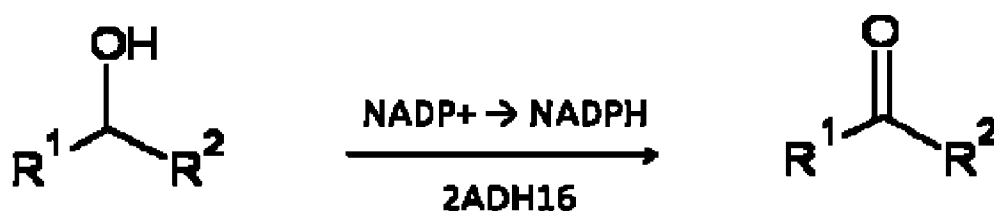


FIG. 35

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Calculated Rate Constants for Oxidation Reactions of 2ADH16



• Oxidation Reaction

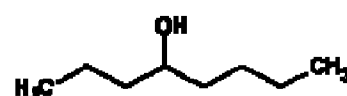
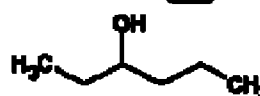
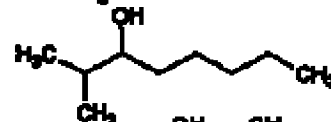
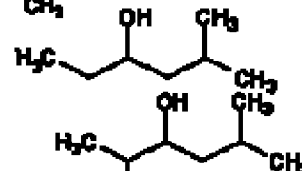
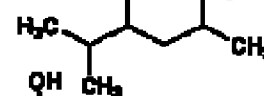
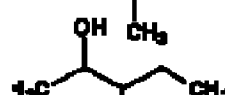
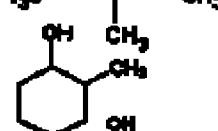
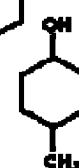
- 4-octanol: $k_{cat}/K_M = 1430 \text{ sec}^{-1}\text{M}^{-1} \pm 200$ - 4-decanol: $k_{cat}/K_M = 1260 \text{ sec}^{-1}\text{M}^{-1} \pm 290$ - cycloheptanol: $k_{cat}/K_M = 198 \text{ sec}^{-1}\text{M}^{-1} \pm 19$ - 3-hexanol: $k_{cat}/K_M = 148 \text{ sec}^{-1}\text{M}^{-1} \pm 16$ - 2-methyl-3-octanol: $k_{cat}/K_M = 123 \text{ sec}^{-1}\text{M}^{-1} \pm 16$ - 5-methyl-3-hexanol: $k_{cat}/K_M = 17.9 \text{ sec}^{-1}\text{M}^{-1} \pm 3.5$ - 2,5-dimethyl-3-hexanol: $k_{cat}/K_M = 1.55 \text{ sec}^{-1}\text{M}^{-1} \pm .24$ - 3-methyl-2-pentanol: $k_{cat}/K_M = 8.54 \text{ sec}^{-1}\text{M}^{-1} \pm 2.04$ - 2-methylcyclohexanol: $k_{cat}/K_M = 97.4 \text{ sec}^{-1}\text{M}^{-1} \pm 14.4$ - 4-methylcyclohexanol: $k_{cat}/K_M = 10.4 \text{ sec}^{-1}\text{M}^{-1} \pm 21.1$ 

FIG. 36

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Alginate Lyases

Protein	Organism	GenBank/ GenPept	
Family 5			
alginate lyase (AlgL)	<u>Azotobacter chroococcum</u> <u>ATCC 4412</u>	<u>AJ223605</u>	<u>CAA11481.1</u>
		<u>AF027499</u>	<u>AAC04567.1</u>
alginate lyase (AlgL)	<u>Azotobacter vinelandii</u>	<u>AF037600</u>	<u>AAC32313.1</u>
alginate lyase (Alg)	<u>Cobetia marina N-1</u>	<u>AB018795</u>	<u>BAA33966.1</u>
alginate lyase (AlgL)	<u>Pseudomonas aeruginosa</u> <u>8830</u>	<u>L14597</u>	<u>AAA71990.1</u>
alginate lyase (AlgL)	<u>Pseudomonas aeruginosa</u> <u>FRD1</u>	<u>U27829</u>	<u>AAA91127.1</u>
	<u>Pseudomonas aeruginosa</u> <u>PAO1</u>	<u>AE004775</u>	<u>AAG06935.1</u>
alginate lyase (AlgL;PA3547)		<u>NC 002516</u>	<u>NP 252237.1</u>
alginate lyase (AlgL)	<u>Pseudomonas sp. QD03</u>	<u>AY380832</u>	<u>AAR23929.1</u>
alginate lyase (AlgL)	<u>Pseudomonas sp. QDA</u>	<u>AY163384</u>	<u>AAN63147.1</u>
alginate lyase (AlgL)	<u>Pseudomonas syringae pv.</u> <u>syringae FF5</u>	<u>AF222020</u>	<u>AAF32371.1</u>
alginate lyase (aly;A1-I/PolyG+PolyM;A1-II/PolyG;A1-III/PolyM)		-	<u>2009330A</u>
	<u>Sphingomonas sp. A1</u>	<u>AB011415</u>	<u>BAB03312.1</u>
Family 6			
alginate lyase (AlyP)	<u>Pseudomonas sp. OS-ALG-9</u>	<u>D10336</u>	<u>BAA01182.1</u>
Family 7			
guluronate lyase (alyPG)	<u>Corynebacterium sp. ALY-1</u>	<u>AB030481</u>	<u>BAA83339.1</u>
poly(-L-guluronate) lyase (AlyA)	<u>Klebsiella pneumoniae subsp.</u> <u>aerogenes</u>	<u>L19657</u>	<u>AAA25049.1</u>
alginate lyase / poly-mannuronate lyase (AlxM)	<u>Photobacterium sp. ATCC</u> <u>43367</u>	<u>X70036</u>	<u>CAA49630.1</u>
	<u>Pseudomonas aeruginosa</u> <u>PAO1</u>	<u>AE004547</u>	<u>AAG04556.1</u>
alginate lyase (PA1167)		<u>NC 002516</u>	<u>NP 249858.1</u>
alginate lyase (A1-II')	<u>Sphingomonas sp. A1</u>	<u>AB120939</u>	<u>BAD16656.1</u>
alginate lyase (aly;A1-I/PolyG+PolyM;A1-II/PolyG;A1-III/PolyM)		-	<u>2009330A</u>
	<u>Sphingomonas sp. A1</u>	<u>AB011415</u>	<u>BAB03312.1</u>

FIG. 37A

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Alginate Lyases

Protein	Organism	GenBank	GenPept
Family 7			
poly(a-L-guluronate) lyase (AlyVGI;AlyVG1)	<u>Vibrio halioticoli IAM14596T</u>	<u>AF114039</u>	<u>AAF22512.1</u>
alginate lyase / poly-mannuronate lyase (AlyVOA)	<u>Vibrio sp. O2</u>	<u>DQ235160</u>	<u>ABB36771.1</u>
alginate lyase / poly-mannuronate lyase (AlyVOB)	<u>Vibrio sp. O2</u>	<u>DQ235161</u>	<u>ABB36772.1</u>
alginate lyase (AlyVI)	<u>Vibrio sp. QY101</u>	<u>AY221030</u>	<u>AAP45155.1</u>
exo-oligoalginate lyase (HdAlex;HdAlex-1)	<u>Haliotis discus hannai</u>	<u>AB234872</u>	<u>BAE81787.1</u>
alginate lyase (HdAly)	<u>Haliotis discus hannai</u>	<u>AB110094</u>	<u>BAC87758.1</u>
polysaccharide lyase acting on glucuronic acid (vAL-1)	<u>Chlorella virus CVK2</u>	<u>AB044791</u>	<u>BAB19127.1</u>
alginate lyase (AlyII)	<u>Pseudomonas sp. OS-ALG-9</u>	<u>AB003330</u>	<u>BAA19848.1</u>
Family18			
alginate lyase	<u>Pseudoalteromonas sp. 272</u>		
alginate lyase (Aly)	<u>Pseudoalteromonas sp. IAM14594</u>	<u>AF082561</u>	<u>AAD16034.1</u>
Family15			
exotype alginate lyase (Atu3025)	<u>Agrobacterium tumefaciens str. C58</u>	<u>AE009232</u>	<u>AAL43841.1</u>
		<u>NC 003305</u>	<u>NP 533525.1</u>
exotype alginate lyase (AGR_L_3558p)	<u>Agrobacterium tumefaciens str. C58 (Cereon)</u>	<u>AE008381</u>	<u>AAK90358.1</u>
		<u>NC 003063</u>	<u>NP 357573.1</u>
oligo alginate lyase (A1-IV)	<u>Sphingomonas sp. A1</u>	<u>AB011415</u>	<u>BAB03319.1</u>
alginate lyase (A1-IV')	<u>Sphingomonas sp. A1</u>	<u>AB176667</u>	<u>BAD90006.1</u>

FIG. 37B

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
Family1			
pectate lyase	<u>Bacillus agaradhaerens</u>	-	<u>AAE59745.</u> <u>1</u>
		-	<u>AAS29292.</u> <u>1</u>
pectate lyase	<u>Bacillus halodurans</u>	-	<u>AAE59748.</u> <u>1</u>
		-	<u>AAS29295.</u> <u>1</u>
pectate lyase (BH3819)	<u>Bacillus halodurans C-125</u>	<u>AP001520</u>	<u>BAB07538.</u> <u>1</u>
		<u>NC 002570</u>	<u>NP 24468</u> <u>6.1</u>
pectate lyase (BH0698)	<u>Bacillus halodurans C-125</u>	<u>AP001509</u>	<u>BAB04417.</u> <u>1</u>
		<u>NC 002570</u>	<u>NP 24156</u> <u>4.1</u>
pectate lyase (PelA)	<u>Bacillus licheniformis</u> <u>14A</u>	-	<u>AAE59746.</u> <u>1</u>
		-	<u>AAN26179</u> <u>.1</u>
		<u>AJ517194</u>	<u>CAD56882</u> <u>.1</u>
		-	<u>AAS29293.</u> <u>1</u>
BLi04129 or BL00947 (PelI)	<u>Bacillus licheniformis</u> <u>DSM 13 ATCC 14580</u>	<u>CP000002</u>	<u>AAU25568</u> <u>.1</u>
		<u>AE017333</u>	<u>AAU42942</u> <u>.1</u>
pectate lyase (Pel)	<u>Bacillus licheniformis</u> <u>RN1</u>	<u>AB428424</u>	<u>BAG12908</u> <u>.1</u>
pectate lyase (PelB)	<u>Bacillus pumilus DKS1</u>	<u>EU652988</u>	<u>ACD11362</u> <u>.1</u>
pectate lyase (fragment)	<u>Bacillus sp. KSM-P7</u>	<u>AB015043</u>	<u>BAA76884.</u> <u>1</u>
pectate lyase	<u>Bacillus sp. AAI12</u>	-	<u>AAE59747.</u> <u>1</u>
		-	<u>AAS29294.</u> <u>1</u>

FIG. 38A

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
pectate lyase	<u>Bacillus sp. I534</u>	-	<u>AAE59749.1</u>
		-	<u>AAR65348.1</u>
		-	<u>AAS29296.1</u>
pectate lyase (Pel-103)	<u>Bacillus sp. KSM-P103</u>	AB015044	<u>BAA76885.1</u>
pectate lyase (Pel-34K)	<u>Bacillus sp. P-2850</u>	AB080666	<u>BAC11008.1</u>
pectate lyase (Pel-4A)	<u>Bacillus sp. P-4-N</u>	AB041769	<u>BAA96477.1</u>
pectate lyase (Pel-4B)	<u>Bacillus sp. P-4-N</u>	AB042100	<u>BAA96478.1</u>
pectate lyase (PI47)	<u>Bacillus sp. TS-47</u>	AB045986	<u>BAB40336.1</u>
pectate lyase (PelK)	<u>Bacillus sp. YA-14</u>	D26349	<u>BAA05383.1</u>
pectate lyase	<u>Bacillus subtilis</u>	AX601431	<u>CAD67509.1</u>
		AX601436	<u>CAD67510.1</u>
		AX601448	<u>CAD67511.1</u>
		AX951870	<u>CAF05441.1</u>
		D86417	<u>BAA22313.1</u>
pectate lyase (Pel)	<u>Bacillus subtilis AC327</u>	D86417	<u>BAA22313.1</u>
pectin lyase (Ppr)	<u>Bacillus subtilis IFO 3134</u>	D83791	<u>BAA12119.1</u>
pectate lyase (Pel)	<u>Bacillus subtilis SO113</u>	X74880	<u>CAA52866.1</u>

FIG. 38B

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
pectate lyase (Pel;BSU07560)	<u>Bacillus subtilis subsp. subtilis str. 168</u>	-	<u>AAR45489</u> .1
		D86417	<u>BAA22313</u> .1
		X74880	<u>CAA52866</u> .1
		Z99108	<u>CAB12585</u> .1
		NC 000964	<u>NP_38863</u> 7.1
pectate lyase (Pel-1;Pel1)	<u>Erwinia carotovora 71</u>	L32171	<u>AAA73933</u> .1
Pel9.5 (fragment)	<u>Erwinia carotovora EC14</u>	X61088	<u>CAA43402</u> .1
pectin lyase (Pnl) (probable fragment)	<u>Erwinia carotovora ER</u>	M65057	<u>AAA24857</u> .1
pectate lyase 1 (Pel1;PelI)	<u>Erwinia carotovora ER / IAM1068 / atroseptica EC / atroseptica C18</u>	M18859	<u>AAA24845</u> .1
		S51490	<u>AAC60423</u> .1
		D00217	<u>BAA00155</u> .1
		X81847	<u>CAA57439</u> .1
pectate lyase 2 (Pel2;PelII)	<u>Erwinia carotovora ER / IAM1068 / atroseptica EC / atroseptica C18</u>	M17364	<u>AAA24848</u> .1
		S51475	<u>AAC60422</u> .1
		X81847	<u>CAA57440</u> .1
ECA4067 (PelA)	<u>Erwinia carotovora subsp. atroseptica SCRI1043</u>	BX950851	<u>CAG76964</u> .1
pectate lyase (PelZ)	<u>Erwinia chrysanthemi 3937</u>	X97119	<u>CAA65785</u> .1
pectate lyase A (PelA)	<u>Erwinia chrysanthemi 3937</u>	M77808	<u>AAA24846</u> .1
pectate lyase B (PelB)	<u>Erwinia chrysanthemi 3937</u>		<u>CAA47821</u> .1
		X67475	<u>S25262</u>

FIG. 38C

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
pectate lyase (PelD)	<u>Erwinia chrysanthemi</u> <u>3937</u>	<u>AJ132101</u>	<u>CAA10570</u> <u>.1</u>
		<u>M33584</u>	<u>AAA24854.</u> <u>1</u>
pectate lyase E (PelE)	<u>Erwinia chrysanthemi</u> <u>3937 / B374</u>	<u>X17284</u>	<u>CAA35175</u> <u>.1</u>
pectate lyase D (PelD)	<u>Erwinia chrysanthemi</u> <u>B374</u>	<u>X17284</u>	<u>CAA35176</u> <u>.1</u>
pectate lyase (PelA)	<u>Erwinia chrysanthemi</u> <u>EC16</u>	<u>M14509</u>	<u>AAA24843.</u> <u>1</u>
		<u>M19411</u>	<u>AAA24849.</u> <u>1</u>
		-	<u>AAR45490</u> <u>.1</u>
pectate lyase (PelC)	<u>Erwinia chrysanthemi</u> <u>EC16</u>	-	<u>AAW11900</u> <u>.1</u>
pectate lyase (PelB; PIB)	<u>Erwinia chrysanthemi</u> <u>EC16</u>	<u>M14510</u>	<u>AAA24847.</u> <u>1</u>
pectate lyase (PelE)	<u>Erwinia chrysanthemi</u> <u>EC16</u>	<u>M14509</u>	<u>AAA24844.</u> <u>1</u>
pectate lyase C (PelC)	<u>Erwinia chrysanthemi</u> <u>strain 3937</u>	<u>AJ132325</u>	<u>CAA10642</u> <u>.1</u>
pectin lyase (PnlA)	<u>Pectobacterium</u> <u>carotovorum Ecc71</u>	<u>M59909</u>	<u>AAA24856.</u> <u>1</u>
pectate lyase III (Pel3; PelC)	<u>Pectobacterium</u> <u>carotovorum Er</u>	<u>D10064</u>	<u>BAA00953.</u> <u>1</u>
		<u>AF278705</u>	<u>AAF86343.</u> <u>1</u>
pectate lyase B (PelB)	<u>Pseudoalteromonas</u> <u>haloplanktis 505</u>	<u>AF278705</u>	<u>AAF86343.</u> <u>2</u>
pectate lyase A	<u>Pseudoalteromonas</u> <u>haloplanktis ANT/505</u>	<u>AF278706</u>	<u>AAF86344.</u> <u>2</u>

FIG. 38D

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
pectate lyase (Pel)	<u>Pseudomonas fluorescens CY091</u>	<u>L41673</u>	<u>AAA93535.1</u>
		<u>L38902</u>	<u>AAB46399.1</u>
pectin lyase (PnL) (fragment)	<u>Pseudomonas marginalis N6301</u>	<u>M84971</u>	<u>AAA92512.1</u>
		<u>D32121</u>	<u>BAA06847.1</u>
pectate lyase (Pel)	<u>Pseudomonas marginalis N6301</u>	<u>S65042</u>	<u>AAC60448.1</u>
		<u>D32122</u>	<u>BAA06848.1</u>
pectate lyase P (PelP)	<u>Pseudomonas syringae pv. lachrymans</u>	<u>U75414</u>	<u>AAB17879.1</u>
pectate lyase (Pel;Pstru-4)	<u>Pseudomonas viridiflava</u>	<u>L38901</u>	<u>AAB46398.1</u>
		<u>L38574</u>	<u>AAC41521.1</u>
		<u>DQ273695</u>	<u>ABB55454.1</u>
		<u>D44611</u>	<u>BAA08077.1</u>
pectate lyase (Pel)	<u>Pseudonocardia sp.</u>	<u>AF002241</u>	<u>AAC38059.1</u>
pectate lyase (SCO2821;SCBAC17F8.12c)	<u>Streptomyces coelicolor A3(2)</u>	<u>AL596030</u>	<u>CAC44284.1</u>
		<u>NC 003888</u>	<u>NP 62705.0.1</u>
pectate lyase (SCO1880;SCI39.27c)	<u>Streptomyces coelicolor A3(2)</u>	<u>AL591322</u>	<u>CAC38815.1</u>
		<u>NC 003888</u>	<u>NP 62614.7.1</u>
pectate lyase A (PelA;TM04358)	<u>Thermotoga maritima</u>	<u>AE001722</u>	<u>AAD35518.1</u>
		<u>NC 000853</u>	<u>NP 22824.3.1</u>
XC_1298	<u>Xanthomonas campestris pv. campestris str. 8004</u>	<u>CP000050</u>	<u>AAY48367.1</u>

FIG. 38E

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
XC_3590	<u>Xanthomonas campestris pv. campestris str. 8004</u>	CP000050	<u>AAV50632.1</u>
pectate lyase (Pel;XCC0645)	<u>Xanthomonas campestris pv. campestris str. ATCC 33913</u>	AE012162 NC_003902	<u>AAM39961.1</u> <u>NP_63603.1</u>
pectate lyase II (PelB;XCC2815)	<u>Xanthomonas campestris pv. campestris str. ATCC 33913</u>	AE012393 NC_003902	<u>AAM42087.1</u> <u>NP_63816.1</u>
pectate lyase (PelB;PI;Pstru-3)	<u>Xanthomonas campestris pv. malvacearum strain B414</u>	L38573	<u>AAC41522.1</u>
pectin lyase (AN2331.2)	<u>Aspergillus nidulans FGSC A4</u>	DQ490478 AACD01000038	<u>ABF50854.1</u> <u>EAA64442.1</u>
pectin lyase (AN2569.2)	<u>Aspergillus nidulans FGSC A4</u>	AACD01000043 DQ490480	<u>EAA64674.1</u> <u>ABF50856.1</u>
pectate lyase (PelA;AN0741.2)	<u>Aspergillus nidulans FGSC A4</u>	U05592 DQ490468 EF452421 AACD01000012	<u>AAA80568.1</u> <u>ABF50844.1</u> <u>ABO38859.1</u> <u>EAA65383.1</u>
pectate lyase (AN7646.2)	<u>Aspergillus nidulans FGSC A4</u>	AACD01000130 DQ490513	<u>EAA61832.1</u> <u>ABF50889.1</u>
pectin lyase A (PelA) - PI1A	<u>Aspergillus niger CBS 120.49 / N400</u>	X55784 X60724	<u>CAA39305.1</u> <u>CAA43130.1</u>
pectin lyase C (PelC)	<u>Aspergillus niger CBS 120.49 / N400</u>	AY839647	<u>AAW03313.1</u>

FIG. 38F

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
pectin lyase F (PelF)	<u>Aspergillus niger CBS</u> <u>120.49 / N400</u>	<u>AJ489943</u>	<u>CAD34589</u> <u>.1</u>
pectate lyase A (PlyA)	<u>Aspergillus niger CBS</u> <u>120.49 / N400</u>	<u>AJ276331</u>	<u>CAC33162</u> <u>.1</u>
pectin lyase B (PelB)	<u>Aspergillus niger CBS</u> <u>120.49 / N400</u>	<u>A12248</u>	<u>CAA01023</u> <u>.1</u>
		<u>X65552</u>	<u>CAA46521</u> <u>.1</u>
An14g04370 (PelA)	<u>Aspergillus niger CBS</u> <u>513.88</u>	<u>AM270321</u>	<u>CAK48529</u> <u>.1</u>
An03g00190 (PelB)	<u>Aspergillus niger CBS</u> <u>513.88</u>	<u>AM270043</u>	<u>CAK37997</u> <u>.1</u>
An15g07160 (PelF)	<u>Aspergillus niger CBS</u> <u>513.88</u>	<u>AM270351</u>	<u>CAK48551</u> <u>.1</u>
An19g00270 (PelD)	<u>Aspergillus niger CBS</u> <u>513.88</u>	<u>AM270415</u>	<u>CAK47350</u> <u>.1</u>
pectate lyase I (PlyA; An10g00870)	<u>Aspergillus niger CBS</u> <u>513.88</u>	<u>AM270216</u>	<u>CAK40523</u> <u>.1</u>
pectin lyase D (PelD)	<u>Aspergillus niger N756</u>	<u>M55657</u>	<u>AAA32701</u> <u>.1</u>
pectin lyase 2 (Pel2)	<u>Aspergillus oryzae</u> <u>KBN616</u>	<u>AB029323</u>	<u>BAB82468</u> <u>.1</u>
pectin lyase 1 (Pel1)	<u>Aspergillus oryzae</u> <u>KBN616</u>	<u>AB029322</u>	<u>BAB82467</u> <u>.1</u>
pectin lyase 1 (Pel1; AO090010000504)	<u>Aspergillus oryzae RIB</u> <u>40</u>	<u>EF452419</u>	<u>ABO38857</u> <u>.1</u>
		<u>AP007175</u>	<u>BAE66352</u> <u>.1</u>
pectin lyase 2 (Pel2; AO090010000030)	<u>Aspergillus oryzae RIB</u> <u>40</u>	<u>AP007175</u>	<u>BAE65949</u> <u>.1</u>
pectate lyase (PelB)	<u>Colletotrichum</u> <u>gloeosporioides</u>	<u>AF052632</u>	<u>AAD09857</u> <u>.1</u>
pectin lyase (PnlA)	<u>Colletotrichum</u> <u>gloeosporioides</u>	<u>L22857</u>	<u>AAA21817</u> <u>.1</u>
pectate lyase 2 (Pel-2)	<u>Colletotrichum</u> <u>gloeosporioides f. sp.</u> <u>malvae</u>	<u>AF156985</u>	<u>AAD43566</u> <u>.1</u>

FIG. 38G

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Pectate Lyases

Protein	Organism	GenBank/GenPept	
pectin lyase (Pnl1;Pnl-1)	<u>Colletotrichum gloeosporioides f. sp. malvae</u>	<u>AF158256</u>	<u>AAF22244.1</u>
pectin lyase 2 (Pnl2;Pnl-2)	<u>Colletotrichum gloeosporioides f. sp. malvae</u>	<u>AF156984</u>	<u>AAD43565.1</u>
pectate lyase 1 (Pel-1)	<u>Colletotrichum gloeosporioides f. sp. malvae</u>	<u>AF156983</u>	<u>AAD43564.1</u>
pectate lyase (LLP-52)	<u>Lilium longiflorum</u>	<u>L18911</u>	<u>AAA33398.1</u>
		<u>EF026017</u>	<u>ABM68553.1</u>
		<u>Z17328</u>	<u>CAA78976.1</u>
pectate lyase (Pel;PI1;MwPI1;Ban17)	<u>Musa acuminata Williams</u>	<u>AF206319</u>	<u>AAF19195.1</u>
		<u>DQ663594</u>	<u>ABG74583.1</u>
		<u>X92943</u>	<u>CAA63496.1</u>
pectate lyase	<u>Nicotiana tabacum</u>	<u>X61102</u>	<u>CAA43414.1</u>
		<u>X67158</u>	<u>CAA47630.1</u>
		<u>X67159</u>	<u>CAA47631.1</u>
pectate lyase	<u>Zinnia elegans</u>	<u>Y09541</u>	<u>CAA70735.1</u>
		<u>AX005936</u>	<u>CAC05181.1</u>

FIG. 38H

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Rhamnogalacturonases

Protein	Organism	GenBank/GenPept	
rhamnogalacturonate lyase (RhiE)	<u>Erwinia chrysanthemi</u> 3937	<u>AJ438339</u>	<u>CAD27359.1</u>
rhamnogalacturonan lyase (RhgB)	<u>Aspergillus aculeatus</u> KSM 510	<u>L35500</u>	<u>AAA64368.1</u>
rhamnogalacturonan lyase (AN6395.2)	<u>Aspergillus nidulans</u> FGSC A4	<u>AACD01000108</u>	<u>EAA58417.1</u>
		<u>DQ490501</u>	<u>ABF50877.1</u>
rhamnogalacturonan lyase (AN7135.2)	<u>Aspergillus nidulans</u> FGSC A4	<u>AACD01000122</u>	<u>EAA61387.1</u>
		<u>DQ490504</u>	<u>ABF50880.1</u>
rhamnogalacturonan lyase (YesW;BSU07050)	<u>Bacillus subtilis</u> subsp. <u>subtilis</u> str. 168	<u>Z99107</u>	<u>CAB12524.1</u>
		<u>NC 000964</u>	<u>NP 388586.1</u>
exo-unsaturated rhamnogalacturonan lyase (YesX;BSU07060)	<u>Bacillus subtilis</u> subsp. <u>subtilis</u> str. 168	<u>Z99107</u>	<u>CAB12525.1</u>
		<u>NC 000964</u>	<u>NP 388587.1</u>
rhamnogalacturonan lyase - Rgl11A	<u>Cellvibrio japonicus</u> (formerly <u>Pseudomonas cellulosa</u>)	<u>AY026755</u>	<u>AAK20911.1</u>
CJA_3559 (rhamnogalacturonan lyase) - Rgl11A	<u>Cellvibrio japonicus</u> Ueda107	<u>CP000934.1</u>	<u>ACE83155.1</u>
rhamnogalacturonan lyase Y - Rgl11Y	<u>Clostridium cellulolyticum</u> ATCC 35319	<u>AF316823</u>	<u>AAG45161.1</u>

FIG. 39A

Rhamnogalacturonate Hydrolases

Protein	Organism	GenBank/GenPept	
<i>GH family 105</i>			
unsaturated rhamnogalacturonyl hydrolase (BSU30120; YteR)	<u>Bacillus subtilis subsp. subtilis str. 168</u>	<u>Z99119</u>	<u>CAB14990.1</u>
unsaturated rhamnogalacturonyl hydrolase (BSU07000; YesR)	<u>Bacillus subtilis subsp. subtilis str. 168</u>	<u>Z99107</u>	<u>CAB12519.1</u>
		NC_000964	NP_388581.1

FIG. 39B

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Pectate Methyl Esterases

Protein	Organism	GenBank/GenPept	
Family 8			
ECA3253 (PemA)	<u>Erwinia carotovora</u> subsp. <u>atroseptica</u> <u>SCRI1043</u>	<u>BX950851</u>	<u>CAG76151.1</u>
ECA0107 (PmeB)	<u>Erwinia carotovora</u> subsp. <u>atroseptica</u> <u>SCRI1043</u>	<u>BX950851</u>	<u>CAG73027.1</u>
pectin methylesterase b	<u>Erwinia</u> <u>chrysanthemi</u> 3937	<u>X84665</u>	<u>CAA59151.1</u>
pectin methylesterase A (PemA;Pem)	<u>Erwinia</u> <u>chrysanthemi</u> 3937 / B374	<u>L07644</u>	<u>AAA24852.1</u>
		-	<u>AAR64146.1</u>
		<u>Y00549</u>	<u>CAA68628.1</u>
pectate lyase A	<u>Pseudoalteromonas</u> <u>haloplanktis</u> <u>ANT/505</u>	<u>AF278706</u>	<u>AAF86344.2</u>
pectin methylesterase (Pme5; Vgd1;At2g47040/F14M4 .13)	<u>Arabidopsis</u> <u>thaliana</u>	<u>AC004411</u>	<u>AAC34240.1</u>
		<u>AY091768</u>	<u>AAM10316.1</u>
		<u>BT001120</u>	<u>AAN64511.1</u>
		<u>AY830948</u>	<u>AAV91508.1</u>
		<u>AJ250430</u>	<u>CAB58974.1</u>
		<u>NM_130272</u>	<u>NP_182227.1</u>
pectin methylesterase (Pme1)	<u>Aspergillus</u> <u>aculeatus</u>	<u>U49378</u>	<u>AAB42153.1</u>
pectin methyl esterase (AN3390.2)	<u>Aspergillus</u> <u>nidulans</u> FGSC A4	<u>DQ490489</u>	<u>ABF50865.1</u>
		<u>AACD01000</u> 055	<u>EAA63358.1</u>
pectin methylesterase (Pme1)	<u>Aspergillus niger</u> <u>RH 5344</u>	<u>A34997</u>	<u>CAA02198.1</u>
		<u>A35006</u>	<u>CAA02201.1</u>
		<u>A35008</u>	<u>CAA02202.1</u>
		<u>X52902</u>	<u>CAA37084.1</u>
		<u>X54145</u>	<u>CAA38084.1</u>
pectin methylesterase (PmeA)	<u>Aspergillus oryzae</u> <u>KBN616</u>	<u>AB011211</u>	<u>BAA75474.1</u>
pectin methylesterase (PmeA;AO09001200074 9)	<u>Aspergillus oryzae</u> <u>RIB 40</u>	<u>AP007161</u>	<u>BAE60873.1</u>
pectin methylesterase (Bcpme2)	<u>Botryotinia</u> <u>fuckeliana</u> Bd90	<u>AJ428403</u>	<u>CAD21438.1</u>

FIG. 40A

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Pectate Methyl Esterases

Protein	Organism	GenBank/GenPept	
pectin methyl esterase (Bcpme1)	<u>Botryotinia fuckeliana T4</u>	<u>AJ309701</u>	<u>CAC29255.1</u>
pectin methylesterase 1.1 (PECS-1.1)	<u>Citrus sinensis</u>	<u>U82973</u>	<u>AAB57667.1</u>
		<u>U82976</u>	<u>AAB57670.1</u>
Protein	Organism	GenBank	GenPept
pectin methylesterase (PME1)	<u>Cochliobolus carbonum</u>	<u>AF159252</u>	<u>AAD43340.1</u>
pectin methylesterase	<u>Daucus carota</u>		
pectinesterase FaPE1	<u>Fragaria x ananassa</u>	<u>AY324809</u>	<u>AAQ21124.1</u>
pectin methyl-esterase (Pef1)	<u>Medicago truncatula</u>	<u>AJ249611</u>	<u>CAB65291.1</u>
pectin methyl-esterase (Per)	<u>Medicago truncatula</u>	<u>AJ249611</u>	<u>CAB65290.2</u>
pectin methylesterase	<u>Nicotiana benthamiana</u>	<u>AY238968</u>	<u>AAO85706.1</u>
pectin methylesterase	<u>Nicotiana plumbaginifolia</u>	<u>Z71752</u>	<u>CAA96434.1</u>
pectin methylesterase (NtPPME1)	<u>Nicotiana tabacum</u>	<u>AY772945</u>	<u>AAX13972.1</u>
pectin methylesterase	<u>Nicotiana tabacum</u>	<u>AJ401158</u>	<u>CAB95025.1</u>
pectin methylesterase (PME1) (fragment)	<u>Orobancha cumana</u>	<u>AY072720</u>	<u>AAL66865.1</u>
pectin methylesterase (fragment)	<u>Orobancha cumana</u>	<u>AF333068</u>	<u>AAG49395.1</u>
pectin methylesterase	<u>Petunia inflata</u>	<u>L27101</u>	<u>AAA33714.1</u>
pectin methyl esterase (PttPME1)	<u>Populus tremula x Populus tremuloides</u>	<u>AJ277547</u>	<u>CAC01624.1</u>
pectin methylesterase PME1 (fragment)	<u>Prunus armeniaca</u>	<u>AF184079</u>	<u>AAG12248.1</u>
pectin methylesterase (SgPME1)	<u>Salix gilgiana</u>	<u>AB029461</u>	<u>BAA89480.1</u>
pectin methylesterase	<u>Sitophilus oryzae</u>	<u>AY841894</u>	<u>AAW28928.1</u>

FIG. 40B

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Pectate Methyl Esterases

Protein	Organism	GenBank/GenPept	
pectin methylesterase 2	<u>Solanum lycopersicum</u>	<u>U50985</u>	<u>AAB67739.1</u>
		-	<u>AAQ71552.1</u>
		<u>A15983</u>	<u>CAA01257.1</u>
		<u>A17011</u>	<u>CAA01315.1</u>
		<u>X07910</u>	<u>CAA30746.1</u>
		<u>X74639</u>	<u>CAA52704.1</u>
pectin methylesterase (PmeU1)	<u>Solanum lycopersicum</u>	<u>U49330</u>	<u>AAD09283.1</u>
		<u>AY046596</u>	<u>AAL02367.1</u>
pectin methylesterase 1 (PME1.9)	<u>Solanum lycopersicum</u>	<u>U50986</u>	<u>AAB67740.1</u>
		<u>A17010</u>	<u>CAA01314.1</u>
		<u>X74638</u>	<u>CAA52703.1</u>
pectin methyl esterase (Pest1)	<u>Solanum tuberosum</u>	<u>AF152171</u>	<u>AAF23891.1</u>
pectin methyl esterase (Pest2)	<u>Solanum tuberosum</u>	<u>AF152172</u>	<u>AAF23892.1</u>
pectin methylesterase isoform alpha (PME2) (fragment)	<u>Vigna radiata</u>	<u>AF229849</u>	<u>AAF35897.1</u>
pectin methylesterase (PME)	<u>Vitis riparia</u>	<u>AF178989</u>	<u>AAD51853.1</u>

FIG. 40C

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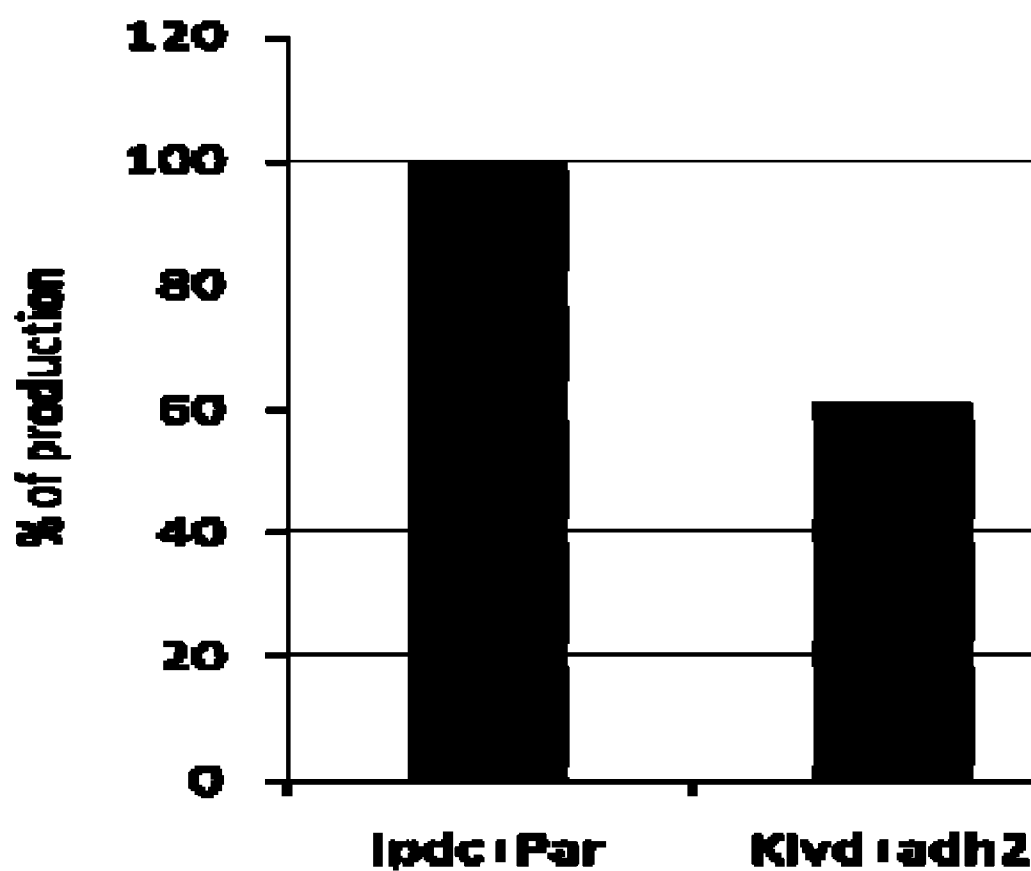
Pectate Acetyl Esterases

Protein	Organism	GenBank/GenPept	
Family12			
acetyl xylan esterase (Rgae;BH1115)	<u>Bacillus halodurans C-125</u>	<u>AP001511</u>	<u>BAB04834.1</u>
		<u>NC_002570</u>	<u>NP_241981.1</u>
cephalosporin C deacetylase	<u>Bacillus sp. KCCM10143</u>	<u>AF184175</u>	<u>AAF25818.1</u>
acetyl xylan esterase (YesT;BSU07020)	<u>Bacillus subtilis subsp. subtilis str. 168</u>	<u>Z99107</u>	<u>CAB12521.1</u>
		<u>NC_000964</u>	<u>NP_388583.1</u>
ECA3252 (PaeY)	<u>Erwinia carotovora subsp. atroseptica SCRI1043</u>	<u>BX950851</u>	<u>CAG76150.1</u>
pectin acetylerase (PaeY)	<u>Erwinia chrysanthemi 3937</u>	<u>Y09828</u>	<u>CAA70971.1</u>
rhamnogalacturonan acetylerase (Rha1)	<u>Aspergillus aculeatus KSM 510</u>	<u>X89714</u>	<u>CAA61858.1</u>
rhamnogalacturonan acetylerase (AN2528.2)	<u>Aspergillus nidulans FGSC A4</u>	<u>DQ490479</u>	<u>ABF50855.1</u>
		<u>AACD01000043</u>	<u>EAA64633.1</u>
pectin acetylerase	<u>Vigna radiata Wilzeck</u>	<u>X99348</u>	<u>CAA67728.1</u>

FIG. 41

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Production of 2-phenyl ethanol (24 hrs)

*FIG. 42A*

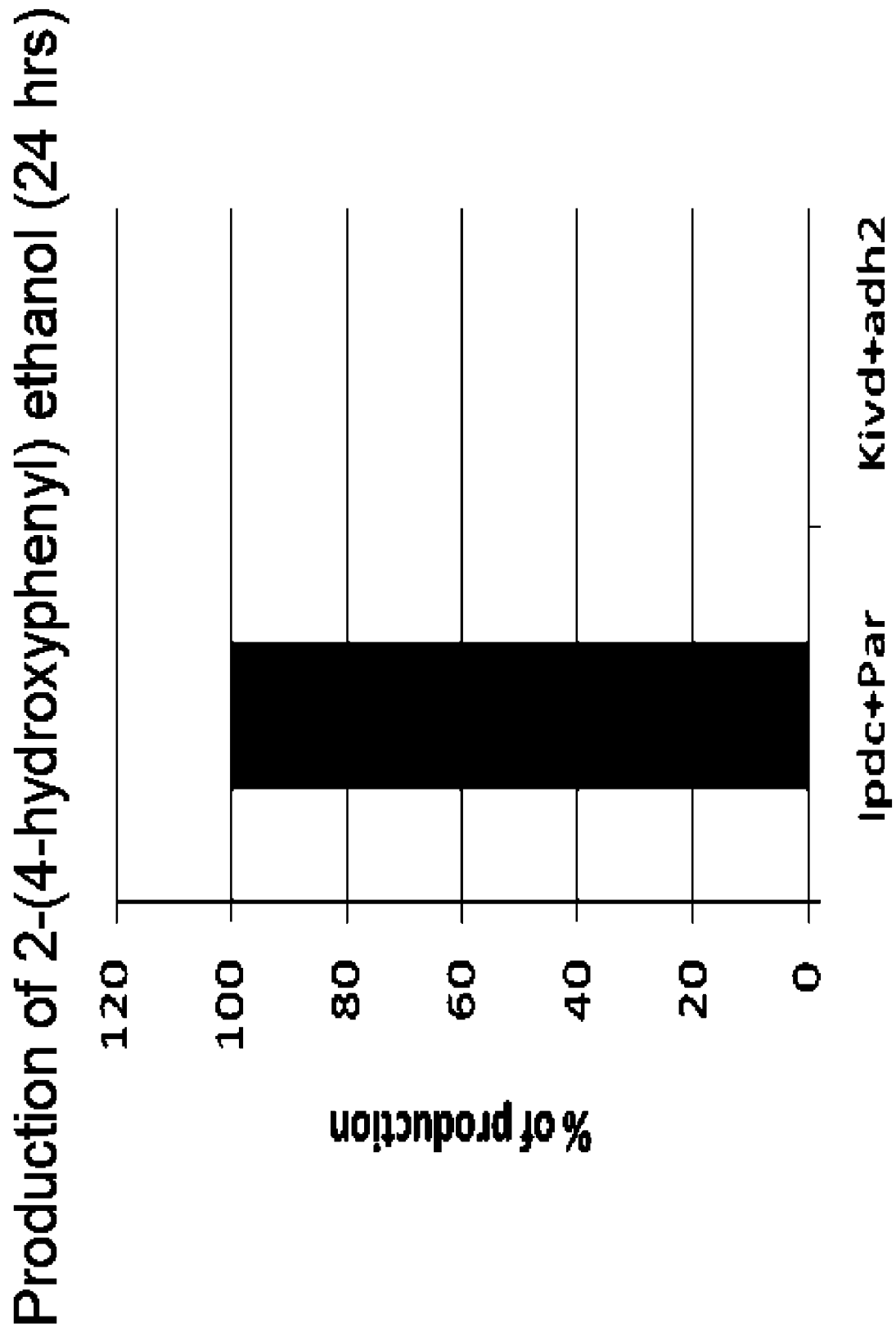


FIG. 42B

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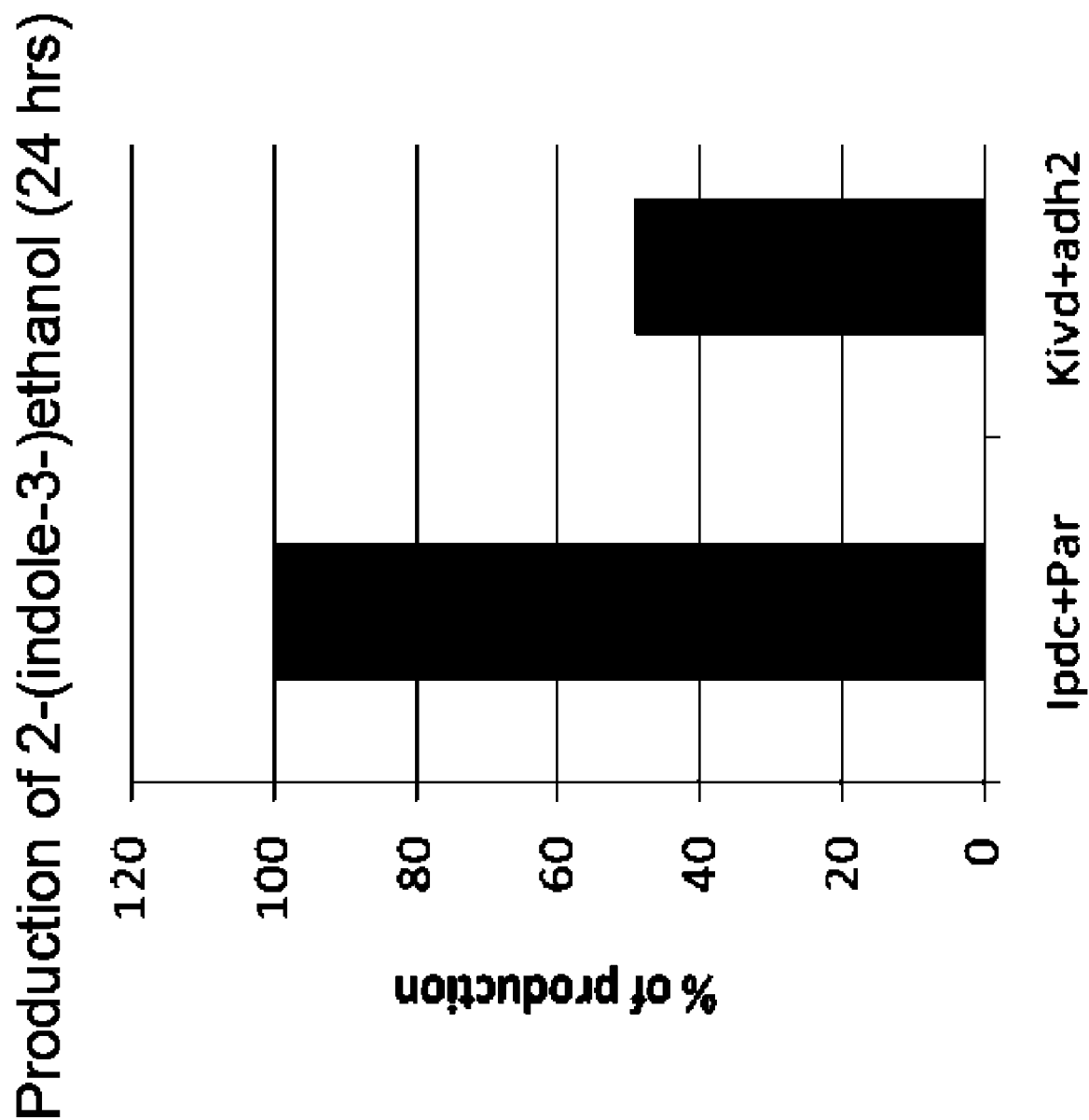


FIG. 42C

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GC-MS chromatogram of control

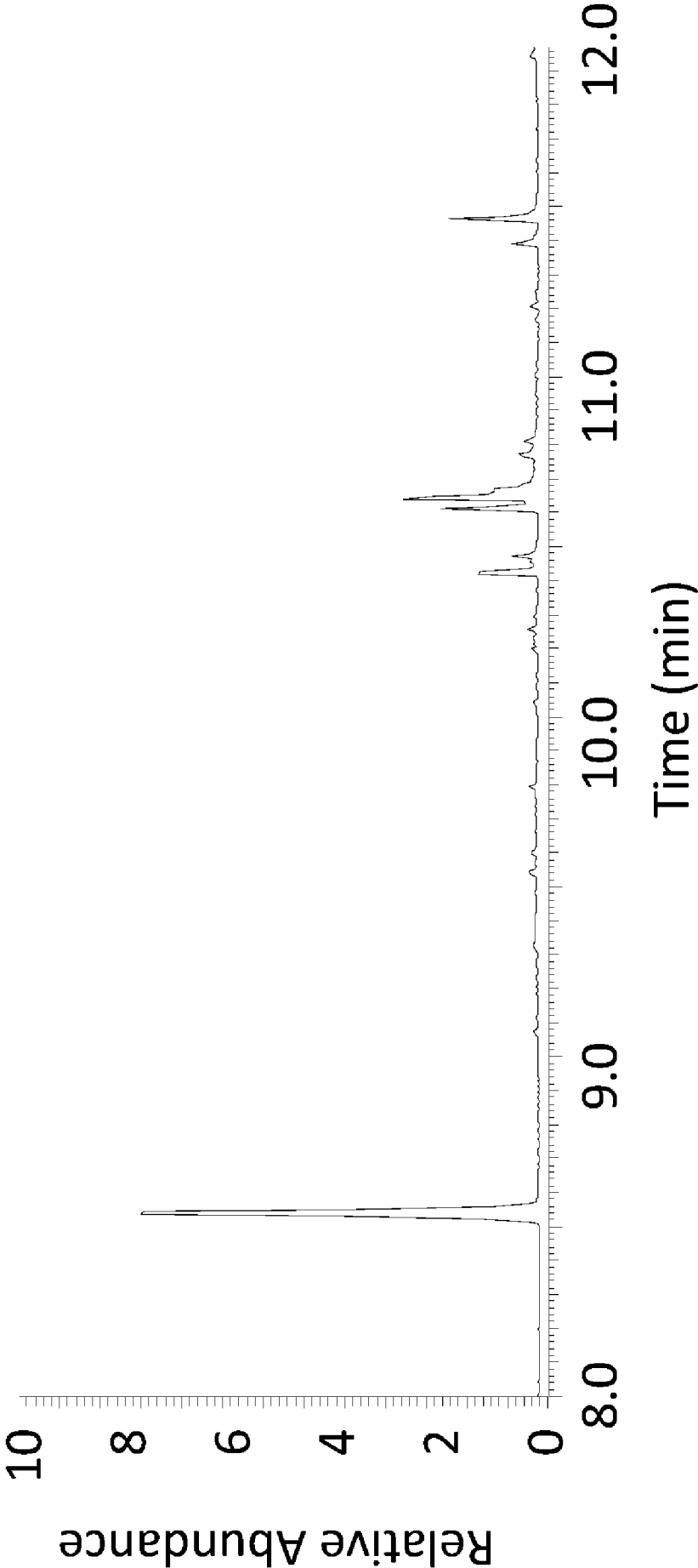
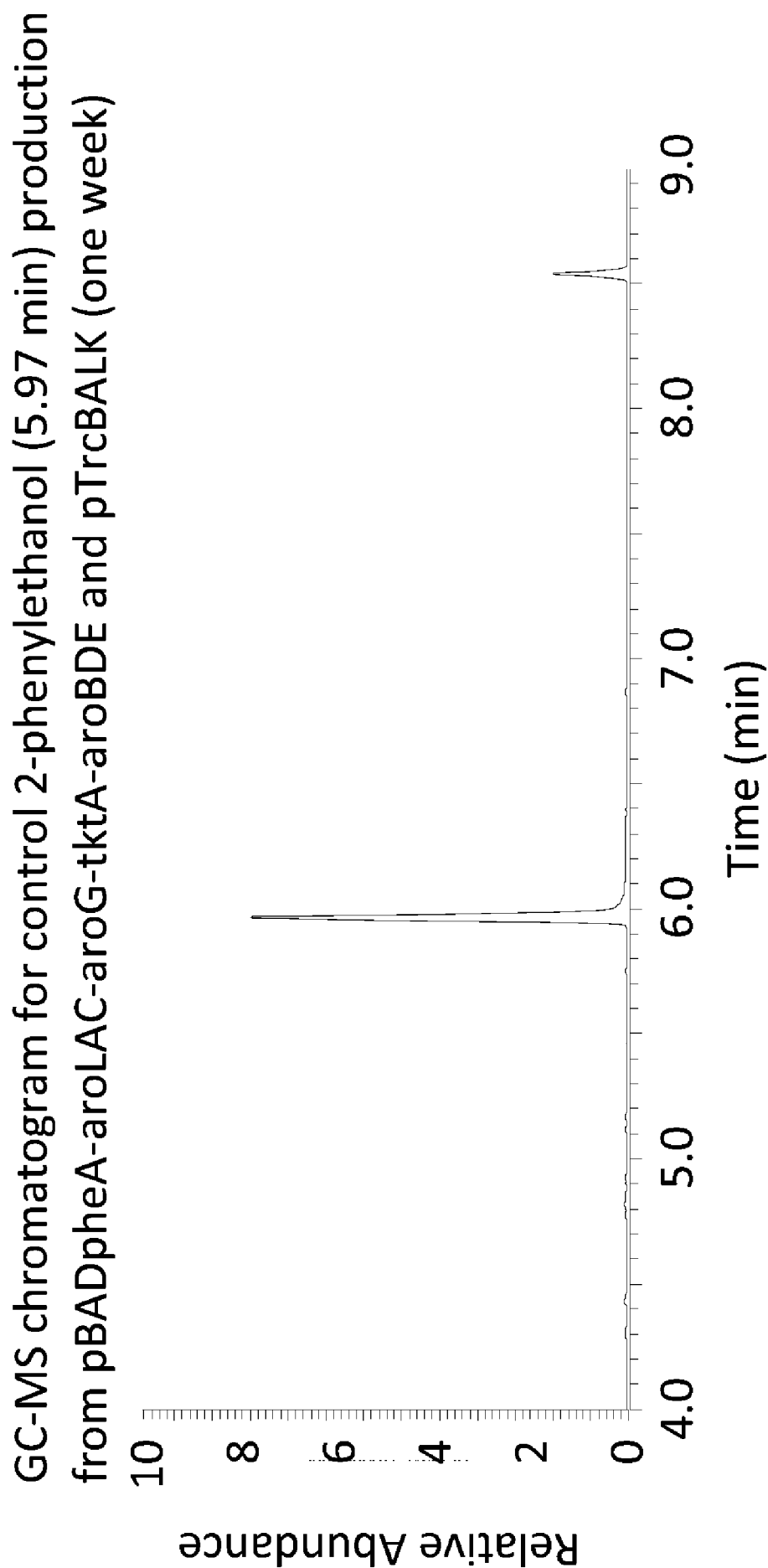


FIG. 43A

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*FIG. 43B*

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GC-MS chromatogram for 2-(4-hydroxyphenyl) ethanol (9.36 min) and 2-(indole-3)
ethanol (10.32 min) production from pBADtyrA-aroLAC-aroG-kttA-aroBDE and
pTrcBALK (one week)

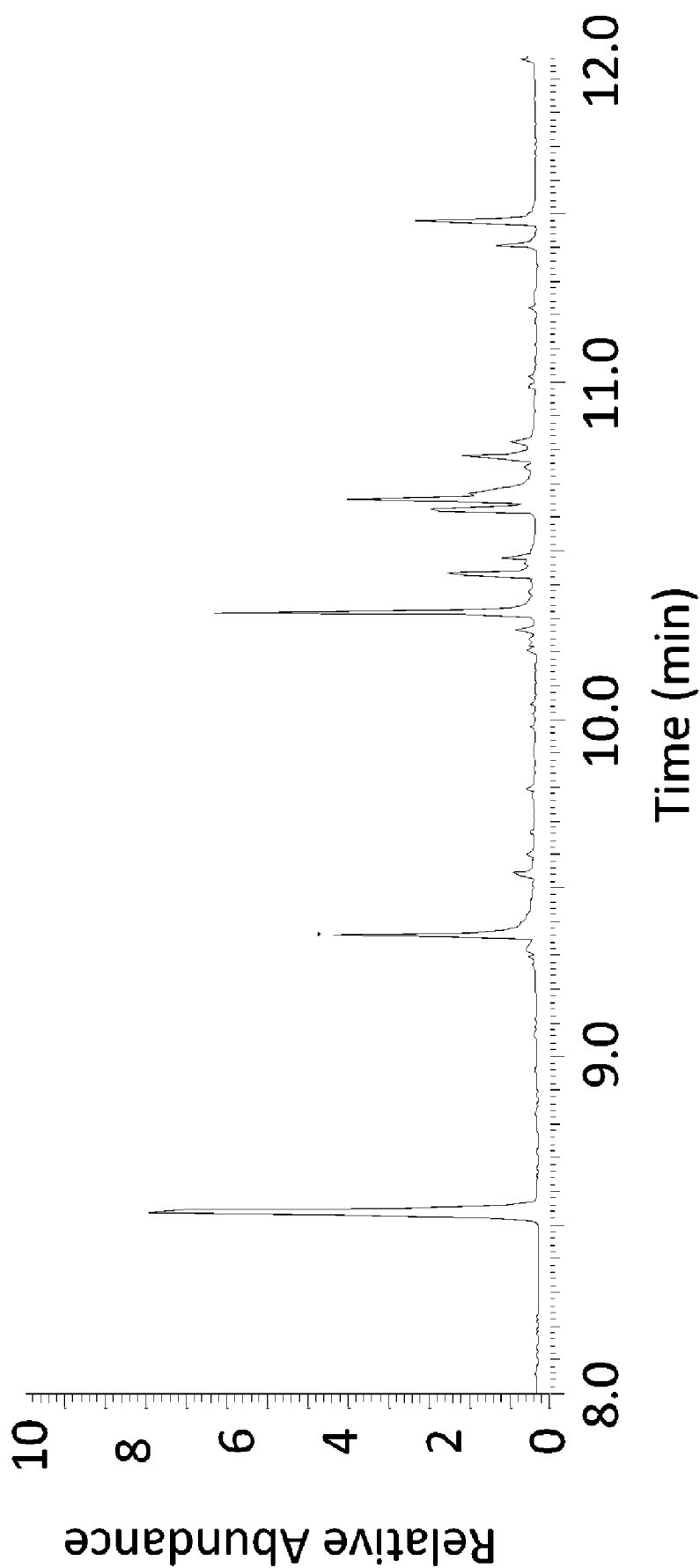
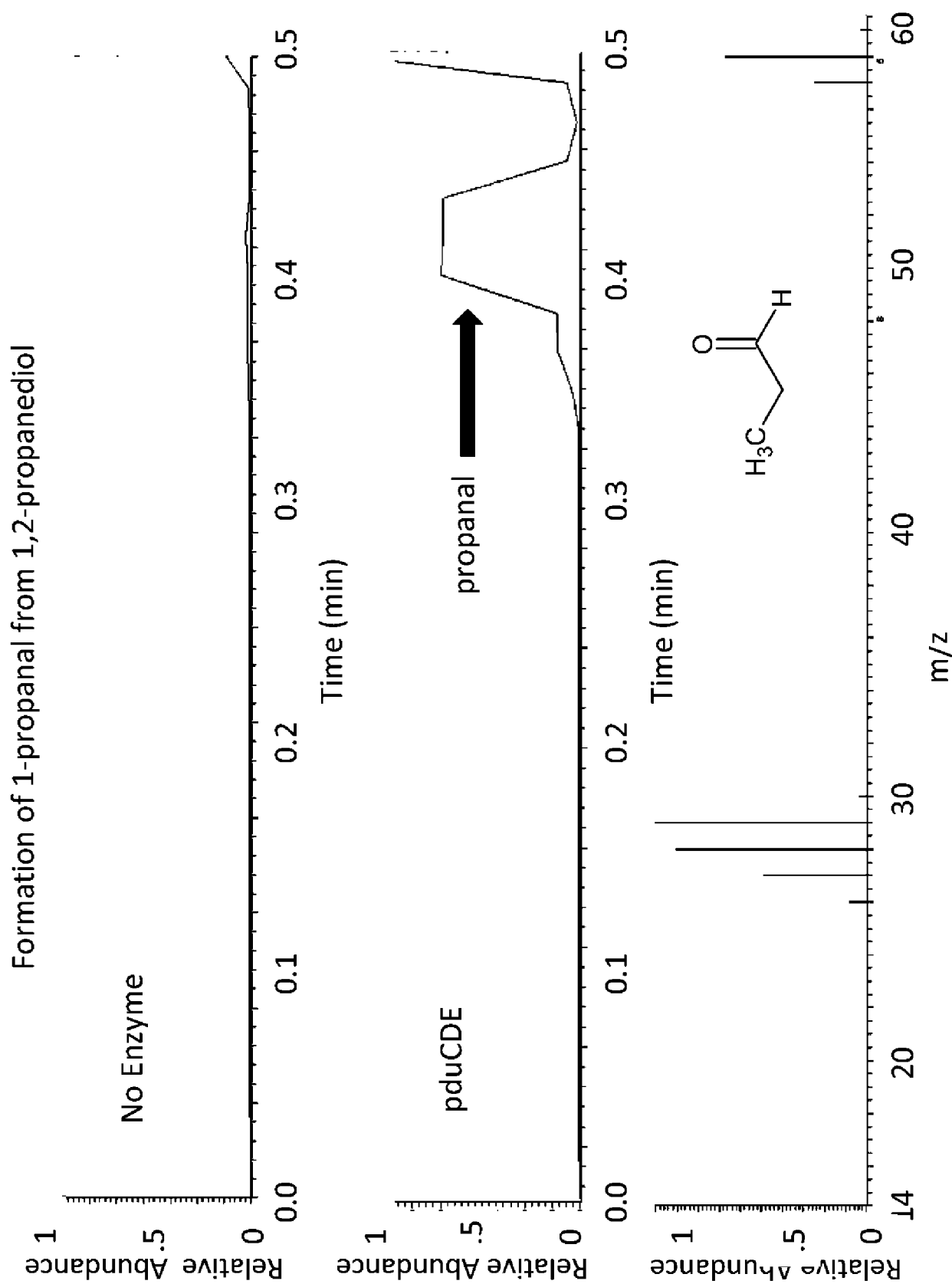


FIG. 44

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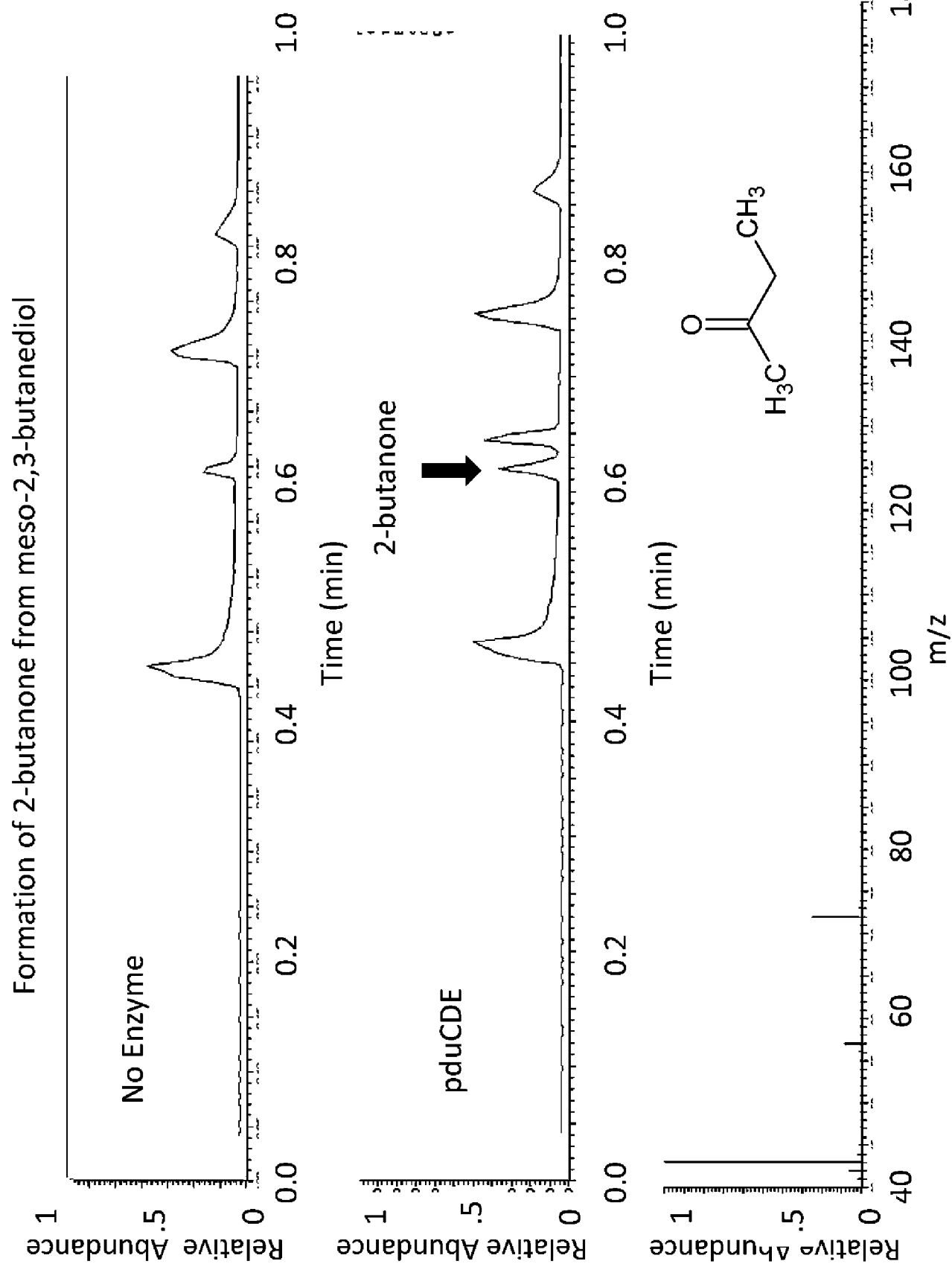


FIG. 45B

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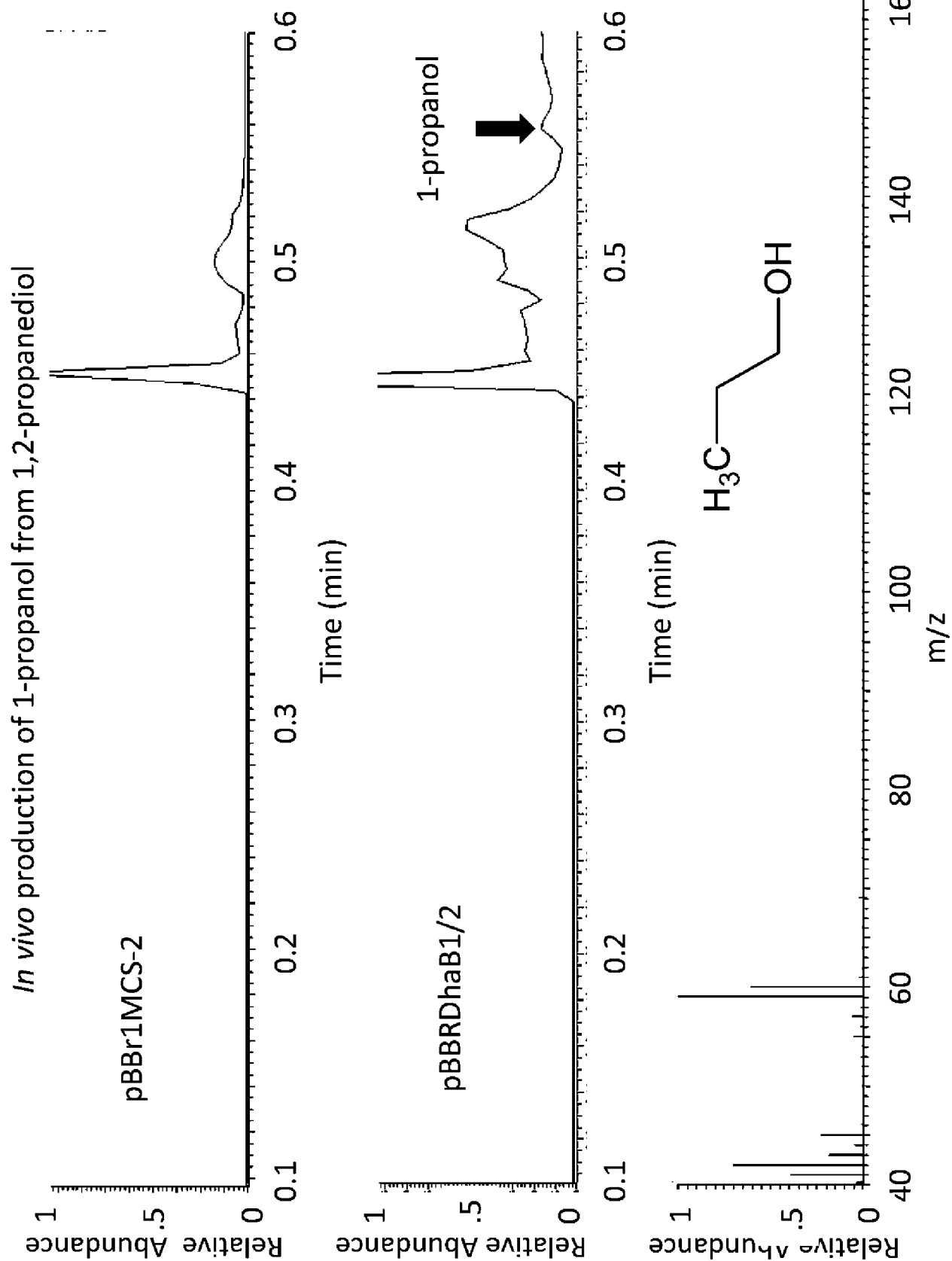
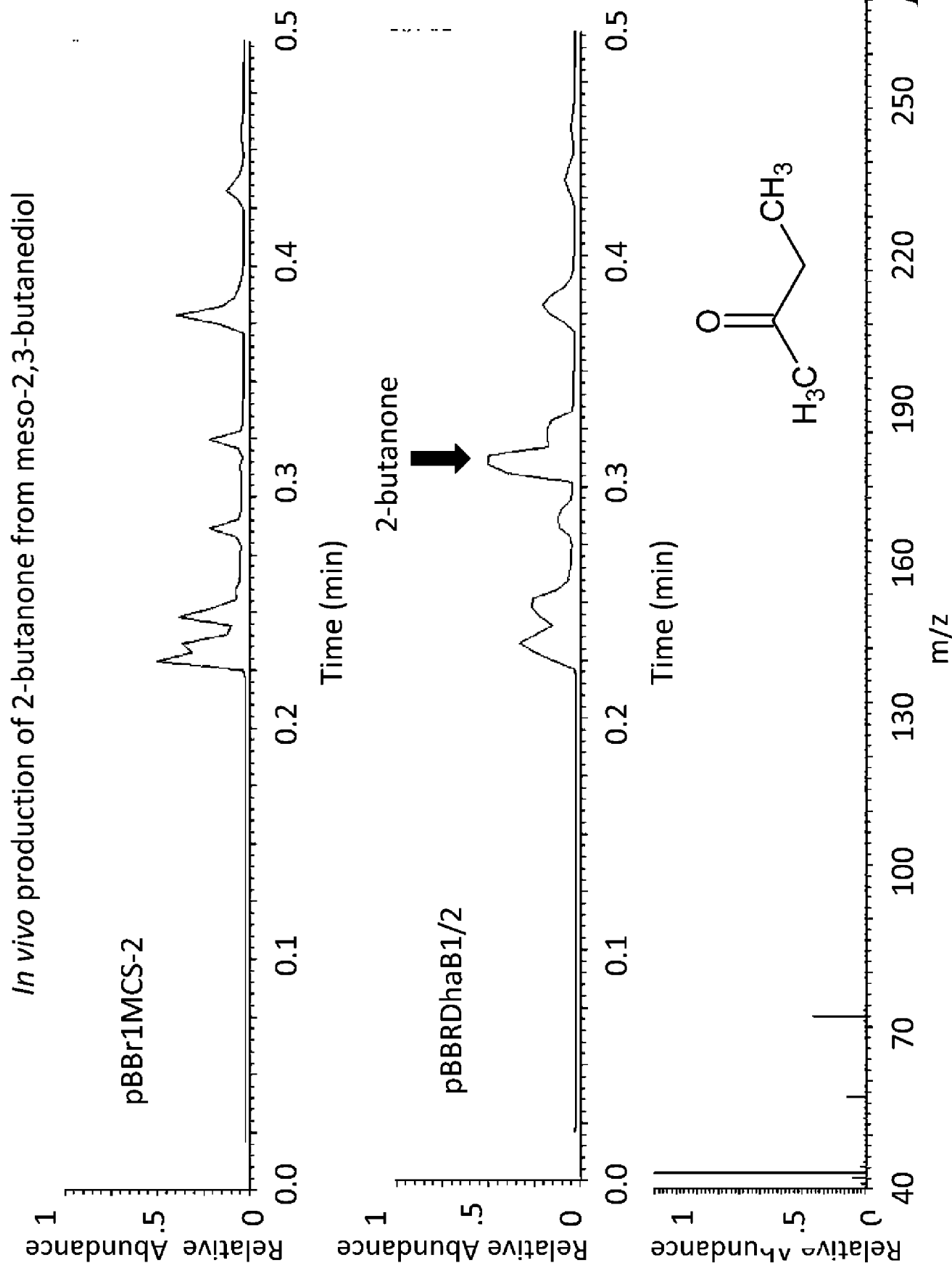
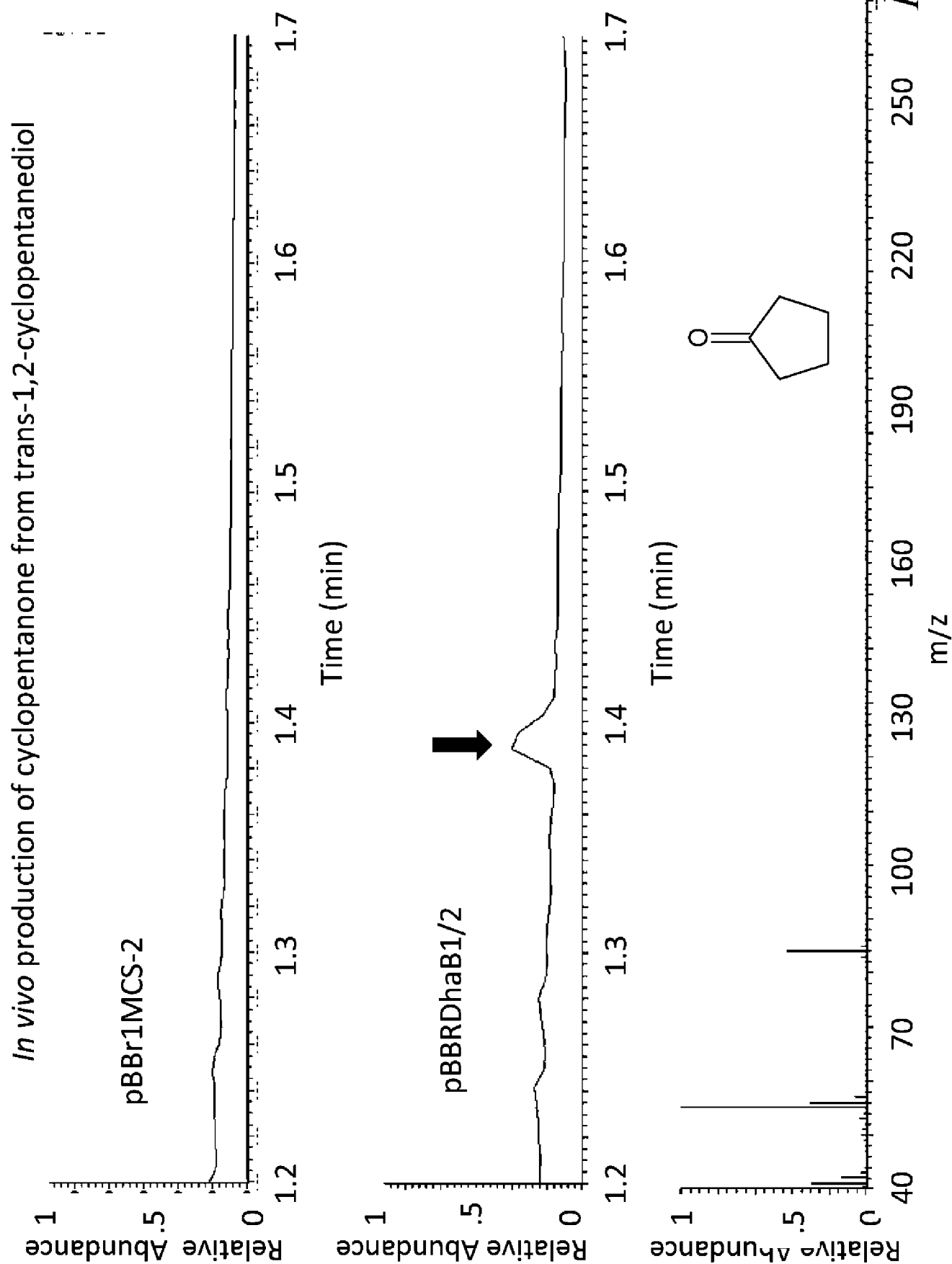


FIG. 46A

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Thiobarbituric acid (TBA) Assay



FIG. 47

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In vivo production of 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone from ligation reaction between acetaldehyde and propionaldehyde catalyzed by BAL enzyme

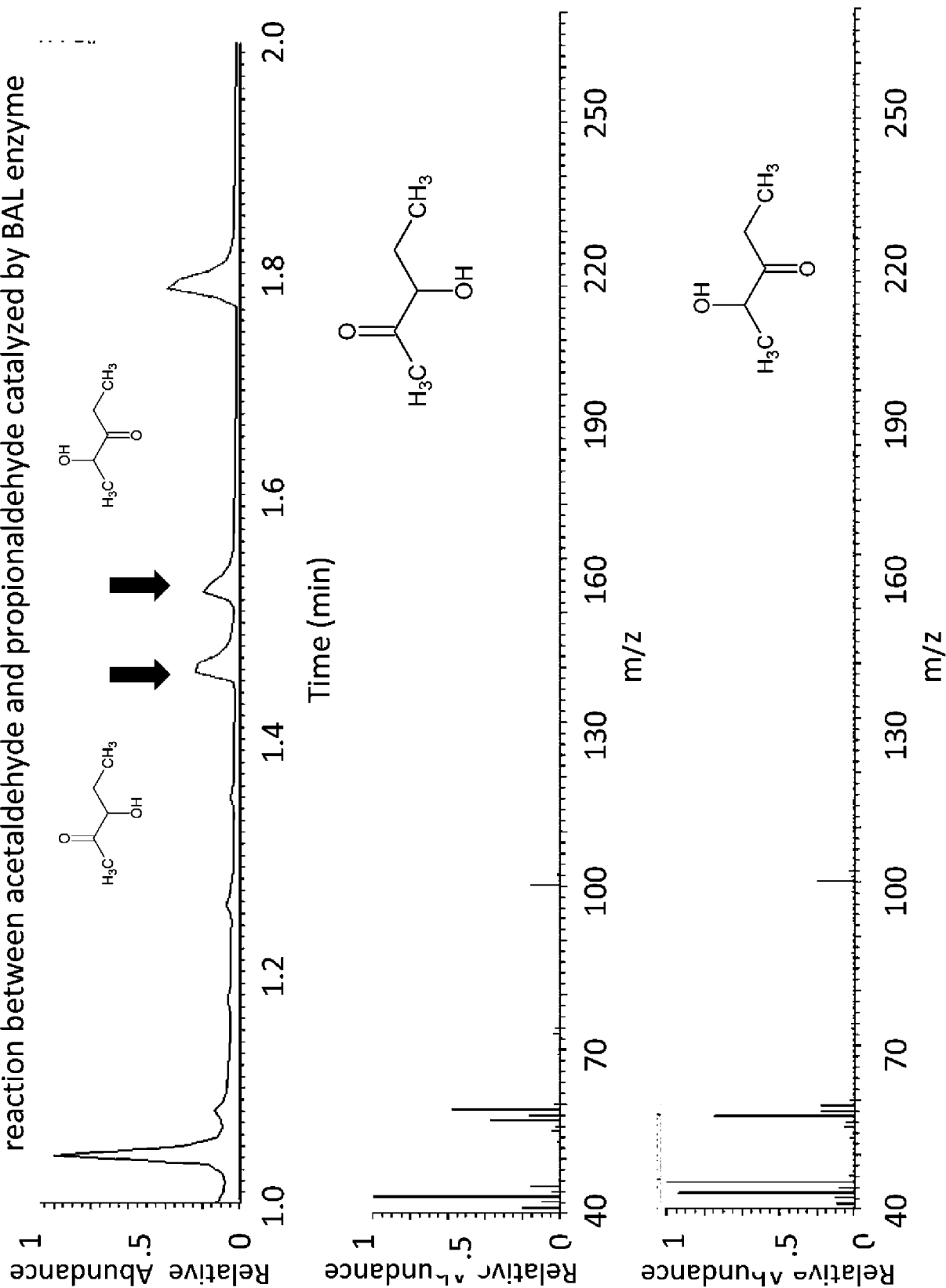


FIG. 48A

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In vivo production of 4-hydroxy-3-heptanone and 3-hydroxy-4-heptanone from ligation reaction between propionaldehyde and butyraldehyde catalyzed by BAL enzyme

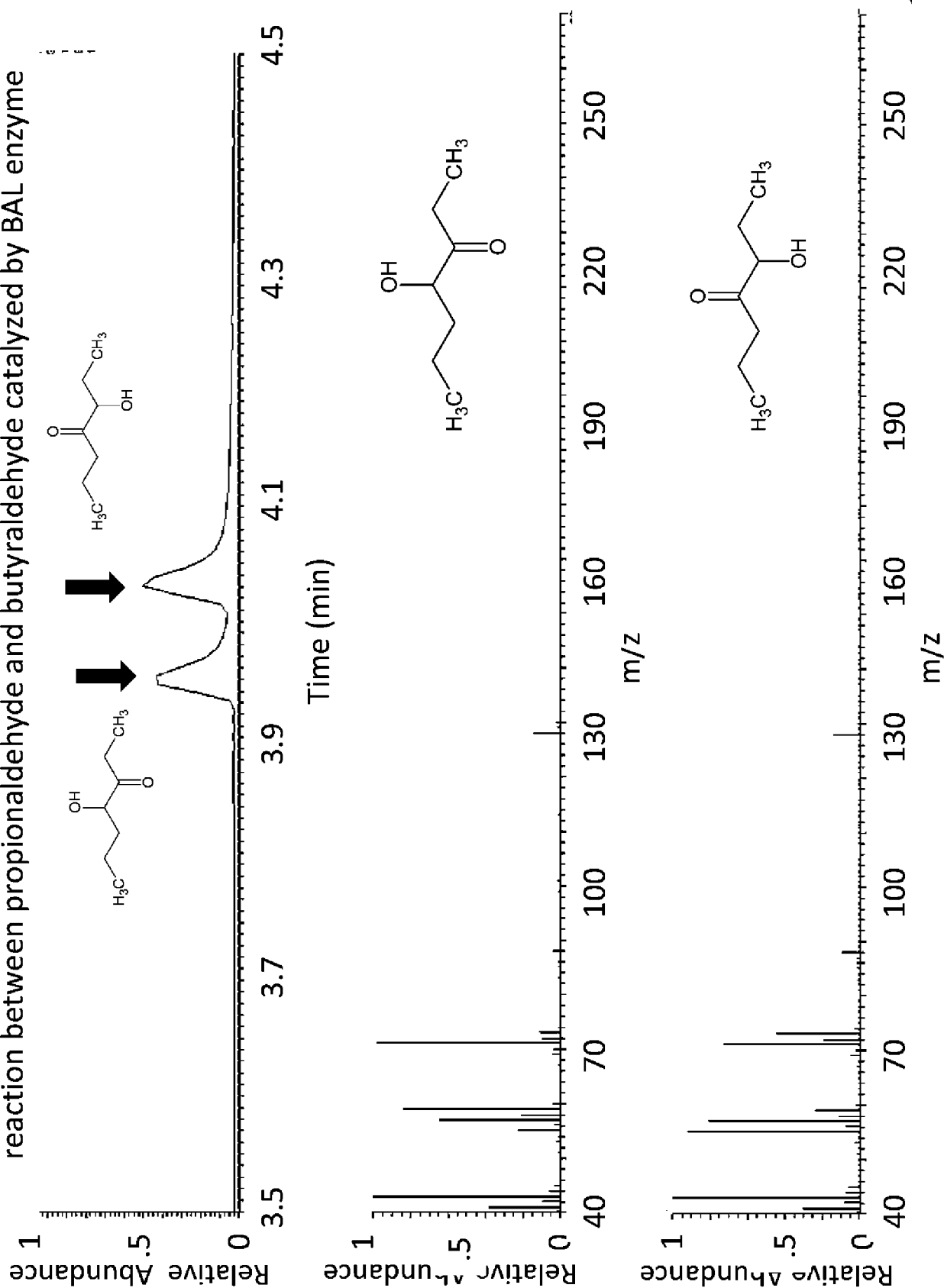


FIG. 48B

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In vivo production of 3-hydroxy-2-heptanone from ligation reaction between
acetaldehyde and pentanaldehyde catalyzed by BAL enzyme

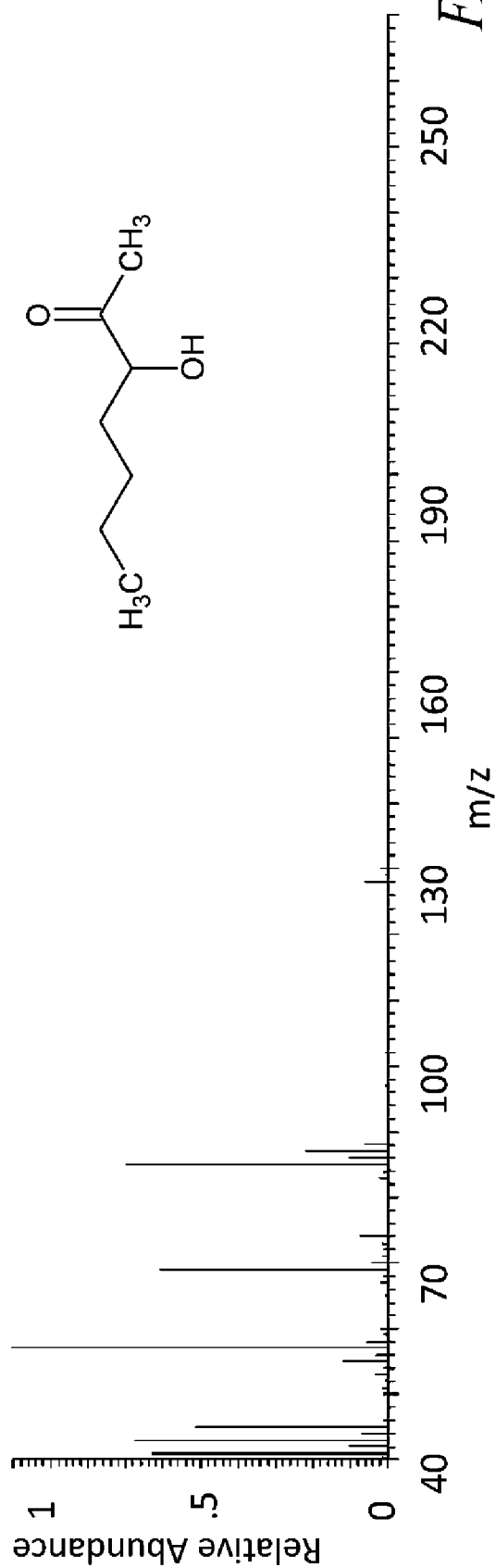
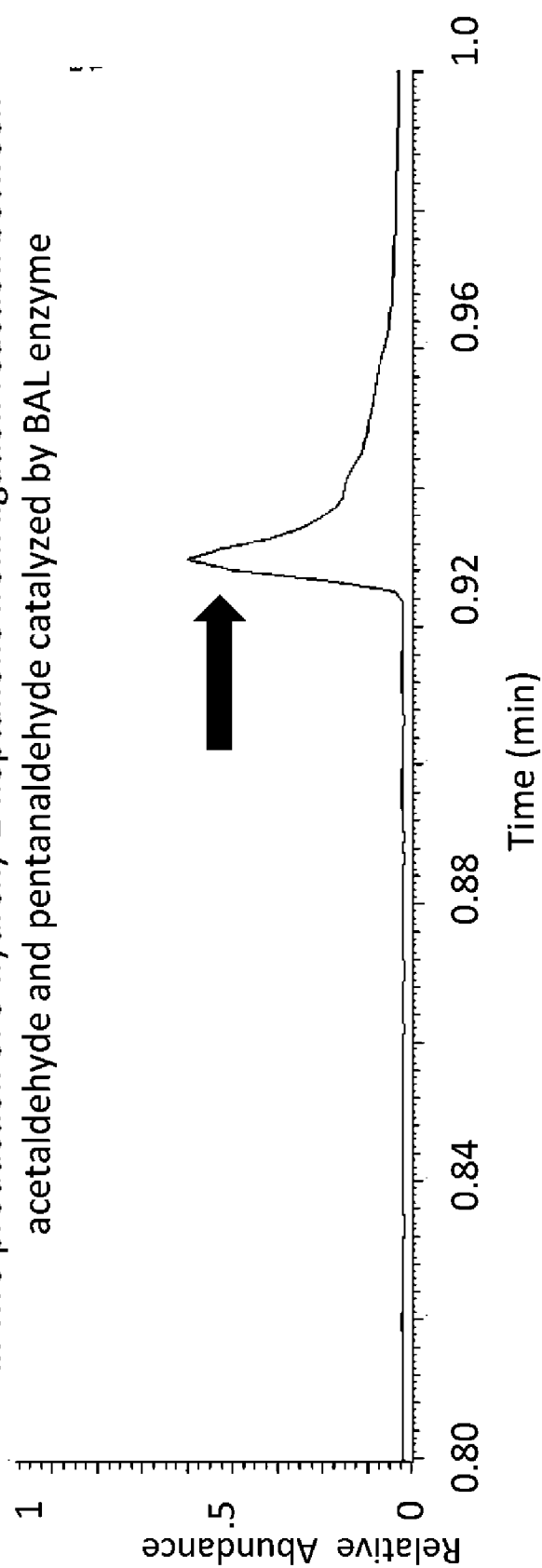


FIG. 49A

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In vivo production of 4-hydroxy-3-octanone and 3-hydroxy-4-octanone from ligation reaction between pentanaldehyde and propionaldehyde catalyzed by the BAL enzyme

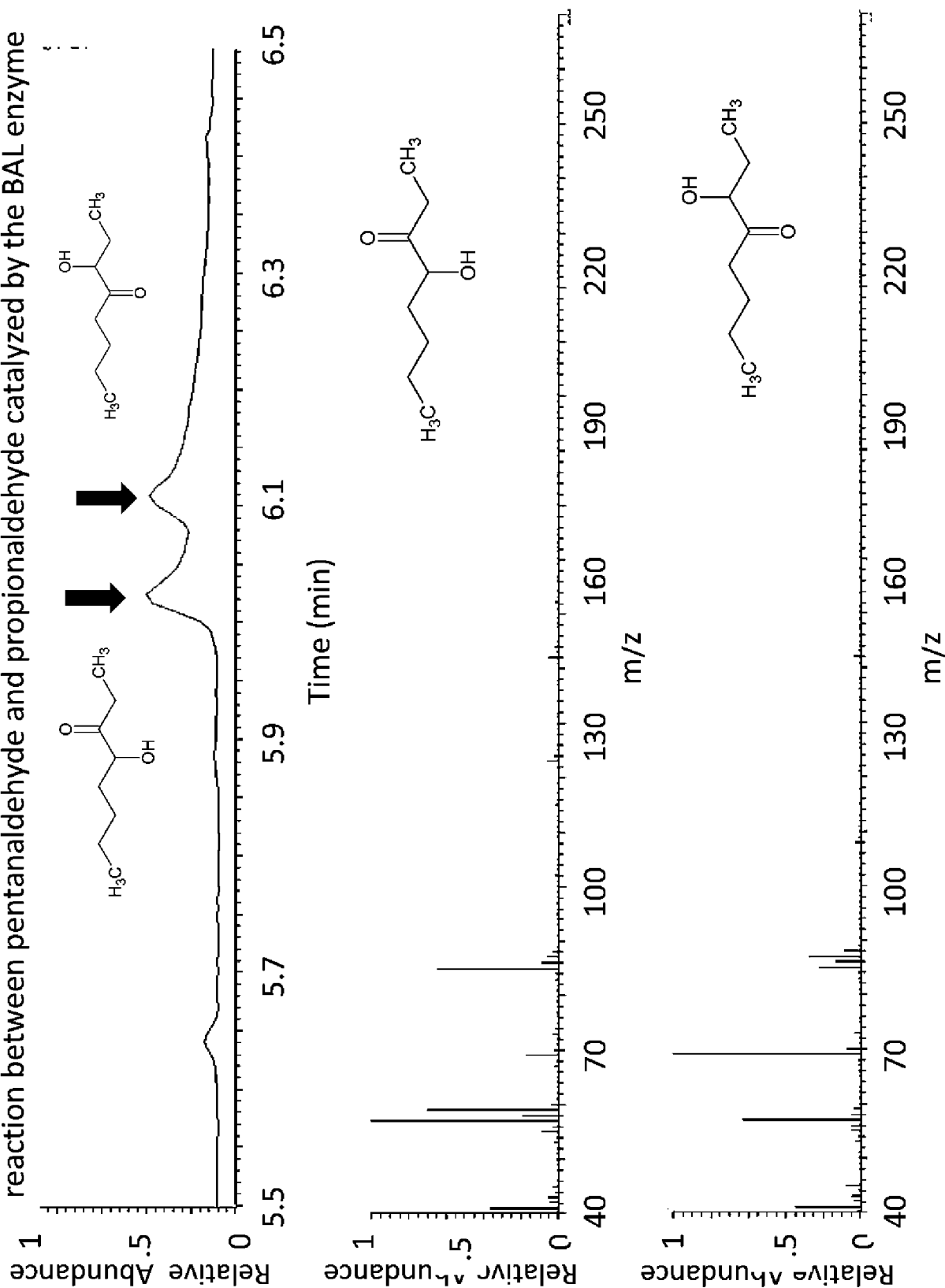


FIG. 49B

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In vivo production of 5-hydroxy-4-nonanone from ligation reaction between butyraldehyde and pentanaldehyde catalyzed by BAL enzyme

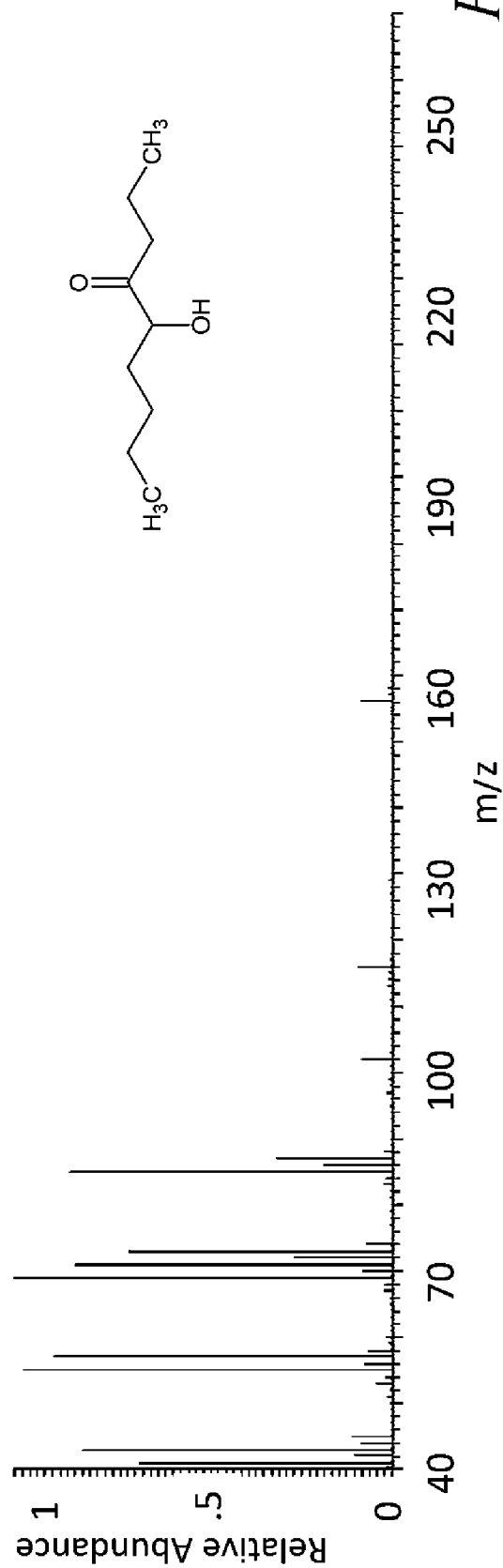
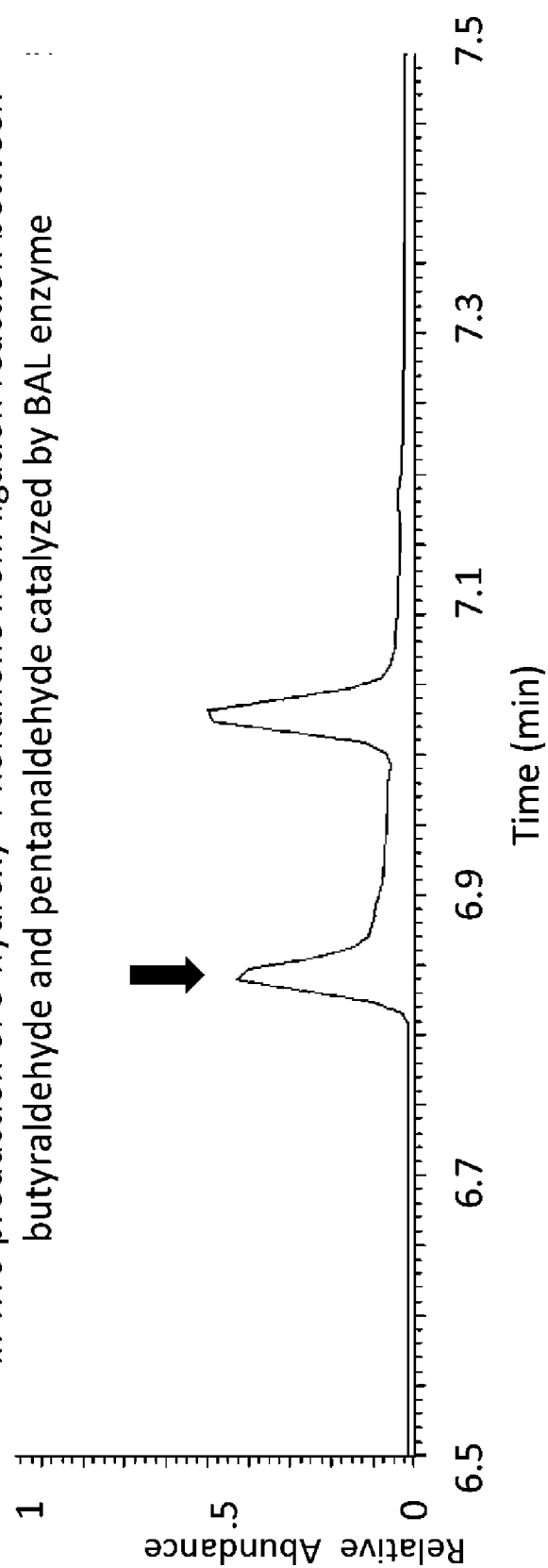


FIG. 50A

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In vivo production of 2-methyl-5-hydroxy-4-decanone and 2-methyl-4-hydroxy-5-decanone from ligation reaction between hexanal and 3-methylbutyraldehyde catalyzed by BAL

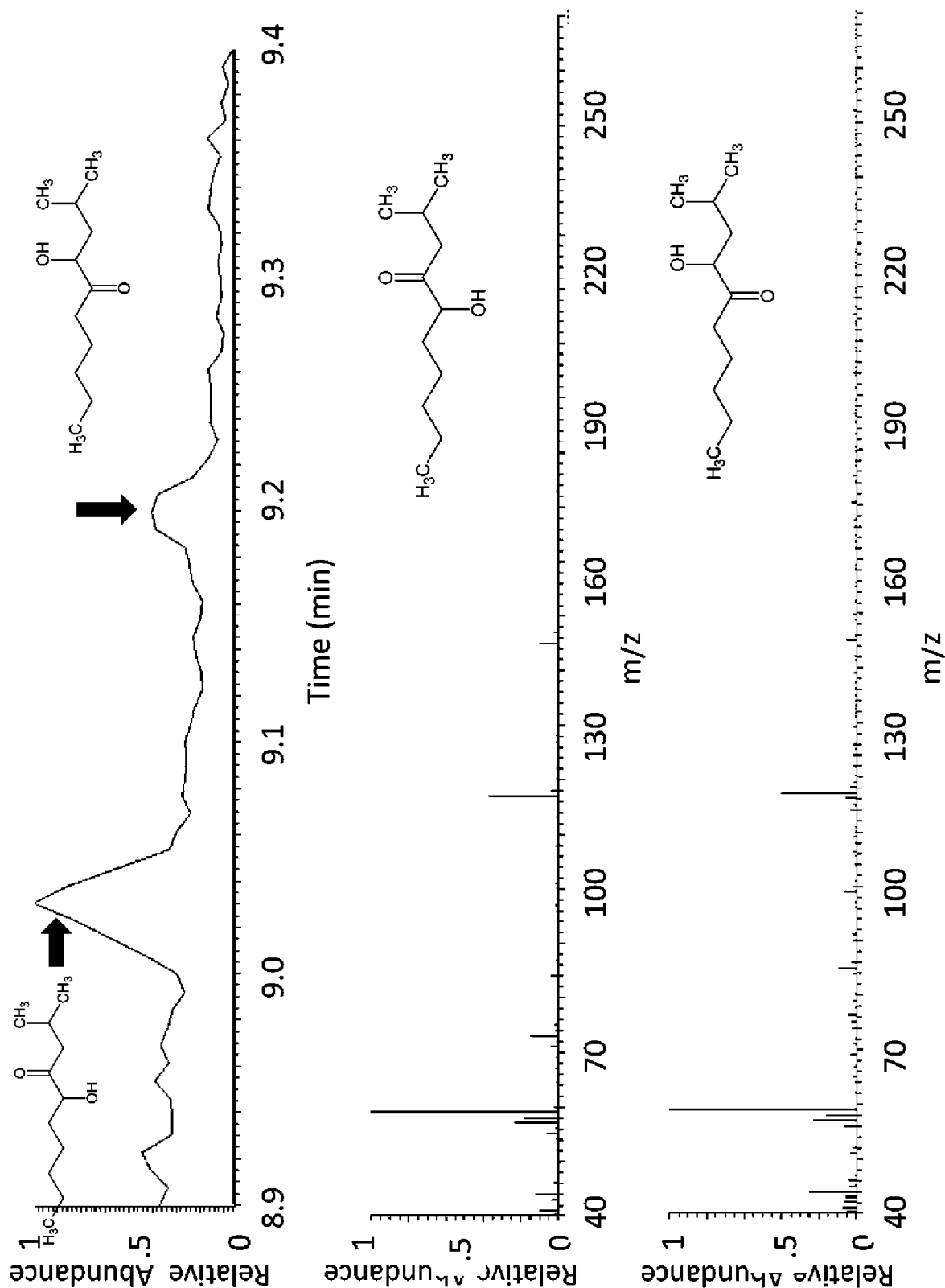


FIG. 50B

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In vivo production of 6-methyl-3-hydroxy-2-heptanone from ligation reaction between acetaldehyde and 4-methylhexanaldehyde catalyzed by BAL enzyme

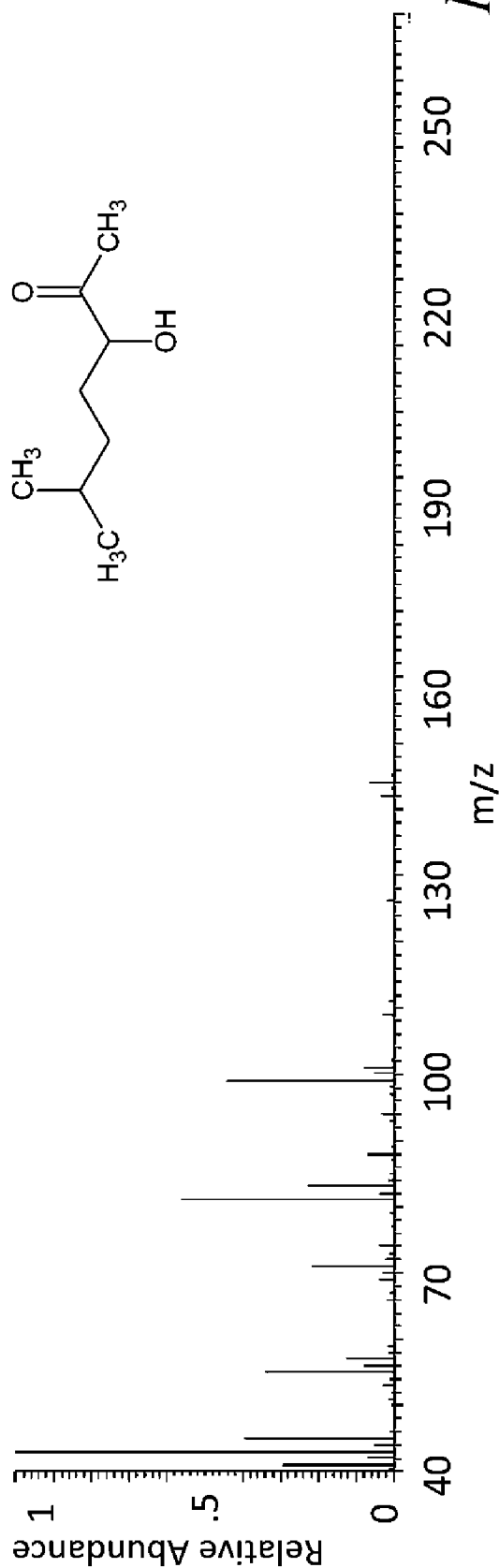
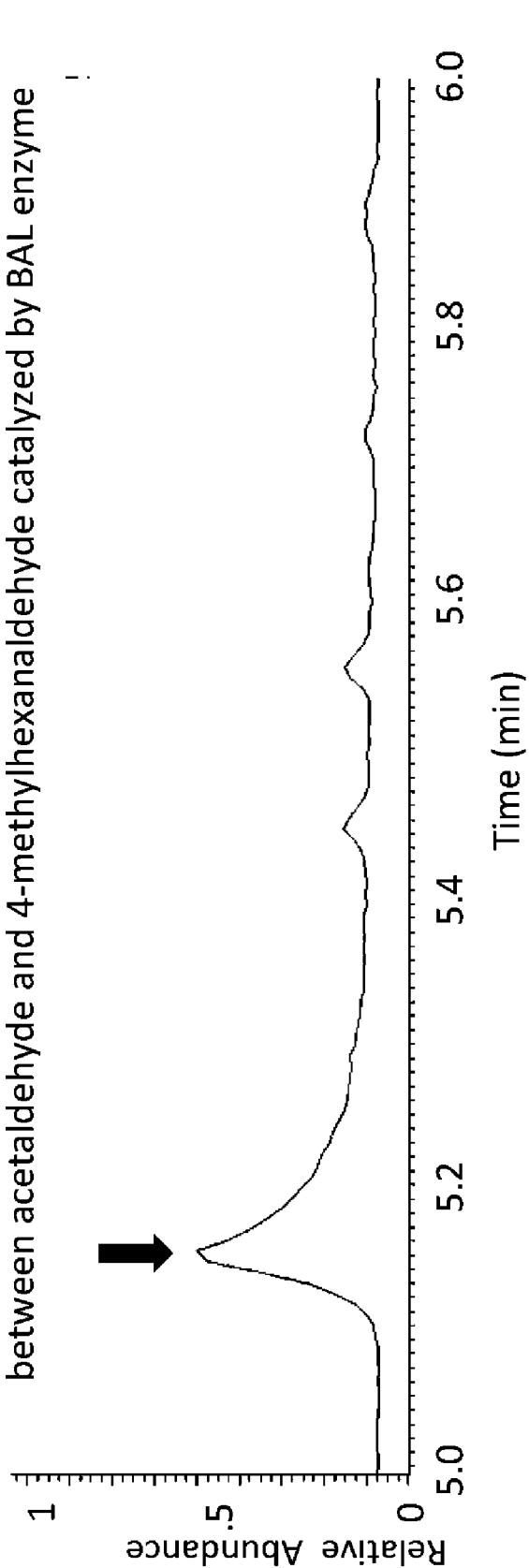


FIG. 51A

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In vivo production of 7-methyl-4-hydroxy-3-octanone from ligation reaction between 4-methylhexanaldehyde and propionaldehyde catalyzed by BAL enzyme

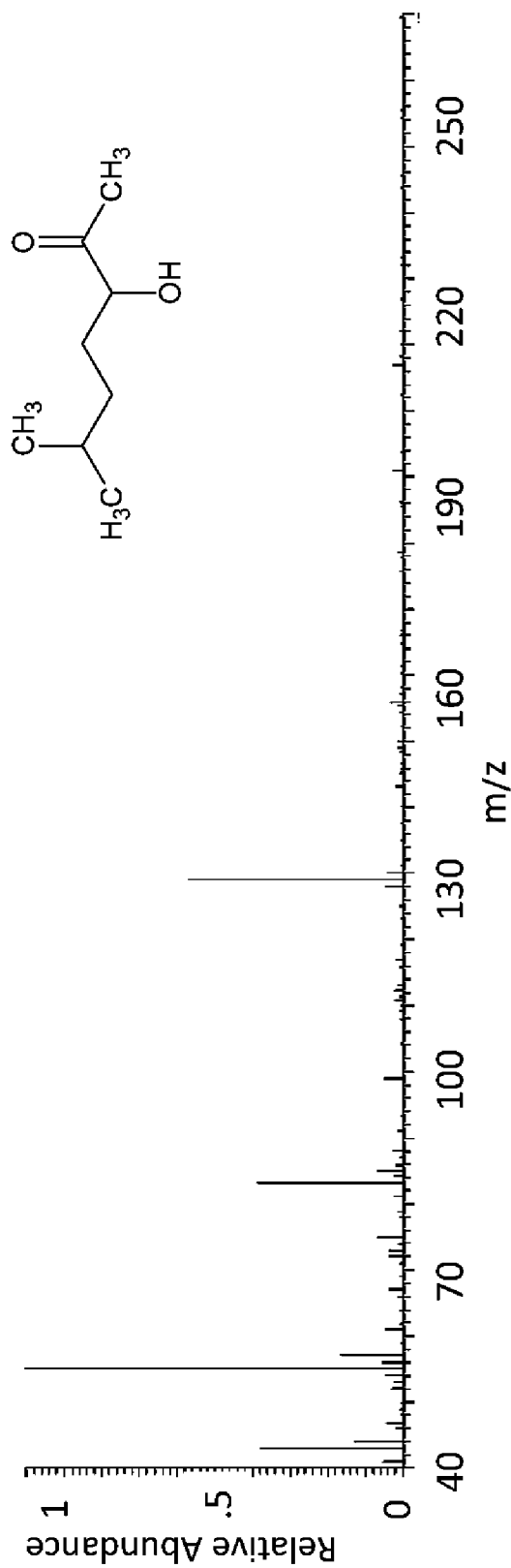
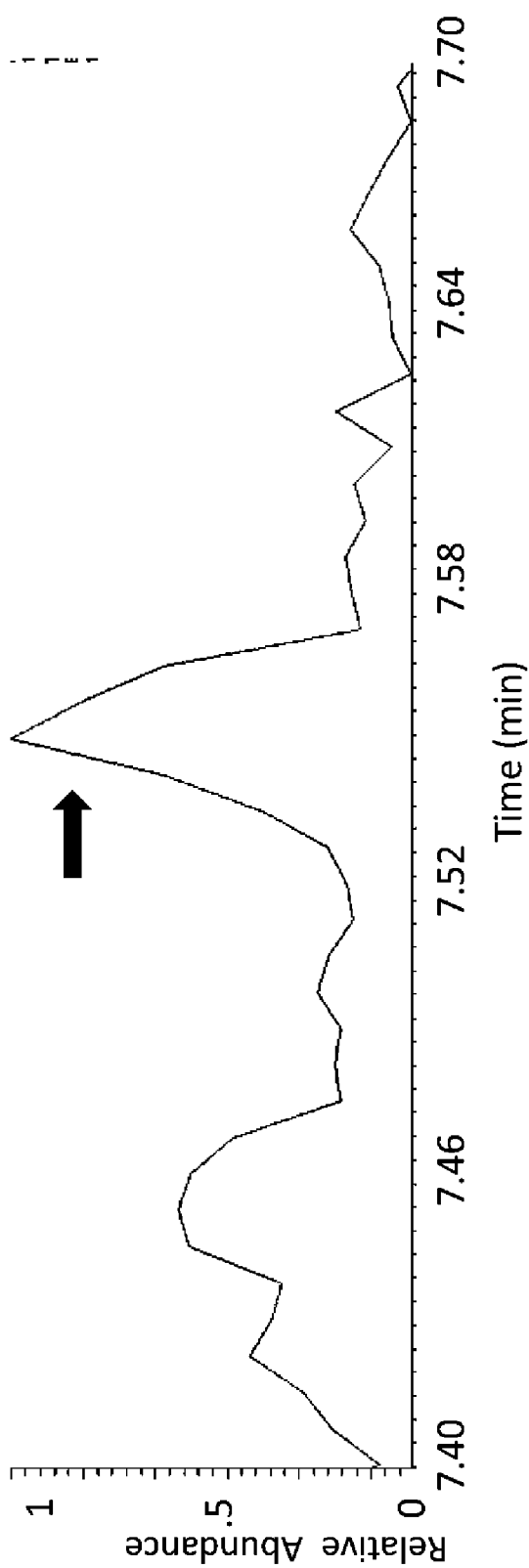


FIG. 51B

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In vivo production of 8-methyl-5-hydroxy-4-nonanone from ligation reaction between 4-methylhexanaldehyde and butyraldehyde catalyzed by BAL enzyme

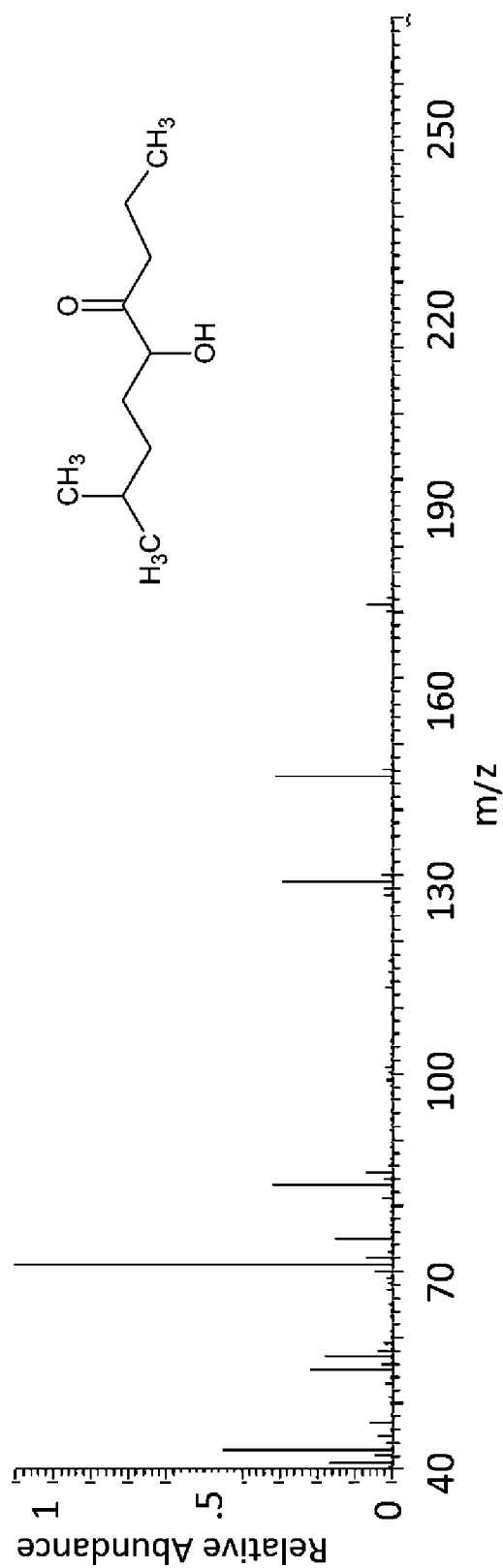
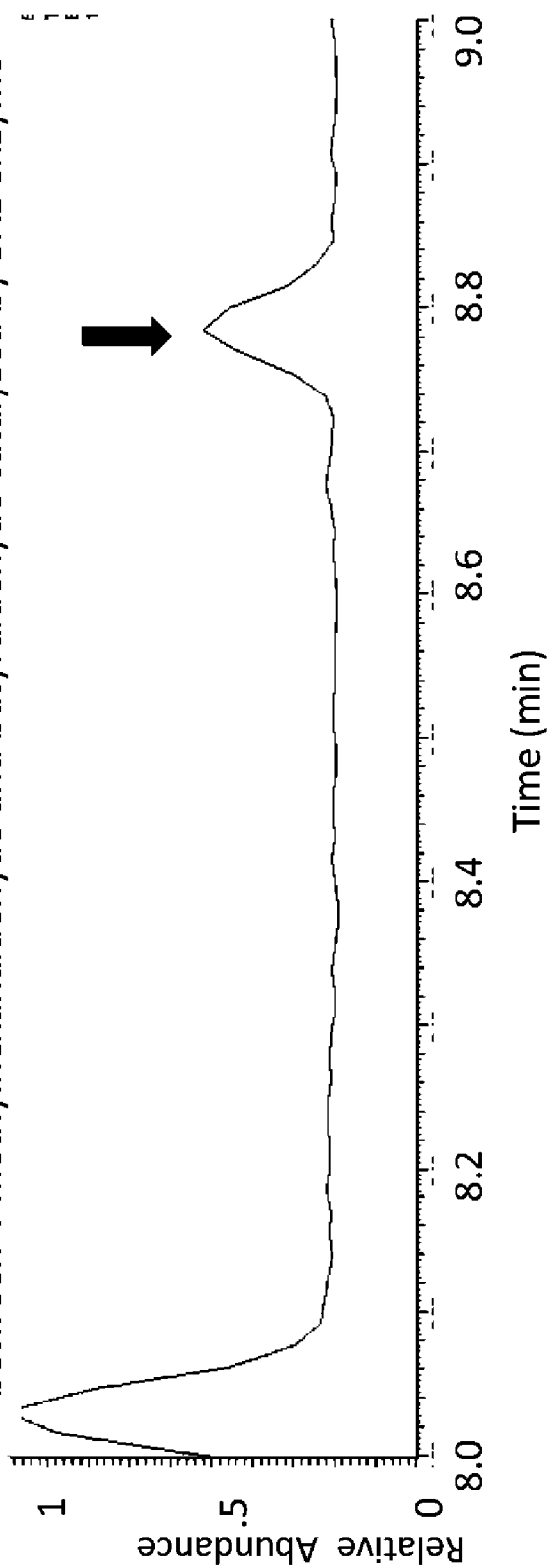


FIG. 52A

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In vivo production 3-hydroxy-2-decanone from ligation reaction between acetaldehyde and octanal catalyzed by BAL

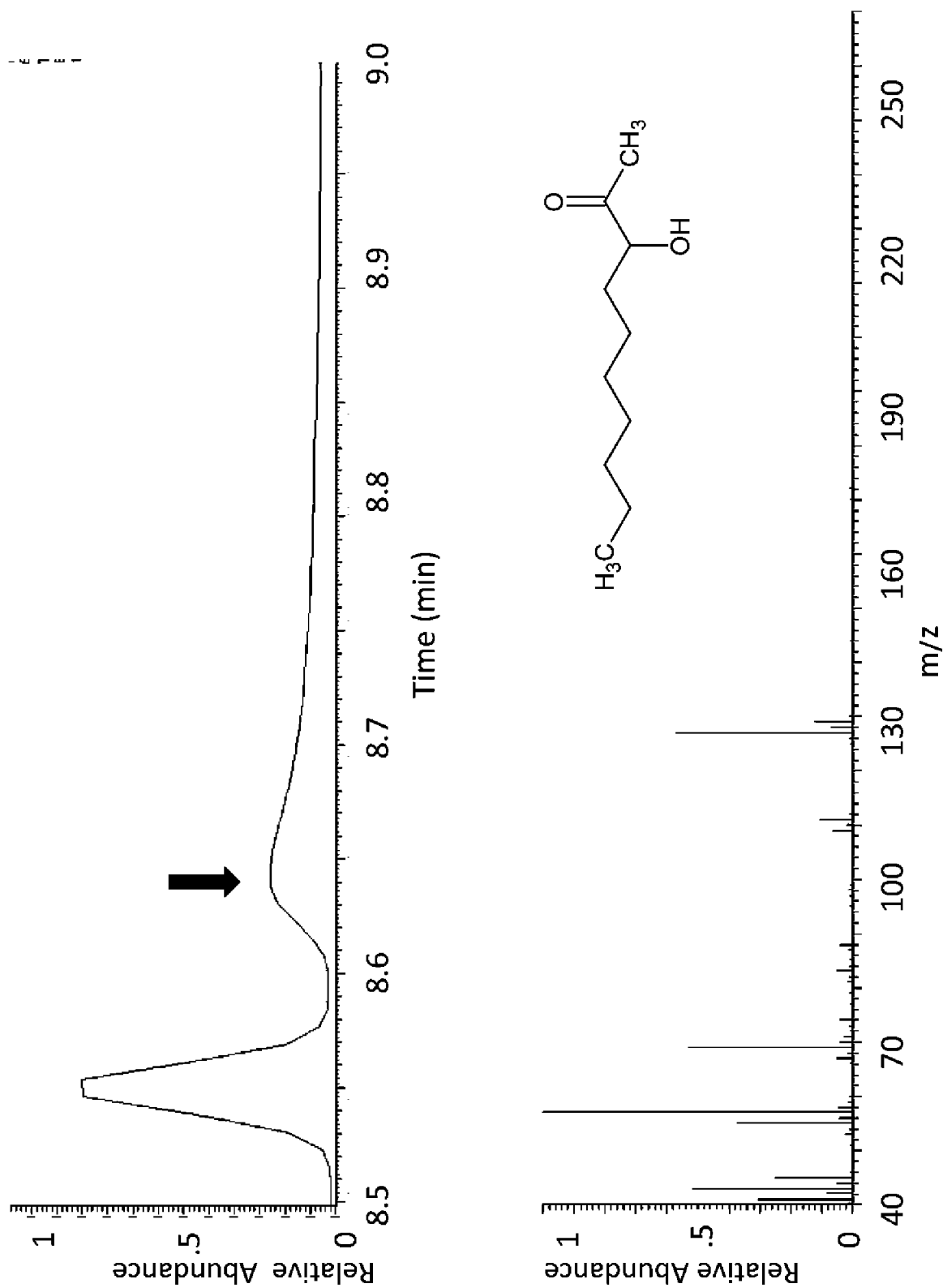


FIG. 52B

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In vivo production 4-hydroxy-3-undecanone from ligation reaction between octanal and propionaldehyde catalyzed by BAL

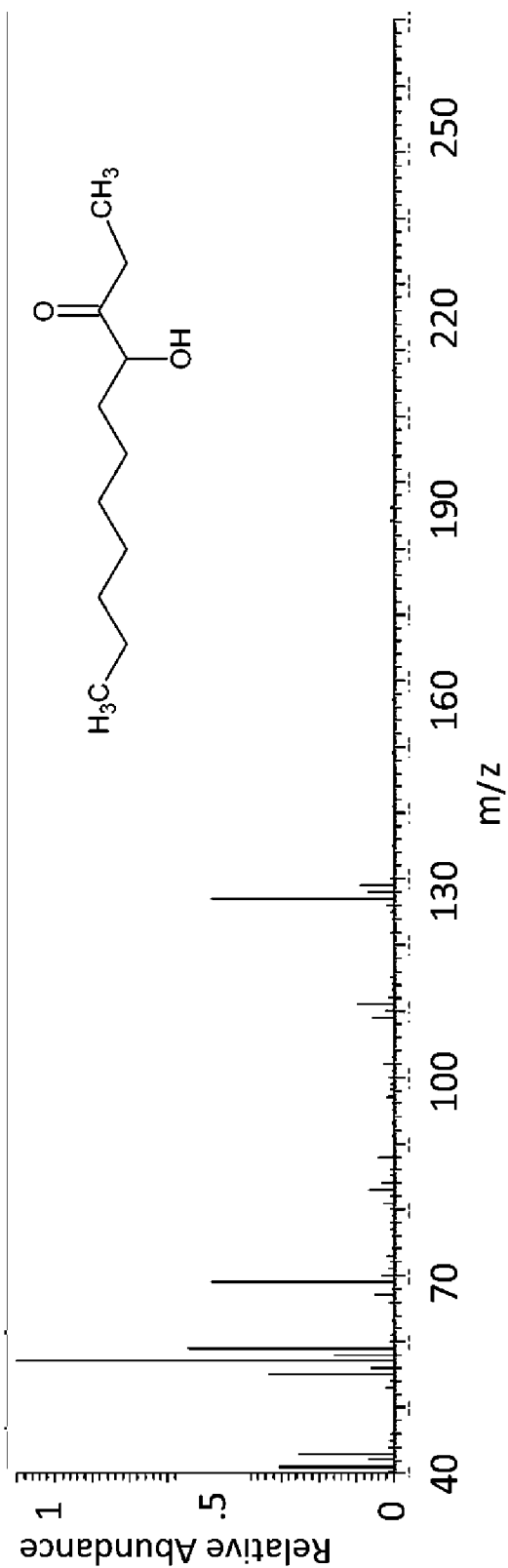
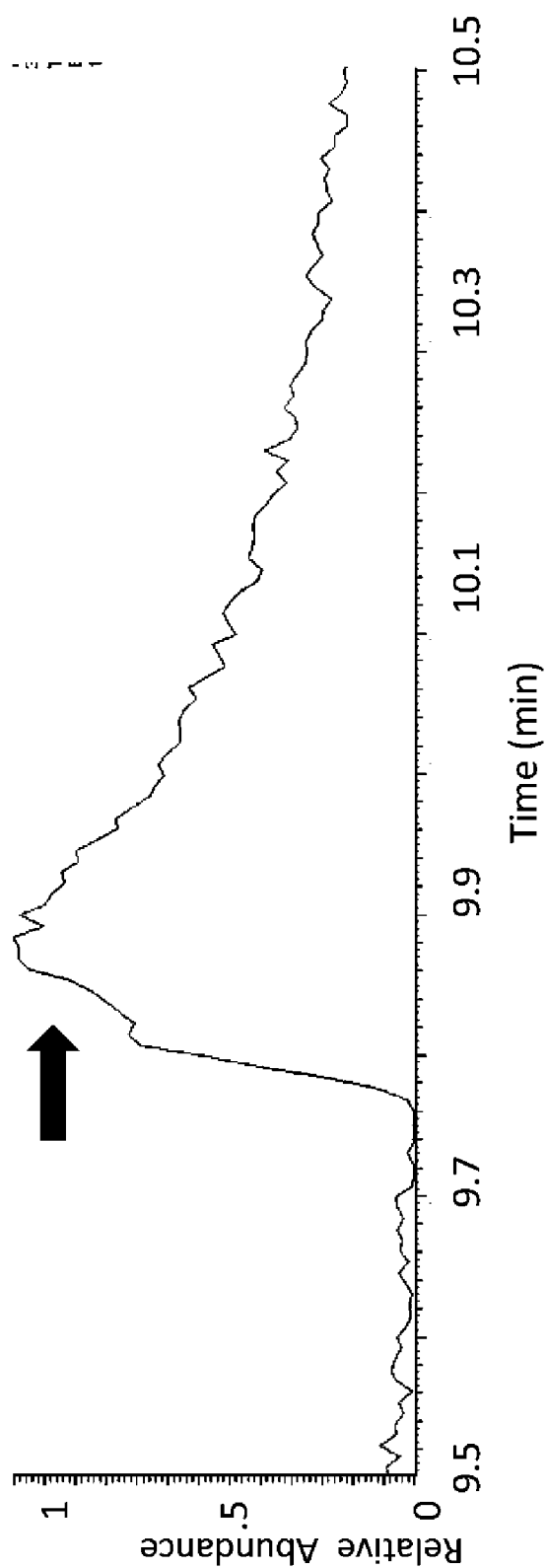


FIG. 53A

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In vivo production 5-hydroxy-4-dodecanone from ligation reaction between octanal and butyraldehyde catalyzed by BAL

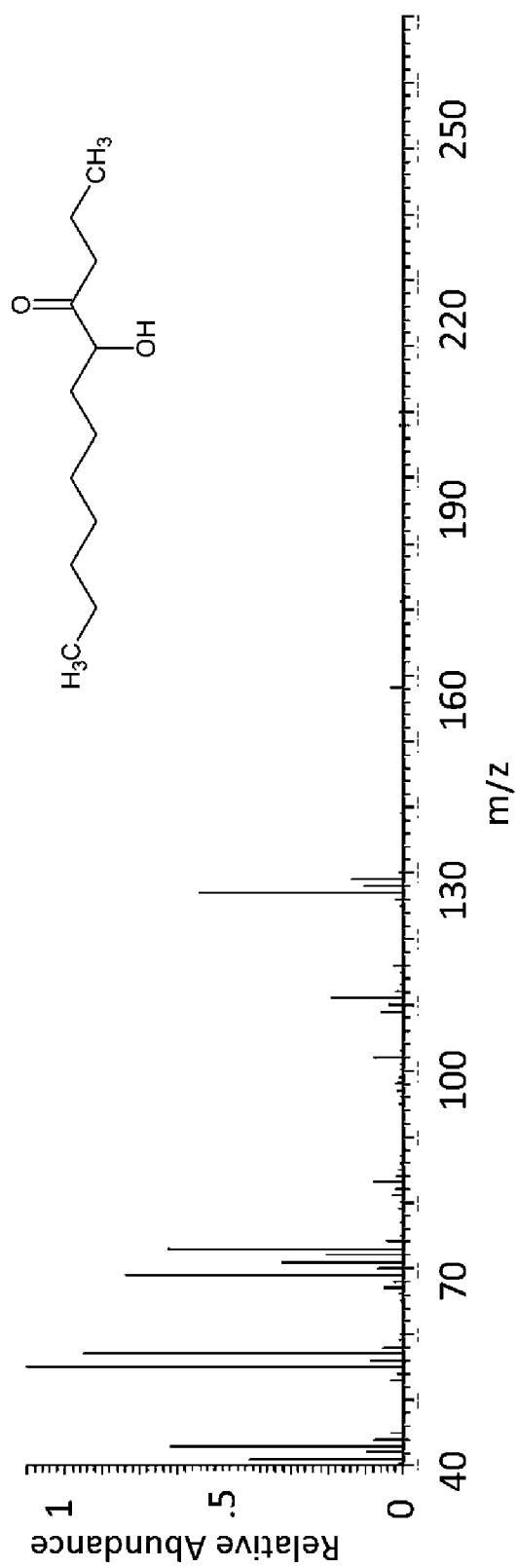
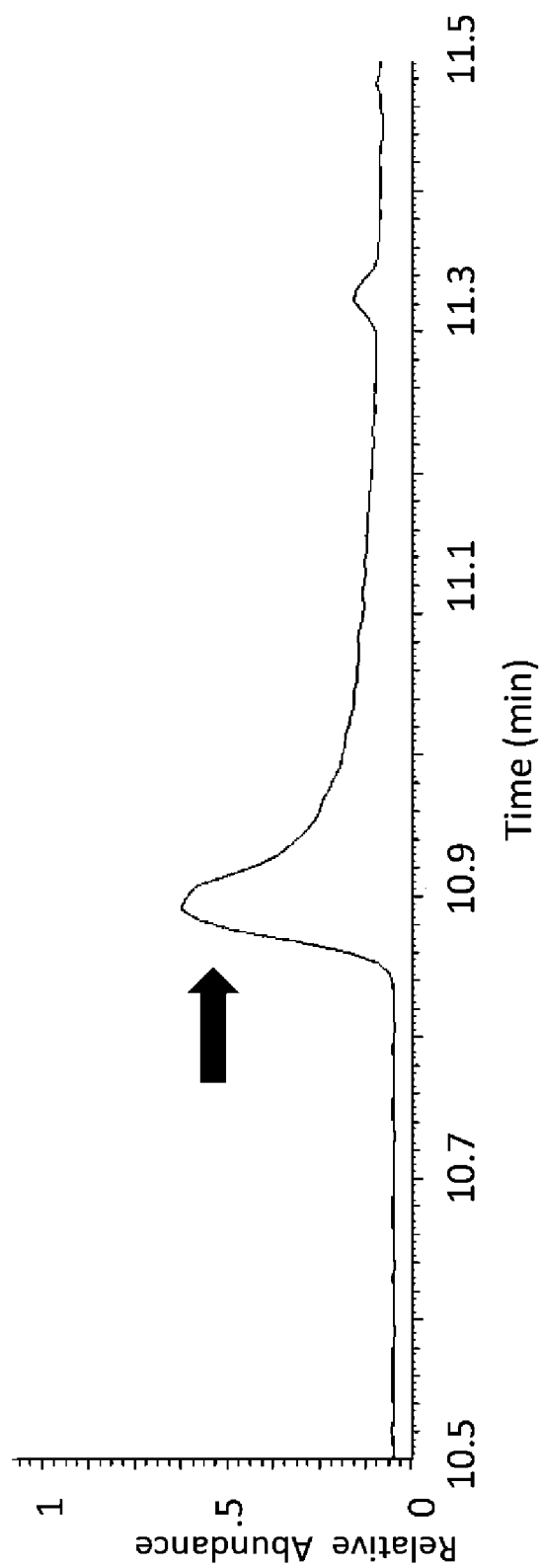


FIG. 53B

In vivo production of 6-hydroxy-5-tridecanone from ligation reaction between octanaldehyde and pentanaldehyde catalyzed by BAL enzyme

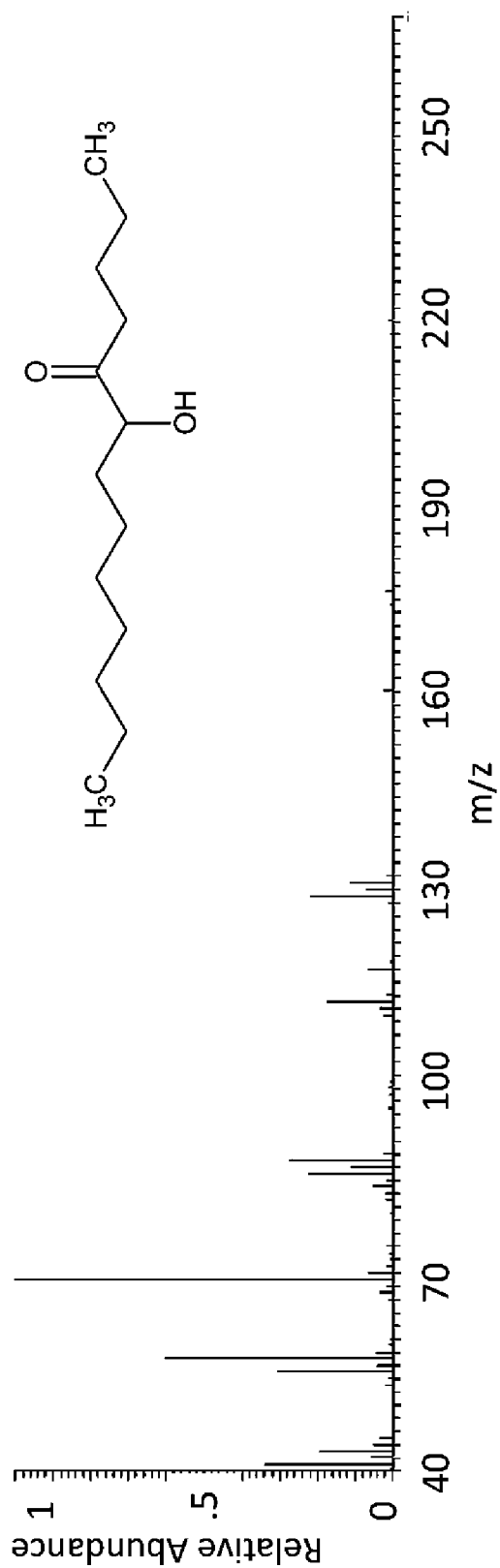
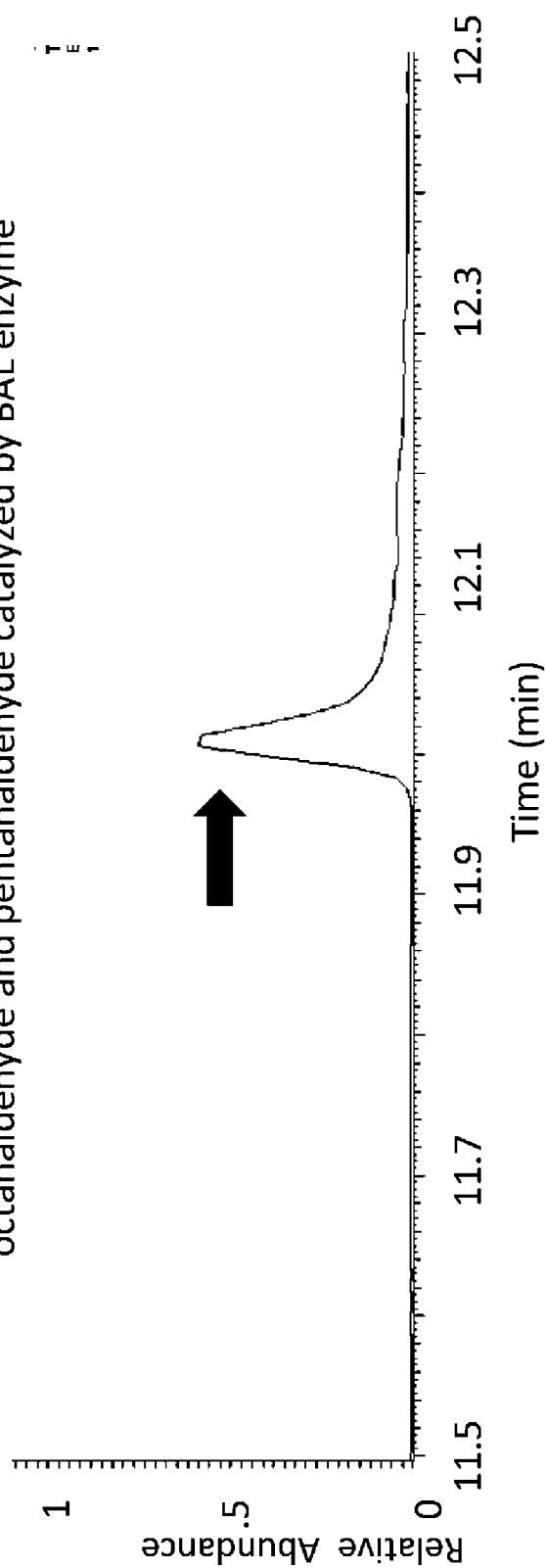


FIG. 54A

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In vivo production 2-methyl-5-hydroxy-4-dodecanone and 2-methyl-4-hydroxy-5-decanone from ligation reaction between octanal and 3-methylbutyraldehyde catalyzed by BAL

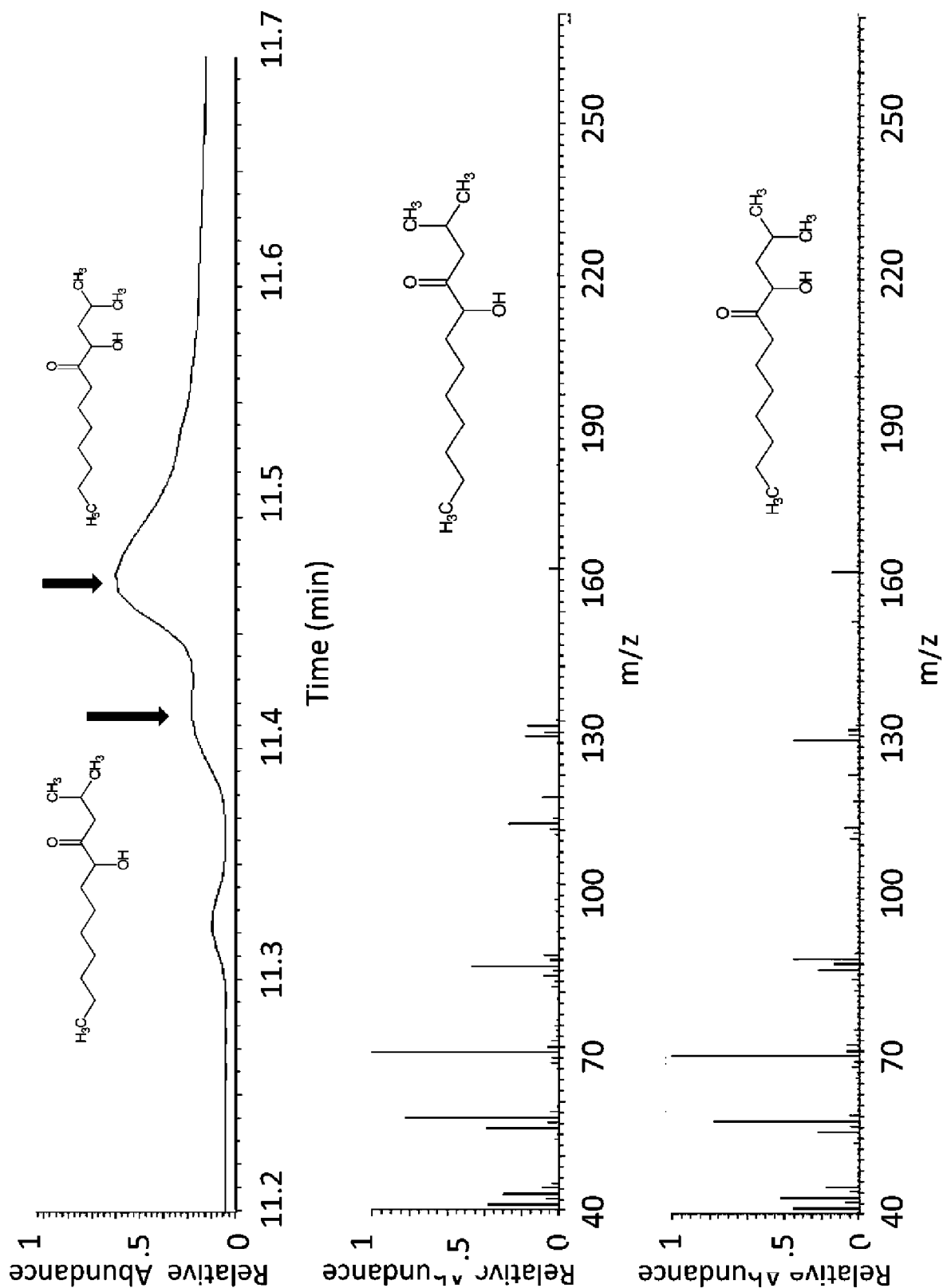


FIG. 54B

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In vivo production 2-methyl-6-hydroxy-5-tridecanone from ligation reaction
between octanaldehyde and 4-methylpentanaldehyde catalyzed by BAL enzyme

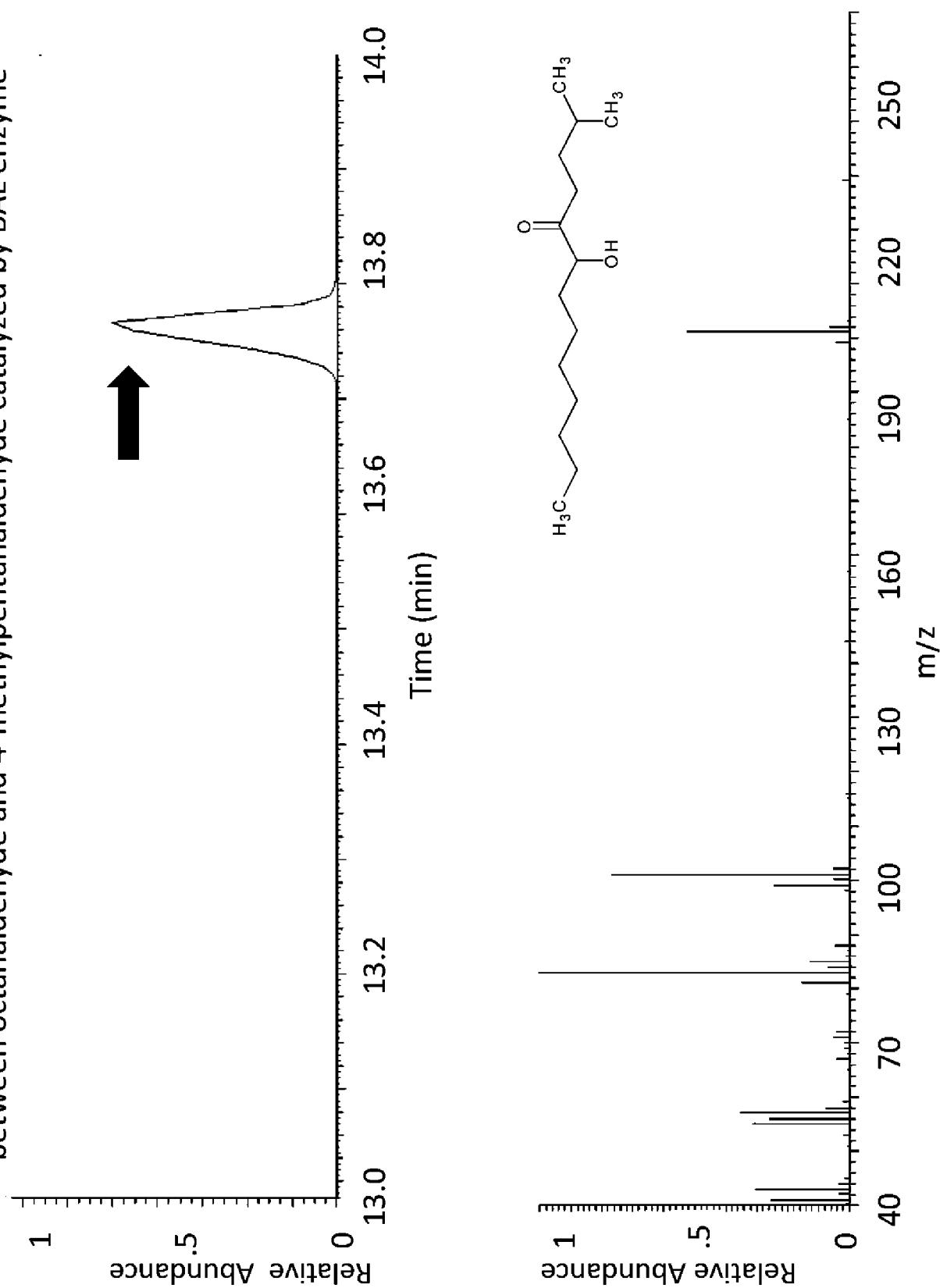
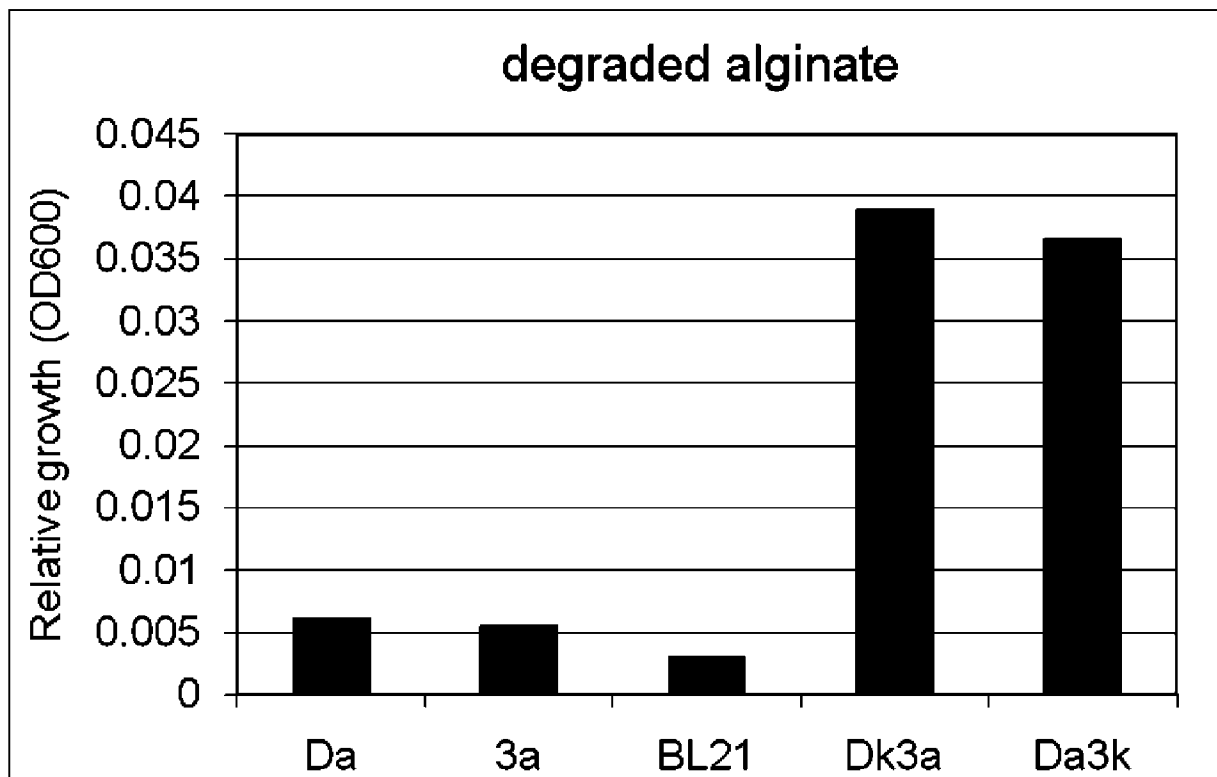
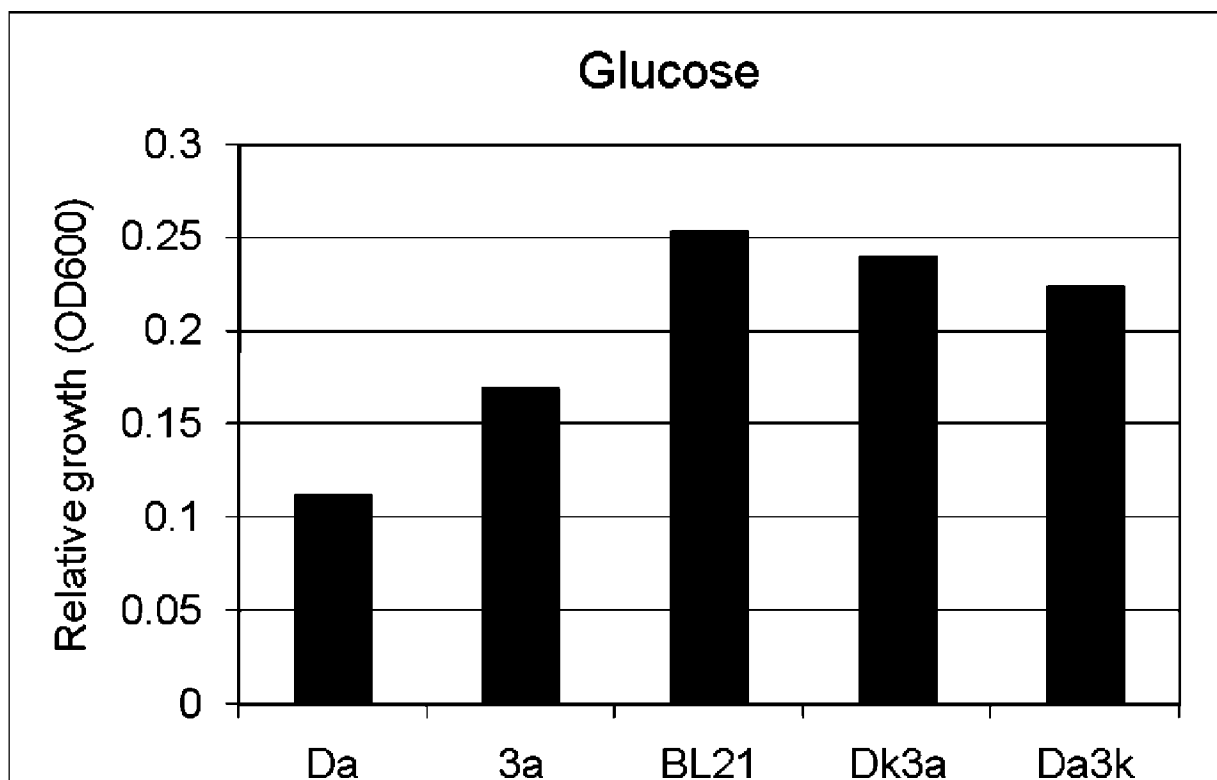


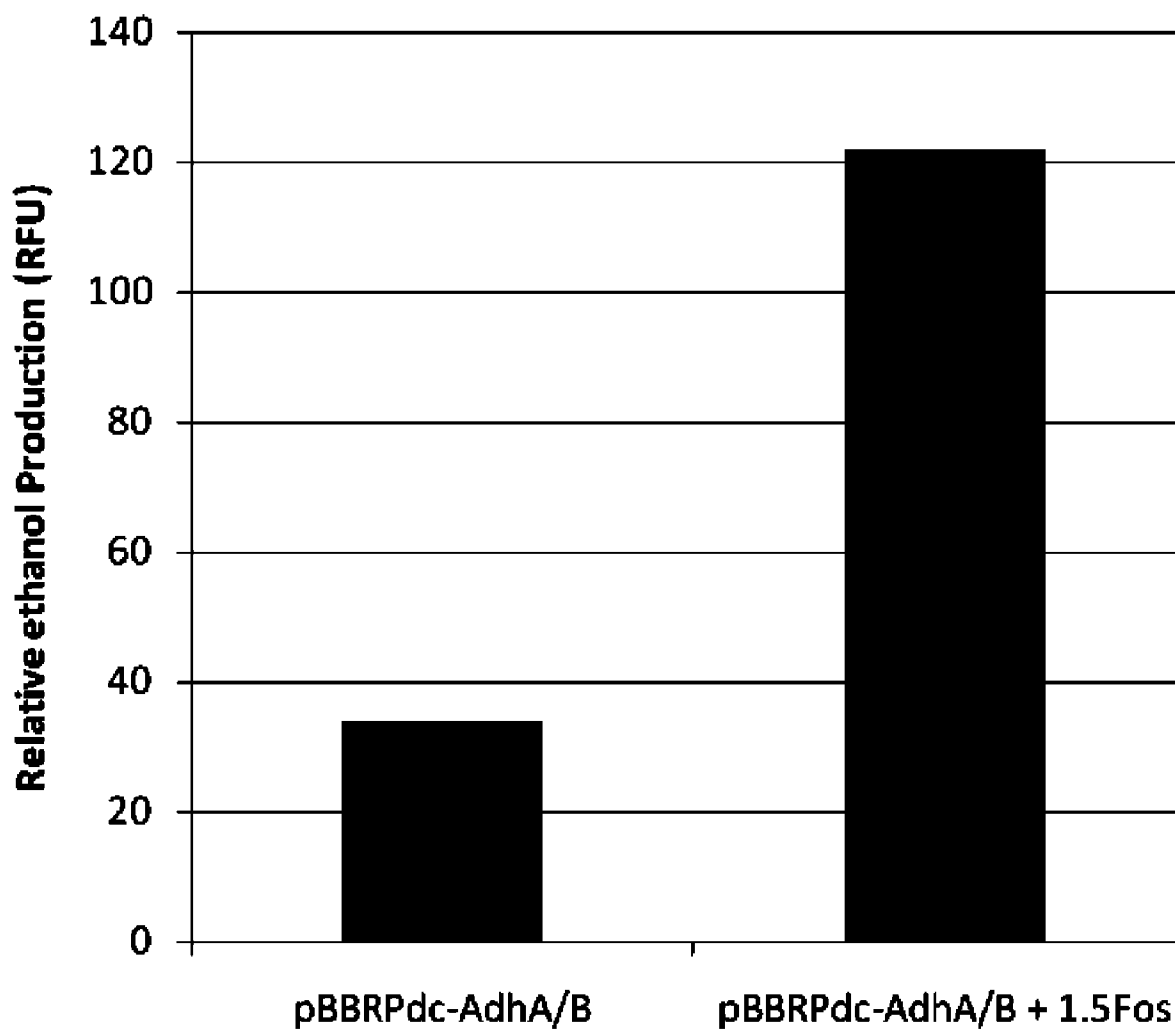
FIG. 55

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*FIG. 56A**FIG. 56B*

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Production of Ethanol from Alginate

*FIG. 57*