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Bio-Based Production of C2–C6 Platform Chemicals

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ABSTRACT: Platform chemicals composed of 2–6 carbons derived from fossil resources are used as important precursors for making a variety of chemicals and materials, including solvents, fuels, polymers, pharmaceuticals, perfumes, and foods. Due to concerns regarding our environment and the limited nature of fossil resources, however, increasing interest has focused on the development of sustainable technologies for producing these platform chemicals from renewable resources. The techniques and strategies for developing microbial strains for chemicals production have advanced rapidly, and it is becoming feasible to develop microbes for producing additional types of chemicals, including non-natural molecules. In this study, we review the current status of the bio-based production of major C2–C6 platform chemicals, focusing on the microbial production of platform chemicals that have been used for the production of chemical intermediates, building block compounds, and polymers.

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KEYWORDS: biomass; building block chemical; platform chemical; polymer; metabolic engineering; systems metabolic engineering

Introduction

Platform chemicals are the ones that serve as the basic starting materials for producing chemical intermediates, building block compounds, and polymers. For example, lactic acid and ethylene are used as building block compounds for the production of polylactide (PLA) and polyethylene (PE), respectively (Fig. 1). Over the past 10 years, bio-plastic PLA has reached the stage of being directly produced on an industrial scale from biomass-derived lactic acid. However, other commercial platform chemicals are still mostly produced by petrochemical processes that use fossil oil as the starting raw material (Erickson et al., 2012; Jung et al., 2010; Lee et al., 2011a; Park et al., 2012; Yang et al., 2010). In recent years, bio-based production of platform chemicals from renewable resources has attracted increasing attention as an alternative to petrochemical production methods, due to the limited nature of fossil oil, increasing oil prices, and global concerns on sustainability and the environment (Kamm, 2007; Lee et al., 2011b; Prather and Martin, 2008).

The key factors that should be addressed for the bio-based production of platform chemicals include the development of natural or engineered microorganisms for efficient production, compatibility with existing industrial infrastructures, optimization of downstream processes, and access to cost-competitive and sustainable resources (Hatti-Kaul et al., 2007; Lee et al., 2011b). The development of efficient microorganisms capable of producing target compounds at a sufficiently high titers, yields, and industrial production levels remains a significant challenge and a major limiting step. The optimization of downstream processes is interrelated with the development of host microorganisms, and both will play important roles in reducing production costs and allowing bio-based processes to compete against the current petrochemical processes.

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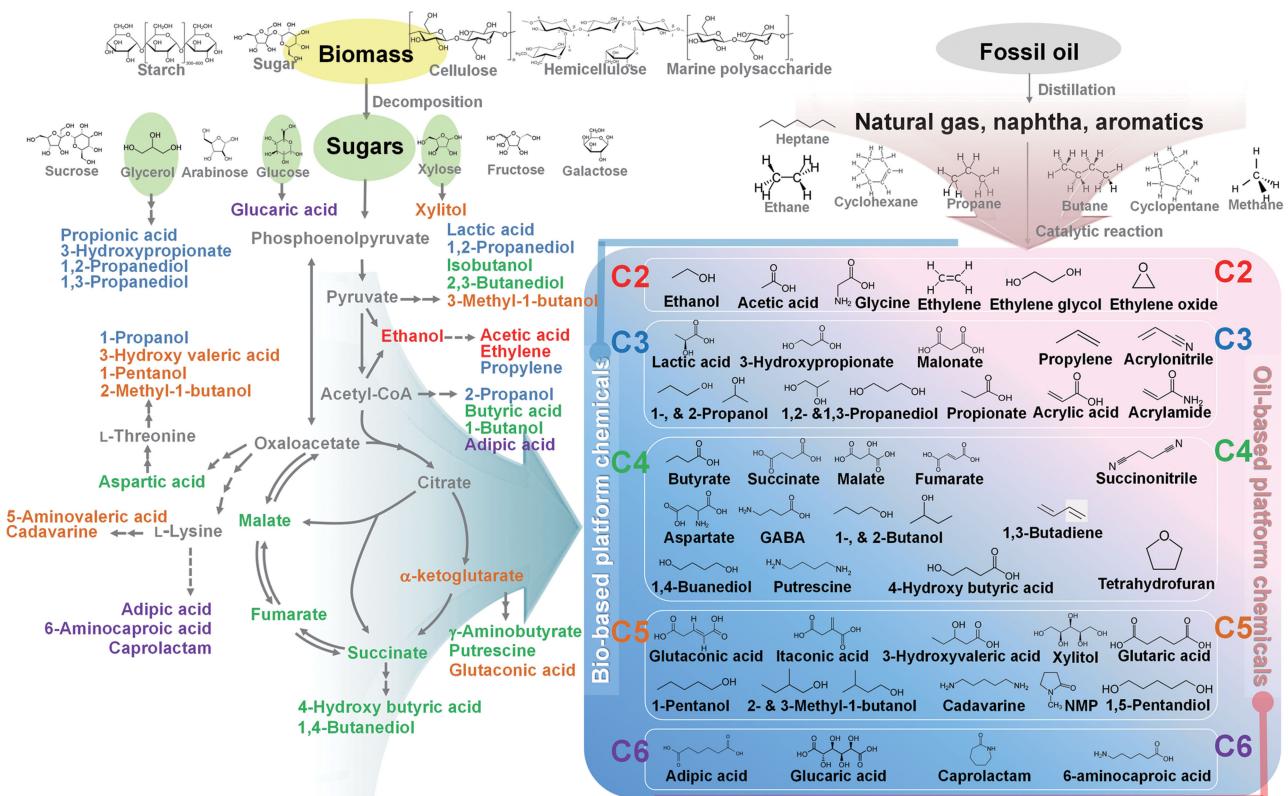


Figure 1. Platform chemical production trends are shifting away from petroleum-based methods to biology-based processes. Representative building blocks and platform chemicals that are currently obtained mostly from fossil oil are shown (right bottom box, not all platform chemicals currently produced are shown). Most of the platform chemicals currently produced are generally derived from petroleum fractions. Chemicals obtained from the refineries are used as precursors for preparing platform chemicals (red arrow). Many efforts are currently being directed toward developing new bio-based technologies (blue arrow, i.e., bio-refineries) capable of producing the same platform chemicals (blue shades replacing red shades) while addressing environmental concerns. Simplified biosynthetic networks (gray arrows) for producing platform chemicals (or corresponding intermediate chemicals) that can occur in a microorganism are illustrated (left portion of the figure). The end products and intermediates produced from various metabolic pathways vary by their carbon numbers. The different font colors represent platform chemicals with different carbon numbers: red, 2 carbons; blue, 3 carbons; green, 4 carbons; orange, 5 carbons; and purple, 6 carbons. The renewable carbon sources (or precursors) that can be used for producing such chemicals include sucrose, glycerol, arabinose, glucose, xylose, fructose, and galactose (green oval), which are obtained by decomposition of starch, sugarcane, plants, and algae (yellow oval).

During the past decade, systems metabolic engineering for the production of platform chemicals has improved significantly, as have bioinformatics and biotechnology (Becker and Wittmann, 2012; Kim et al., 2008; Krivoruchko et al., 2011; Lee et al., 2011b, 2012b; Nielsen and Pronk, 2012; Park and Lee, 2008; Tan and Liao, 2012; Wahrheit et al., 2011). As a result, biological production of some platform chemicals, including succinic acid, 1,4-butanediol, isobutanol, and isoprene, are currently in line for industrial-scale production (Erickson et al., 2012). In this communication, we review the current development status of the bio-based platform chemicals used in the polymer and chemical industries.

Platform Chemicals and Their Applications in Industry

Although most of the platform chemicals shown in Figure 1 are directly produced from fossil oil, some could be replaced

by bio-based chemicals at this time (Carbonell et al., 2012; Curran and Alper, 2012; Hong and Nielsen, 2012; Murphy, 2011; Papagianni, 2012; Ye and Bhatia, 2012). Bio-based platform chemicals are derived from renewable resources, such as agricultural products, lignocellulosics, and marine biomass, making them carbon neutral and sustainable (Hatti-Kaul et al., 2007; Jang et al., 2012b). Biological production by metabolically engineered microorganisms has been reported for a number of platform chemicals, including carboxylic acids, hydroxy acids, amino carboxylic acids, alcohols, amines, and aromatics (Fig. 1).

Building block compounds and platform chemicals possess chemical moieties that serve as linkages during polymerization or chemical reactions (Fig. 2). For example, diamines and dicarboxylic acids serve as building block compounds by offering amine and carboxylic groups, respectively, to form amide bonds during the polymerization stages of polyamide production (Fig. 2). Dicarboxylic acids (oxalic, malonic, succinic, glucaric, adipic, fumaric, and malic acids), diamines (ethylenediamine, cadaverine,

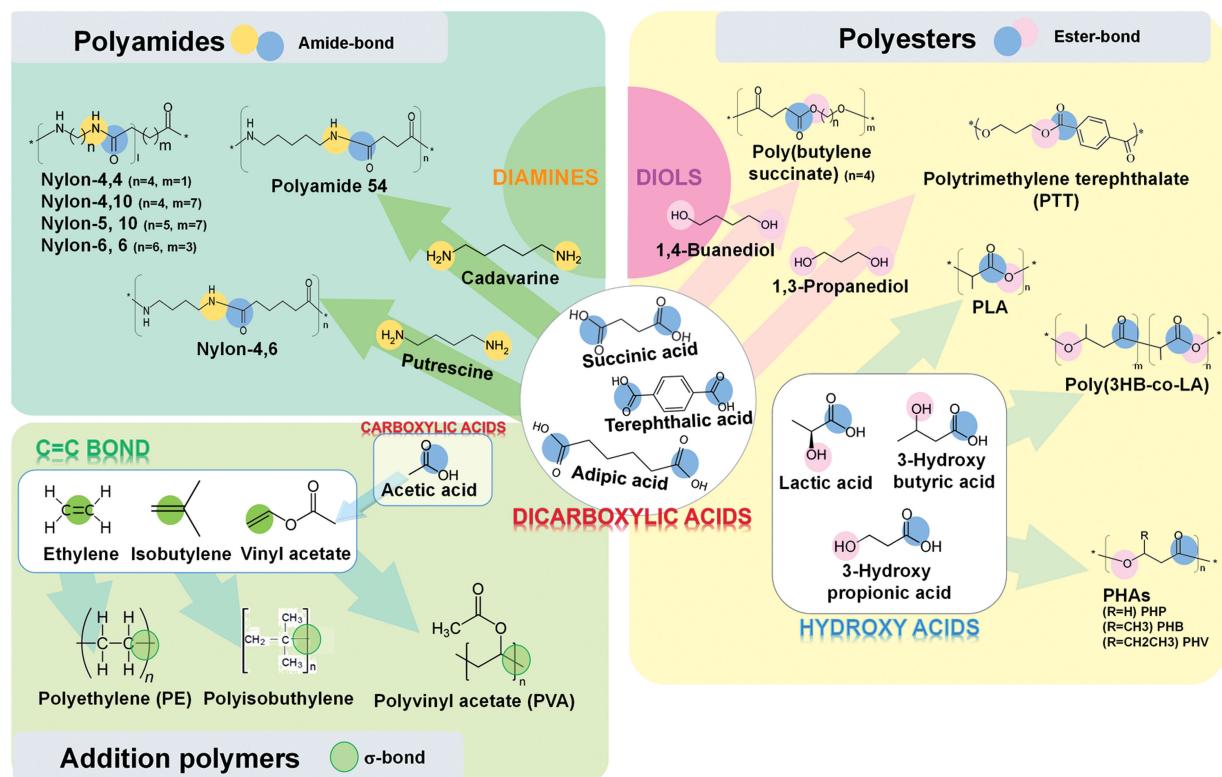


Figure 2. Various polymerization schemes for generating the platform and building block chemicals. The carboxylic groups (blue ellipses) of platform chemicals can be reacted with the amino groups (yellow ellipses) of other platform chemicals to produce highly durable polyamides through the formation of amide bonds (yellow ellipses conjugated with blue ellipses). The dicarboxylic acids can also be polymerized with different alcohols (pink ellipses) to produce numerous polyester fabrics and fibers by forming ester bonds (blue ellipses conjugated with pink ellipses). Polymerization of acetic acid derivatives that have unsaturated carbon–carbon bonds (green-outlined ellipses) generates plastic materials (e.g., PE) by forming new carbon–carbon bonds (green-outlined ellipses) resulting from the addition polymerization. Polymerization of hydroxy acids produces polymers (e.g., PLA or PHAs) by the formation of ester bonds. Building block chemicals biologically produced by fermentation can be used for producing various synthetic polymers, just as if they had been produced from petroleum. Further development of biotechnology and infrastructure for the economical production of such chemicals will ultimately lower the demand for petroleum-based production methods.

and putrescine), diols (ethylene glycol, propanediols, and butanediols), and aldehydes (formaldehyde) are the most common building block compounds used in condensation polymerization reactions (Odian, 2004). See Figure 2 for details on combining building block compounds to produce polymers, and the resulting linkages that are formed. Another building block chemical, ethylene, has a carbon–carbon double bond that serves as a π -bond to make a σ -bond linkage to an adjacent ethylene molecule during the polymerization stage of PE production (Fig. 2). Building block compounds containing carbon-to-carbon double bonds (e.g., ethylene, isobutylene, acrylonitrile, vinyl chloride, styrene, methyl methacrylate, vinyl acetate, and isoprene) are typically used for addition polymerization (Odian, 2004; Fig. 2). Some platform chemicals, such as mono-carboxylic acids (acetic, propionic, butyric, petanoic, and hexanoic acids) and mono-ols (ethanol, propanol, *n*-/iso-butanol, and pentanols) are used as precursors to form polymers, followed by chemical reactions that convert them into building block compounds (Fig. 2). In the next sections, we review the biological production of C2–C6 platform chemicals. The sections are arranged by the functional group

chemicals and then divided into subsections covering different platform chemicals with the same carbon numbers. The platform chemicals are all summarized in Table I.

Production of C2 Chemicals

In this section, we cover the bio-based production of representative C2 platform chemicals, including ethanol, ethylene, ethylene glycol, and acetic acid. Biologically produced ethanol can be chemically processed into ethylene (C2), ethylene glycol (C2), and propylene (C3), which are used as building blocks for consumer products. Biological methods were used for acetic acid production in the past, but this process has been replaced by chemical methods in all but the food industry.

Ethanol and Its Derivatives, Ethylene, and Ethylene Glycol

Currently, ethanol is biologically produced from corn starch and sugarcane sucrose on sufficiently large scales in the USA

Table I. Current status of the production of platform chemicals using microorganisms.

Carbon length	Platform chemical	Leading host	Substrates and/or conditions	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)	Refs.
C2	Ethanol	<i>S. cerevisiae</i>	Ammonia fiber expansion (AFEX)-corn stover (CS)-hydrolysates, batch fermentation	40	0.46	0.8	Lau and Dale (2009)
		<i>S. cerevisiae</i>	Cellbiose, xylose, and glucose, batch fermentation	48	0.37	0.8	Ha et al. (2011)
	<i>Z. mobilis</i>		Glucose an xylose, batch fermentation	62	0.46	1.29	Joachimsthal et al. (1999)
	<i>E. coli</i>		Xylose, batch fermentation	23.5	0.48	— ^a	Wang et al. (2011b)
	<i>A. aceti</i>		Ethanol, batch fermentation	111.7	—	0.6	Nakano et al. (2006)
	<i>P. acidipropionici</i>		Glycerol, fed-batch fermentation in fibrous bed bioreactor	106	0.56	0.035	Zhang and Yang (2009)
C3	Lactic acid	<i>Sporolactobacillus</i>	Glucose, 30 L fed-batch supplemented with 40 g/L peanut meal	207	0.93	3.8	Wang et al. (2011a)
		<i>E. coli</i>	Glucose, fed-batch fermentation	138	0.86	3.5	Zhu et al. (2007)
		<i>K. pneumonia</i>	Glycerol, fed-batch fermentation	16.0	—	.01	Ashok et al. (2011)
	3-Hydroxypropionic acid	<i>E. coli</i>	Glycerol, fed-batch fermentation	38.7	0.34	0.53	Rathnasingh et al. (2009)
	Propanol	<i>E. coli</i>	Glucose, flask culture	3.9	—	0.04	Atsumi and Liao (2008)
	Iso-propanol	<i>C. acetobutylicum</i>	Glucose, anaerobic flask culture	5.1	—	—	Lee et al. (2012a)
C4	1,2-Propanediol	<i>E. coli</i>	Glucose, fed-batch fermentation	13.6	0.15	0.28	Jojima et al. (2008)
		<i>C. thermosaccharolyticum</i>	Glucose, anaerobic batch fermentation	9.1	0.20	0.35	Sánchez-Riera et al. (1987)
	1,3-Propanediol	<i>E. coli</i>	Glycerol, batch fermentation	5.6	0.21	0.077	Clomburg and Gonzalez (2011)
		<i>C. acetobutylicum</i>	Glycerol, anaerobic fed-batch fermentation	83.6	0.54	1.70	Gonzalez-Pajuelo et al. (2005)
		<i>E. coli</i>	Glucose, fed-batch 10 L fermentation	135	0.51	3.5	Nakamura and Whited (2003)
	Butyric acid	<i>C. tyrobutyricum</i>	Glucose, fed-batch fermentation (Δ ack/ Δ pta)	32.5-41.7	0.38-0.42	0.24-0.68	Zhu et al. (2010), Liu et al. (2006)
C5		<i>C. tyrobutyricum</i>	Glucose, repeated fed-batch fermentation by immobilized cells in a fibrous bed bioreactor	86.9	0.46	1.1	Jiang et al. (2011)
	Succinic acid	Engineered rumen bacteria	Glucose, anaerobic fed-batch fermentation	52-106	0.76-0.88	1.8-2.8	Guettler et al. (1996), Lee et al. (2006)
		<i>E. coli</i>	Glucose, fed-batch fermentation	73-87	0.8-1.0	0.7-0.9	Jantama et al. (2008a,b), Thakker et al. (2012)
		<i>C. glutamicum</i>	Glucose, fed-batch fermentation	140-146	0.92-1.1	1.9-2.5	Litsanov et al. (2012a,b), Okino et al. (2008a)
	Malic acid	<i>Aspergillus flavus</i>	Glucose, batch fermentation	113	0.95	0.59	Battat et al. (1991)
		<i>S. cerevisiae</i>	Glucose, fed-batch fermentation	59	0.31	0.19	Zelle et al. (2008)
C6	Fumaric acid	<i>R. arrhizus NRRL 2582</i>	Glucose, two-stage fermentation	33.9	0.47	1.06	Zhang et al. (2011)
		<i>L. brevis NCL912</i>	Glucose, batch fermentation	97.7	0.81	1.02	Kenearly et al. (1986)
	GABA	<i>C. glutamicum</i>	Glucose and glutamate, fed-batch fermentation	103.7	—	—	Li et al. (2010)
		<i>E. coli</i>	Glucose, batch fermentation	2.2	—	0.01	Shi and Li (2011)
	1-Butanol	<i>C. acetobutylicum</i>	Glucose, anaerobic batch fermentation (Δ buk)	16.7	—	0.31	Harris et al. (2000)
	Isobutanol	<i>E. coli</i>	Glucose, batch cultivation	20	0.33-0.36	0.20-0.29	Dellomonaco et al. (2011), Shen et al. (2011)
C7	1,4-Butanediol	<i>C. glutamicum</i>	Glucose, fed-batch fermentation	13.0	0.20	0.33	Atsumi et al. (2008b)
		<i>E. coli</i>	Glucose, microaerobic fed-batch fermentation	18	—	0.15	Blombach et al. (2011b)
	2,3-Butanediol	<i>K. pneumonia SDM</i>	Glucose, fed-batch fermentation	150	0.48	4.21	Yim et al. (2011)
		<i>S. marcescens</i>	Glucose, fed-batch fermentation	152	0.46	2.67	Ma et al. (2009)
C8	Putrescine	<i>E. coli</i>	Glucose, fed-batch culture	24.2	—	0.75	Zhang et al. (2010)
						Qian et al. (2009)	

C5	Itaconic acid	<i>Aspergillus terreus</i> IFO-6365	Glucose and corn steep, flask and 100 L batch fermentations	82–85	0.54
			Glucose, flask batch culture	—	0.06
			Glucose and levulinic acid, flask batch cultivation	5.3	—
			Glucose and threonine, flask batch cultivation	1.3	—
			Glucose, flask batch cultivation	0.81	—
			Glucose	0.5	—
			Glucose	1.25	0.17
			Glucose	1.28	0.11
			Xylose, oxygen-limited condition with cell recycling	182	12.0
			Glucose and xylose, fed-batch fermentation	38	—
C6	3-Hydroxyvalerate	<i>E. coli</i> <i>P. putida</i>	Glucose, fed-batch fermentation	9.61	0.12
			Glucose, flask culture	2.5	—
			Glucose, fed-batch cultivation	14	0.41
			Glucose, flask batch culture	0.14	0.006
			3-Dehydroshikimate (DHS)	4.2	0.12
			Cadaverine	—	—
			Glucaric acid	—	—
			Anthranilic acid	—	—
			Phenol	—	—
			Catechol	—	—

^aNot reported.

and Brazil, respectively. It is mainly used as a fuel (Geddes et al., 2011; Jang et al., 2012b), or as a platform chemical for the production of ethylene and ethylene glycol via chemical catalysis.

In this pathway, microorganisms form pyruvate from sugars; pyruvate decarboxylase (Pdc) or pyruvate-formate lyase (Pfl) and acetaldehyde dehydrogenase (ActDH) remove carbon dioxide from pyruvate to form acetaldehyde; and then alcohol dehydrogenase (Adh) coupled with NADH reduce acetaldehyde to ethanol (Fig. 3A). Two microorganisms, *Saccharomyces cerevisiae* (Gray et al., 2006; Lin and Tanaka, 2006; Mielenz, 2001; Service, 2007) and *Zymomonas mobilis* (Deanda et al., 1996; Zhang et al., 1995), are known for their ability to produce ethanol from hexoses. Pentose, which makes up 20–30% of the lignocellulosic biomass, has recently emerged as a promising renewable feedstock for bioethanol production, but these two strains are unable to utilize pentose sugars (Gray et al., 2006; Lin and Tanaka, 2006; Mielenz, 2001; Service, 2007). Numerous metabolic engineering strategies (e.g., the introduction of fungal/bacterial xylose catabolic pathways and xylose-specific transporters, protein engineering to support balanced cofactor usage, and adaptive evolution) have been used in an effort to develop strains that can utilize both hexose and pentose sugars (Chen, 2011; Chu and Lee, 2007; Hahn-Hagerdal et al., 2007; Jeffries, 2006; Jeffries and Jin, 2004). For example, an engineered *S. cerevisiae* strain produced 40 g/L of ethanol with a 0.46 g/g yield and 0.8 g/L/h productivity from ammonia fiber expansion (AFEX)-corn stover (CS)-hydrolysates containing a mixture of 57.5 g/L glucose and 28.1 g/L xylose in batch fermentation (Lau and Dale, 2009). An engineered *Z. mobilis* strain produced 62 g/L ethanol with a 0.46 g/g yield and 1.29 g/L/h productivity from a mixture of 75 g/L glucose and 75 g/L xylose in batch fermentation (Joachimsthal et al., 1999). Ha et al. (2011) sought to use cellobiose, which is the major form of hexose in lignocellulosic biomass hydrolysates. The engineered *S. cerevisiae* produced 48 g/L of ethanol with a 0.37 g/g yield and 0.8 g/L/h productivity from a mixture containing 80 g/L cellobiose, 40 g/L xylose, and 10 g/L glucose in batch fermentation (Ha et al., 2011). In the wider context of ethanol production from pentose, *Escherichia coli* (which naturally uses a wide spectrum of sugars, including pentose) was developed as a host strain for ethanol production (Geddes et al., 2011; Jarboe et al., 2007; Kim et al., 2007; Wang et al., 2011b; Zhou et al., 2005, 2008). One engineered *E. coli* strain showed ethanol production with a titer of 23.5 g/L and a yield of 0.48 g/g from xylose batch fermentation (Wang et al., 2011b). Nonetheless, the co-utilization of pentose and glucose or cellobiose remains a challenge for ethanol producers. Furthermore, the processes for biomass pretreatment must be optimized to minimize inhibitor formation, or strains should be engineered to resist inhibition.

Ethylene and ethylene glycol can be chemically converted from bioethanol, and may be broadly considered bio-based platform chemicals. Ethylene is widely used in the chemical

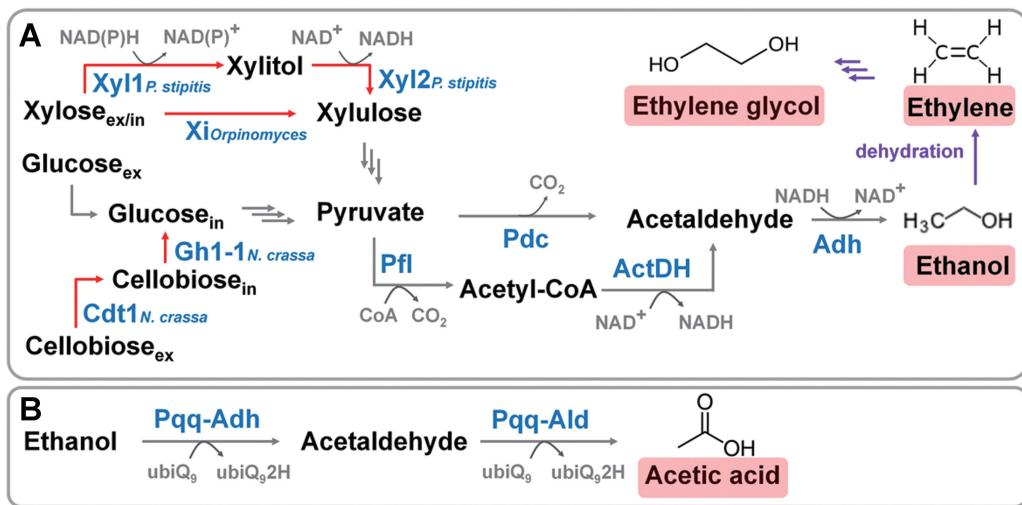


Figure 3. Representative pathways for the production of C2 platform chemicals. **A:** Metabolic pathways that produce ethanol from xylose, glucose, and cellobiose. **B:** Ethanol fermentation pathways that produce acetic acid. Major metabolic intermediates and enzymes with their common sources are shown in black and blue, respectively, next to the corresponding reactions. Multiple arrows indicate multistep reactions. Reactions mediated by heterologous enzymes and chemical catalysis are shown in red and purple, respectively. Final products are highlighted in red boxes with their chemical structures. Metabolites: Pqq-Adh, alcohol dehydrogenase coupled with pyrroloquinoline quinone; Pqq-Ald, aldehyde dehydrogenase coupled with pyrroloquinoline quinone; ubiQ₉, ubiquinone. Enzymes: Adh, alcohol dehydrogenase; ActDH, acetaldehyde dehydrogenase; Cdt1, cellobextrin transporter; Gh1-1, β -glucosidase; Pdc, intracellular pyruvate decarboxylase; Pfl, pyruvate formate lyase; Xi, xylose isomerase; Xyl1, xylose reductase; Xyl2, xylitol dehydrogenase.

and polymer industries, and its worldwide production of 120 million tons in 2008 (Seddon, 2010) exceeded that of any other carbon-based chemical. Bio-based ethylene and ethylene glycol can be used as building block compounds for the production of PE and polyethylene terephthalate (PET; Fig. 2), and coca-cola currently uses so-called “Plant Bottles” made from bioethanol-derived PE and PET. However, we do not yet have a biotechnological process for directly producing ethylene and ethylene glycol. Thus, we need to continue our efforts to engineer microorganisms for biological one-step production of ethylene and ethylene glycol.

C2 Carboxylic Acid: Acetic Acid

Acetic acid is an important C2 platform chemical used in the production of vinyl acetate, acetic anhydride, and cellulose acetate (Fig. 2). Vinyl acetate and acetic anhydride are building block compounds for polymers such as polyvinyl acetate (PVA) and cellulose acetate, which are used to produce vinyl plastics and photographic films, respectively (Fig. 2). Worldwide annual production of acetic acid was estimated to be 7 million tons in 2007 (Sauer et al., 2008). Until the late 19th century, all acetic acid was produced by the fermentation of sugar to ethanol, followed by a second-stage microbial oxidation to acetic acid. Currently, acetic acid is produced by chemical methods, such as methanol carbonylation, acetaldehyde oxidation, and butane liquid-phase oxidation processes.

The biological production of acetic acid is performed by bacteria such as *Acetobacter*, *Gluconacetobacter*, or *Gluconobacter* via the acetoous fermentation of ethanol (Yamada and Yukphan, 2008). These acetic acid bacteria use Adh and aldehyde dehydrogenase (Ald) to produce 50–120 g/L acetic acid from ethanol (Raspor and Goranovic, 2008; Fig. 3B). Adh and Ald are coupled with pyrroloquinoline quinone (Pqq) and linked to the respiratory chain in the cytoplasmic membrane, permitting *Acetobacter* to grow on ethanol (Fig. 3B). The major strain improvement strategies for the production of acetic acid by *Acetobacter aceti* include amplifying the enzymes that participate in acetic acid production (e.g., Adh), and enhancing the acetic acid exporter, which is involved in reducing acetic acid stress. The 75 kDa subunit of the *Acetobacter polyoxogenes* Ald was introduced into *A. aceti*, causing the productivity and titer to increase from 1.8 g/L/h and 68.4 g/L to 4.0 g/L/h and 96.6 g/L, respectively (Fukaya et al., 1992). An ATP-binding cassette (ABC) transporter encoded by the *aatA* gene was identified through proteomic studies, and its overexpression in *A. aceti* improved the titer of acetic acid from 103.7 to 111.7 g/L in batch fermentation (Nakano et al., 2006).

The complete genome sequences of *Gluconacetobacter oxydans* (Prust et al., 2005), *Acetobacter pasteurianus* (Azuma et al., 2009), and *Gluconacetobacter europaeus* (Andres-Barrao et al., 2011) were recently published, and genome sequencing of *A. aceti* is in progress (<http://genome.jgi.doe.gov/genome-projects>). These studies will provide valuable information for the extensive, systems-level metabolic engineering of host strains toward improving

acetic acid production and resistance. Finally, although acetogenic bacteria are not covered in this section (they have lower production levels than acetic acid bacteria), acetic acid production from gasification products (e.g., CO, CO₂, and H₂) by anaerobic acetogens is a promising process that may be developed for use on a variety of biomasses.

Production of C3 Chemicals

In this section, we cover the bio-based production of representative C3 platform chemicals, including carboxylic acid (propionic acid), hydroxy acids (lactic and 3-hydroxypropionic acids), alcohols (1-propanol and isopropanol), and diols (1,2 or 1,3-propandiol). Among them, bio-based 1,3-propandiol has recently been commercialized by DuPont Tate and Lyle BioProducts.

C3 Carboxylic Acid: Propionic Acid

Propionic acid has many application areas, including the production of cellulose fibers, herbicides, perfumes, and pharmaceuticals (Boyaval and Corre, 1995). In industry, propionic acid is mainly produced through petrochemical routes, using ethylene as a starting material (Hohenschutz, 1974).

Fermentative biological production of propionic acid has been investigated in various bacterial organisms, especially the genera *Propionibacterium*, and using various carbon sources, including glucose, glycerol, lactate, and sucrose (Coral et al., 2008; Feng et al., 2010; Zhang and Yang, 2009; Zhu et al., 2010). Glycerol was recently proven to be a promising carbon source for propionic acid production, based on higher yields and reduced byproduct formation (Liu et al., 2012). The metabolic pathway used to produce propionic acid from glycerol is shown in Figure 4A. In glycerol fermentation, propionic acid is mainly produced through succinate. A metabolically engineered *Propionibacterium acidipropionici* strain produced 106 g/L propionic acid with a 0.56 g/g yield and 0.035 g/L/h productivity from glycerol during fed-batch fermentation in a fibrous-bed bioreactor (Zhang and Yang, 2009). However, many hurdles must be overcome (toxicity, byproduct formation, low productivity, and redox imbalance) before propionic acid can be economically produced on a large scale.

C3 Hydroxy Acids: Lactic and 3-Hydroxypropionic Acids

Lactic and 3-hydroxypropionic acids, both of which containing a hydroxyl group and a carboxyl group, are versatile block compounds that are particularly useful for the production of biodegradable polyesters, such as PLA and

poly(3-hydroxypropionate) (Fig. 2). At present, lactic acid is produced by the fermentation of carbohydrates; the worldwide lactic acid production volume in 2007 was estimated to be 150,000 tons (Sauer et al., 2008). Current 3-hydroxypropionate production relies mainly on chemical synthesis routes, including the oxidation of 1,3-propanediol, the oxidation of 3-hydroxypropionaldehyde, and the hydration of acrylic acid.

Lactic acid is produced directly from pyruvate by the lactate dehydrogenase (Ldh) of various microorganisms, including *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Fig. 4B). Production of lactic acid by microorganisms has the advantage of producing a more optically pure lactic acid compared to chemical synthesis via hydrolysis of lactonitrile. L-, D-, or DL-Lactic acid may be biologically produced by utilizing lactic acid dehydrogenases (Ldh) with different stereospecificities (Okino et al., 2008b; Wu et al., 2011). Recently, an engineered *Sporolactobacillus* produced 207 g/L D-lactate from 223 g/L of glucose with a productivity of 3.8 g/L/h and an optical purity of 99.3% in 30-L fed-batch fermentation supplemented with 40 g/L of peanut meal as a nitrogen source (Wang et al., 2011a). Lactic acid may also be produced by metabolically engineered *E. coli* strains (Zhu et al., 2007). A fed-batch fermentation of a metabolically engineered *E. coli* strain showed a production of 138 g/L of D-lactic acid with an overall yield of 0.86 g/g of glucose and a productivity of 3.5 g/L/h (Zhu et al., 2007). Although bio-based production methods for lactic acid have advanced enough to be used at an industrial level, further improvements of their economy could be achieved by developing inexpensive carbon substrates and reducing the requirement of lactic acid bacteria for complex nitrogen sources.

Klebsiella pneumoniae is a promising microorganism for 3-hydroxypropionic acid production from sugars such as glycerol (Ashok et al., 2011). 3-Hydroxypropionate can be produced from glycerol via 3-hydroxypropionic aldehyde (3-HPA; Fig. 4C). Recently, the metabolic pathway of *K. pneumoniae* was engineered to direct carbon flux from glycerol to 3-hydroxypropionic acid by the overexpression of endogenous glycerol dehydratase (DhaB) and γ -glutamyl- γ -aminobutyraldehyde dehydrogenase (PuuC; Fig. 4C). Additionally, the *dhaT* gene (encoding 1,3-propanediol oxidoreductase; DhaT) was deleted to eliminate a competing pathway (Fig. 4C). A fed-batch fermentation of this engineered strain produced 16.0 g/L 3-hydroxypropionic acid from glycerol with a productivity of 0.66 g/L/h (Ashok et al., 2011). However, a recombinant *E. coli* strain harboring α -ketoglutaric semialdehyde dehydrogenase (Sadh) from *Azospirillum brasiliense*, along with DhaB and glycerol dehydratase reactivase from *K. pneumoniae*, produced 38.7 g/L of 3-hydroxypropionic acid with a yield of 0.34 g/g and productivity of 0.53 g/L/h in glycerol fed-batch fermentation (Rathnasingh et al., 2009). Unlike *K. pneumoniae*, *E. coli* lacks the coenzyme B12 metabolism required for DhaB activity; therefore, addition of coenzyme B12 to the culture

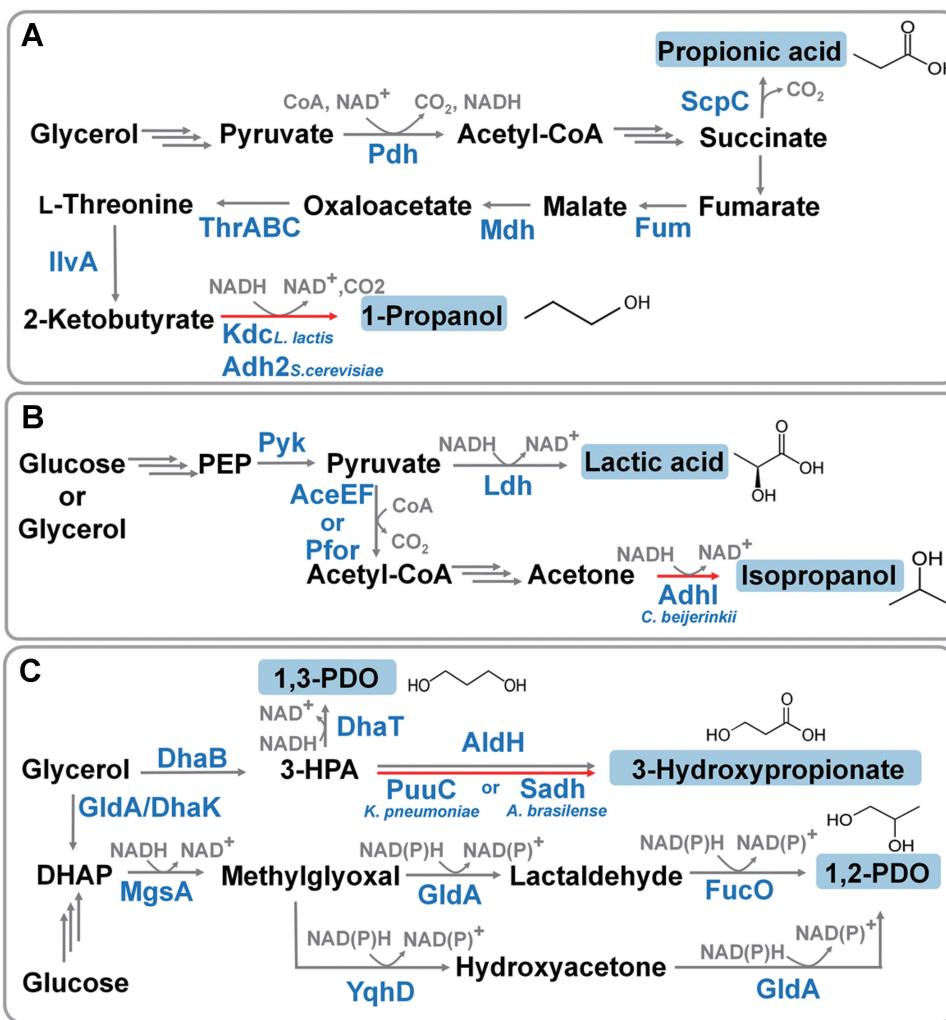


Figure 4. Representative pathways for the production of C3 platform chemicals. **A:** Metabolic pathways that produce propionic acid and 1-propanol from glycerol. **B:** Metabolic pathways that produce lactic acid, 1,2-propanediol (1,2-PDO), and isopropanol from glycerol. **C:** Metabolic pathways that produce 1,2-PDO, 1,3-propanediol (1,3-PDO), and 3-hydroxypropionate from glycerol. Major metabolic intermediates and enzymes with their common sources are shown in black and blue, respectively, next to the corresponding reactions. Multiple arrows indicate multistep reactions. Reactions mediated by heterologous enzymes and chemical catalysis are shown in red and purple, respectively. Final products are highlighted in blue boxes with their chemical structures. Metabolites: DHAP, dihydroxypropionyl phosphate; G3P, glyceraldehyde-3-phosphate; 3-HP, 3-hydroxypropionate; 3-HPA, 3-hydroxypropionyl aldehyde; 1,2-PDO, 1,2-propanediol; 1,3-PDO, 1,3-propanediol; PEP, phosphoenolpyruvate. Enzymes: AceE, pyruvate dehydrogenase component 1; AceF, pyruvate dehydrogenase component 2; Adh1, secondary alcohol dehydrogenase; Adh2, alcohol dehydrogenase; AldH, aldehyde dehydrogenase; DhaB, glycerol dehydratase; GldA, glycerol dehydrogenase; DhaT, 1,3-propanedione dehydrogenase; IlvA, threonine dehydratase; Kdc, ketoacid decarboxylase; Ldh, lactate dehydrogenase; Mdh, malate dehydrogenase; MgsA, methylglyoxal synthase; Pfor, pyruvate:ferredoxin oxidoreductase; PuuC, γ -glutamyl- γ -aminobutyraldehyde dehydrogenase; Pyk, pyruvate kinase; Sadh, α -ketoglutaric semialdehyde dehydrogenase from *Azospirillum brasilense*; ScpC, propionyl-CoA:succinate-CoA transferase; ThrABC, aspartokinase complex; TpiA, triosephosphate isomerase; YqhD, aldehyde oxidoreductase.

medium could increase the cost in large-scale production. However, utilizing glycerol for the biological production of 3-hydroxypropionic acid could be a promising sustainable process because glycerol is a major byproduct of biodiesel production.

C3 Alcohols: 1-Propanol and Iso-Propanol

1-Propanol and iso-propanol can be used in place of methanol as esterifying reagents, and the formed esters show

reduced crystallization at low temperatures (Hanai et al., 2007). Chemically, 1-propanol is produced from ethane, carbon monoxide, and hydrogen, while iso-propanol is produced by a hydration reaction between water and propene (Jain and Yan, 2011).

2-Ketobutyrate, a component of the amino acid biosynthesis pathways, was used as a precursor of 1-propanol in a metabolically engineered *E. coli* strain (Atsumi et al., 2008a; Fig. 4B) by sequential reactions of *Lactococcus lactis* 2-ketoacid decarboxylase (Kdc) and *S. cerevisiae* Adh2 (Fig. 4B). The production of 1-propanol was further

improved by increasing the precursor availability using evolved *Methanococcus jannaschii* citramalate synthase (CimA) to enhance the conversion of pyruvate to 2-ketobutyrate (Atsumi and Liao, 2008). The engineered *E. coli* strain produced 3.9 g/L 1-propanol from glucose with a productivity of 0.04 g/L/h in flask culture (Atsumi and Liao, 2008).

Several microorganisms have been reported for isopropanol production, including wild-type *Clostridium beijerinckii* (George et al., 1983), engineered *E. coli* (George et al., 1983; Hanai et al., 2007), and engineered *Clostridium acetobutylicum* (Lee et al., 2012a). The strategy for the microbial production of isopropanol is derived from the *C. beijerinckii* pathway for converting acetyl-CoA to isopropanol via acetone (George et al., 1983; Fig. 4B). However, due to the limited metabolic information and tools, the maximum titer from the native producer was only 1.8 g/L (George et al., 1983). More recently, a *C. acetobutylicum* ATCC 824 (pIPA3) strain harboring the secondary AdhI of *C. beijerinckii* NRRL B593 and a synthetic acetone operon (*adc-ctfA-ctfB*) of *C. acetobutylicum* produced 5.1 g/L of isopropanol in anaerobic flask culture (Lee et al., 2012a). Metabolically engineered *E. coli* harboring four genes (*thl-adc-ctfA-ctfB*) involved in the acetone-producing pathway of *C. acetobutylicum* ATCC 824 and the gene encoding AdhI of NRRL B593 achieved a higher titer of 13.6 g/L with a 0.15 g/g yield and 0.28 g/L/h productivity in glucose fed-batch fermentation (Jojima et al., 2008).

C3 Diols: 1,2 or 1,3-Propandiol

1,2-Propanediol (PDO) and 1,3-propanediol, which are linear aliphatic glycols with two hydroxyl groups, are versatile building block compounds for polyesters (Fig. 2). In particular, 1,3-PDO is widely used as a monomer for producing polymers ranging from terephthalate to aromatic polyester polytrimethylene terephthalate (PTT; Fig. 2). 1,2-PDO and 1,3-PDO are mainly produced from propylene oxide and propenal, respectively, by chemical synthesis (Corma et al., 2007). 1,3-PDO is also manufactured biologically, in a process that has been commercialized by DuPont Tate and Lyle BioProducts (<http://www.duponttateandlyle.com>).

1,2-Propanediol can be produced from the glycolytic pathway via dihydroxyacetonephosphate (DHAP; Fig. 4C). Wild-type *Clostridium thermosaccharolyticum* is capable of producing up to 9.1 g/L of 1,2-PDO from glucose with a yield of 0.20 g/g and a productivity of 0.35 g/L/h in anaerobic batch fermentation (Sánchez-Riera et al., 1987). Recently, more tractable microorganisms, such as *S. cerevisiae* and *E. coli*, have been engineered for the production of 1,2-PDO. An engineered *S. cerevisiae* strain that produced small amounts of 1,2-PDO from glucose was generated by expressing the *E. coli* *mgsA* and *gldA* genes, which encode methylglyoxal synthase (MgsA) and glycerol dehydrogenase (GldA), respectively (Lee and Dasilva, 2006; Fig. 4C). A

recombinant *E. coli* strain overexpressing MgsA, GldA, and aldehyde oxidoreductase (YqhD), along with the dihydroxyacetone kinase (DhaK) from *Clostridium freundii* as an alternative for native DhaK, produced 1,2-propanediol up to 5.6 g/L with a yield of 0.21 g/g and a productivity of 0.08 g/L/h from glycerol in batch fermentation (Clomberg and Gonzalez, 2011; Fig. 4C).

1,3-PDO can be produced in glycerol fermentation with various microbes, including *Clostridium*, *Enterobacter*, *Klebsiella*, and *Lactobacillus* species (Gungormusler et al., 2010; Oh et al., 2012; Wilkens et al., 2012). In these organisms, the production of 1,3-PDO helps maintain the balance between NADH and NAD⁺ during anaerobic glycerol fermentation (Fig. 4C). Metabolic Explorer Inc. reported a 1,3-PDO production of ~83.6 g/L from glycerol with a yield of 0.54 g/g and a volumetric productivity of 1.70 g/L/h using metabolically modified *C. acetobutylicum* DG1 (pSPD5) in fed-batch fermentation (Gonzalez-Pajuelo et al., 2005). This process has been in pilot-scale operation since 2010 in France, and commercialization is expected in 2012. DuPont and Genecor International Inc. also reported that they successfully engineered an *E. coli* strain to produce 1,3-PDO up to 135 g/L with a yield of 0.51 g/g and a productivity of 3.5 g/L/h in glucose fed-batch 10-L fermentation (Nakamura and Whited, 2003). Recently, DuPont Tate and Lyle BioProducts commercialized several bio-based 1,3-PDOs, including Zemea® and Susterra® propanediol (<http://www.duponttateandlyle.com>).

Production of C4 Chemicals

In this section, we cover the bio-based production of representative C4 platform chemicals, including carboxylic acid (butyric acid), dicarboxylic acids (succinic, malic, and fumaric acids), amino (di)carboxylic acids (aspartic and γ -aminobutyric acids), alcohols (1-butanol and iso-butanol), diols (1,4-butanediol and 2,3-butanediol), diamine (putrescine), and diene (butadiene).

C4 Carboxylic Acid: Butyric acid

Butyric acid is a 4-carbon aliphatic fatty acid that is widely used in the food, perfume, and polymer industries (Zhang and Yang, 2009). It is a starting material for the formation of cellulose acetate butyrate, a polymer used for the manufacture of photographic films and eyeglass frames. Butyric acid is produced either by chemically oxidizing butane and butyraldehyde, or via sugar fermentation (Lah, 2003).

Fermentative production of butyric acid was first detected by Louis Pasteur. Since then, several bacteria belonging to the genera *Clostridium* (van Andel et al., 1985; Wu and Yang, 2003), *Butyrivibrio* (Bryant and Small, 1956), and *Enterococcus* (Centeno et al., 1999) have been shown to produce butyric acid. Butyric acid can be produced from the glycolytic pathway via condensation of acetyl-CoA to acetoacetyl-CoA (Fig. 5A). Recent studies on the production

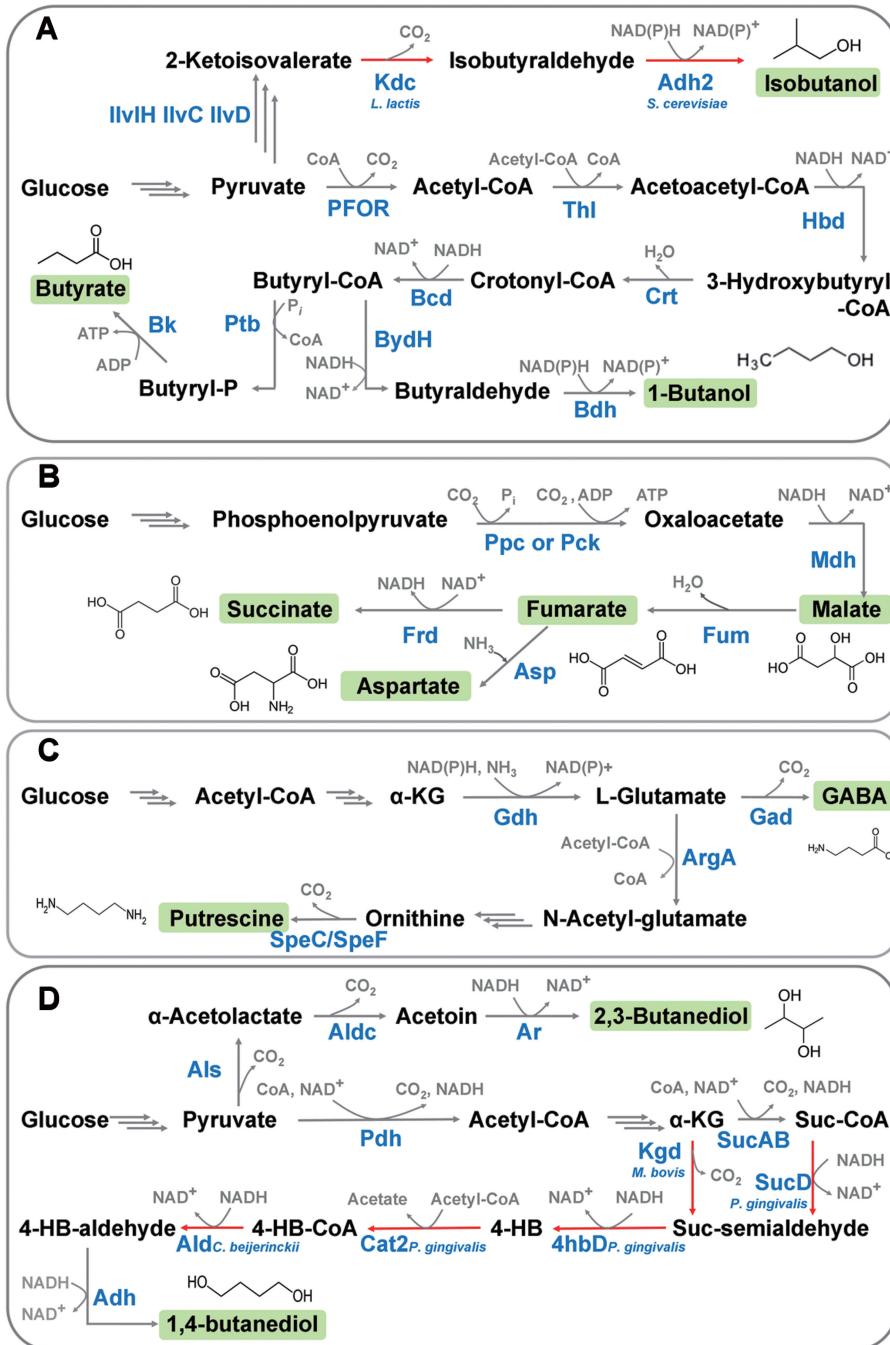


Figure 5. Representative pathways for the production of C4 platform chemicals. **A:** Metabolic pathways that produce butyrate, 1-butanol through the *Clostridial* butanol biosynthesis pathway, and isobutanol through the amino acid biosynthesis pathway. **B:** Metabolic pathways that produce succinate, fumarate, malate, and aspartate from glucose through the anaplerotic pathway. **C:** Metabolic pathways that produce putrescine and γ -aminobutyric acid (GABA) from TCA cycle intermediates. **D:** Metabolic pathways that produce 1,4- and 2,3-butanediol. Major metabolic intermediates and enzymes with their common sources are shown in black and blue, respectively, next to the corresponding reactions. Multiple arrows indicate multistep reactions. Reactions mediated by heterologous enzymes and chemical catalysis are shown in red and purple, respectively. Final products are highlighted in green boxes with their chemical structures. Metabolites: GABA, γ -aminobutyric acid; Butyryl-P, butyryl phosphate; 4-HB, 4-hydroxybutyrate; 4-HB-aldehyde, 4-hydroxybutyryl aldehyde; 4-HB-CoA, 4-hydroxybutyryl-CoA; α -KG, α -ketoglutarate; Suc-CoA, succinyl-CoA; Suc-semialdehyde, succinate semialdehyde. Enzymes: Adh, alcohol dehydrogenase; Ald, aldehyde dehydrogenase; Aldc, α -acetolactate decarboxylase; Als, α -acetolactate synthase; ArgA, *N*-acetyl-glutamate synthase; Asp, aspartase; Ar, acetoin reductase; Bcd, butyryl-CoA dehydrogenase; Bdh, butanol dehydrogenase; Bk, butyrate kinase; BydH, butyraldehyde dehydrogenase; Cat2, 4-hydroxybutyryl-CoA transferase; Crt, crotonase; Frd, fumarate reductase; Fum, fumarase; Gad, glutamate decarboxylase; Gdh, glutamate dehydrogenase; Hbd, 3-hydroxybutyryl-CoA dehydrogenase; IlvC, ketol-acid reductoisomerase; IlvD, dihydroxyacid dehydratase; IlvH, acetolactate synthase; Kdc, 2-ketoacid decarboxylase; Kgd, α -ketoglutarate decarboxylase; Mdh, malate dehydrogenase; Pck, phosphoenolpyruvate carboxylase; Ptb, phosphate butyryltransferase; SpeC/SpeF, ornithine decarboxylase; SucAB, α -ketoglutarate dehydrogenase; SucD, succinyl semialdehyde dehydrogenase; Thl, thiolase; 4hbD, 4-hydroxybutyrate dehydrogenase.

of butyric acid have been conducted using *Clostridium tyrobutyricum*, which produces high titers of butyric acid via butyryl-CoA and forms acetic acid as a byproduct (Fig. 5A). To reduce byproduct formation, researchers have used metabolic engineering strategies (e.g., deletion of the acetate-producing pathways) to enhance butyric acid yield and production titers. Individual inactivation of two genes in the acetate-producing pathways, *pta* and *ack*, which encode phosphotransacetylase and acetate kinase, respectively, improved butyric acid production, yield, and productivity to comparable degrees. The two engineered strains produced butyric acid with yields of 0.38–0.42 g/g and titers of 32.5–41.7 g/L in glucose fed-batch fermentations (Liu et al., 2006; Zhu et al., 2010). Higher titers were achieved by optimizing the fermentation processes (Huang et al., 2011; Jiang et al., 2009, 2010, 2011; Jo et al., 2008, 2009; Mitchell et al., 2009; Wu and Yang, 2003; Zhu and Yang, 2003, 2004). For instance, a significantly higher titer of 86.9 g/L with a yield of 0.46 g/g from glucose was achieved in repeated fed-batch fermentation with immobilized *C. tyrobutyricum* in a fibrous bed bioreactor (FBB; Jiang et al., 2011). However, tolerance issues prevented continuous production of butyric acid as the titer increased. Thus, the butyric acid tolerance of *C. tyrobutyricum* should be improved to achieve the higher titers, yields, and productivities required for commercial production.

C4 Dicarboxylic Acids: Succinic, Malic, and Fumaric Acids

Succinic, malic, and fumaric acids are naturally occurring C4 building blocks that are found as major metabolic intermediates in most prokaryotic and eukaryotic microorganisms (Fig. 5B). The current commercial production of these chemicals is mostly dependent on petrochemical synthesis routes in which these C4 dicarboxylic acids are polymerized into polyamides and polyesters together with diamines and diols, respectively (Fig. 2). Their diverse application areas include the pharmaceutical, agricultural, food, synthetic resin, and polymer industries (Tsao et al., 1999; Zeikus et al., 1999). C4 dicarboxylic acids and their derivatives (e.g., 1,4-butanediol, tetrahydrofuran, γ -butyrolactone, and adipic acid) make up a \$15 billion market of bulk chemicals (McKinlay et al., 2007).

The major metabolic pathway for succinate production utilizes the reductive branch of the TCA cycle (Fig. 5B), and the biological production of succinic acid through metabolic engineering has been extensively investigated in rumen bacteria, such as *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, and *Mannheimia succiniciproducens* (Guettler et al., 1999; Lee et al., 2002; Samuelov et al., 1991). These rumen bacteria produced 52–106 g/L succinic acid with yields of 0.76–0.88 g/g glucose, using a CO₂-fixing anaplerotic pathway in anaerobic fed-batch fermentations (Guettler et al., 1996; Lee et al., 2006; Meynial-Salles et al., 2008; Okino et al., 2008a). Other host

organisms have been examined, as well. *E. coli* strains developed by combining rational metabolic engineering and adaptive evolution produced 73–87 g/L of succinic acid in glucose fermentations (Jantama et al., 2008a,b; Thakker et al., 2012), while engineered *Corynebacterium glutamicum* strains produced 140–146 g/L of succinic acid with high yields of 0.92–1.1 g/g glucose and overall volumetric productivities of 1.9–2.5 g/L/h (Litsanov et al., 2012a,b; Okino et al., 2008a). Both of these hosts have been used for many industrial purposes, suggesting that these systems will contribute to the future industrialization of bio-succinic acid production. The succinic acid market has grown in recent years, increasing the interest for the commercialization of bio-succinic acid production. Several companies, including Myriant (<http://www.myriant.com>) and BioAmber (<http://www.bio-amber.com>), have already reached the stage of industrialization. These companies produce succinic acid from glucose using metabolically engineered organisms, and their systems are competitive in terms of cost, function, and environmental concerns (Erickson et al., 2012).

The bio-based production of malic acid is superior to its chemical synthesis in terms of stereo-selectivity (the current chemical process produces malic acid in a racemic mixture). Malic acid is also a key intermediate of the TCA cycle, making it amenable to native production (i.e., in some fungal species) or rational metabolic engineering strategies in many microorganisms (Fig. 5B). *Aspergillus flavus* can produce 113 g/L L-malic acid with a yield of 0.95 g/g glucose and a productivity of 0.59 g/L/h in batch fermentation (Battat et al., 1991). However, the scaling up of fungal fermentation to an industrial scale has been complicated by problems with oxygen transfer, morphology, and potential toxin production. As an alternative, researchers are seeking to produce L-malic acid using well-characterized microorganisms, such as yeast and *E. coli*. In the case of yeast, the overexpression of native pyruvate carboxylase and malate dehydrogenase in the Pdc-negative *S. cerevisiae*, plus the introduction of a malate transporter from *Schizosaccharomyces pombe*, yielded a strain that could produce 59 g/L of L-malic acid with a yield of 0.31 g/g on glucose (Zelle et al., 2008). In the case of *E. coli*, an engineered succinic acid-producing strain was further engineered to increase accumulation of L-malic acid by deleting the malate metabolism-related genes encoding fumaric acid reductase (FrdBC), malic enzyme (SfcA and MaeB), and fumarase (FumB and FumAC). The resulting strain showed 33.9 g/L L-malic acid production with a productivity of 0.47 g/L/h and a yield of 1.06 g/g on glucose (Zhang et al., 2011).

Bio-based production of fumaric acid was previously used in the USA, but over the past, this has been replaced by chemical synthesis from petrochemical feedstocks. *Rhizopus* species are known to natively produce fumaric acid as an end-product of the TCA cycle (Fig. 5B). One study reported a titer of 97.7 g/L with a yield of 0.81 g/g on glucose and a volumetric productivity of 1.02 g/L/h (Kenealy et al., 1986), while a bioprocess study significantly improved the yield to

0.85 g/g glucose and the productivity to 4.3 g/L/h, using a fermentation system equipped with a rotary biofilm contactor and an adsorption column (Cao et al., 1996). To date, there have been few efforts to improve fumaric acid production through metabolic engineering of *Rhizopus* sp., suggesting that there are still potential opportunities in this area. Alternatively, other microorganisms, such as *S. cerevisiae* and *E. coli*, could be used as hosts for fumaric acid production through rational metabolic engineering.

C4 Amino Carboxylic Acids: γ -Aminobutyric and Aspartic Acids

γ -Aminobutyric acid (GABA) is a non-standard amino acid that has many applications in drugs and food additives (Hayakawa et al., 2004; Inoue et al., 2003; Jakobs et al., 1993; Okada et al., 2000). It is also used as a precursor of pyrrolidone, which is a monomer of Nylon-4 (Le Vo et al., 2011). Aspartic acid is an amino dicarboxylic acid that can be used for manufacturing artificial sweeteners (aspartame), polyesters, polyamides, and other chemical derivatives. The polymeric form of aspartic acid, polyaspartic acid, is also widely used in cleaning compounds (Tsao et al., 1999). Currently, GABA and aspartic acid are produced by chemical and enzymatic processes, respectively (Kim et al., 2009).

In microorganisms, GABA can be produced from the decarboxylation of L-glutamate by L-glutamate decarboxylase (Shelp et al., 1999; Fig. 5C). Under optimized culture conditions, 103.7 g/L of GABA was produced from 40 g/L glucose and 214 g/L glutamate in fed-batch fermentation of *Lactobacillus brevis* NCL912 (Li et al., 2010). A metabolic engineering strategy for the one-step synthesis of GABA from glucose has been developed in *C. glutamicum*. The engineered *C. glutamicum*, which harbors the L-glutamate decarboxylase from *L. brevis*, produced 2.2 g/L GABA from 160 g/L of glucose in batch culture (Shi and Li, 2011). For the economic production of GABA from biomass, it will be crucial to increase the lactic acid bacterial conversion of GABA from L-glutamate by constructing active pathways that directly convert GABA from a single carbon source.

Although strategies for producing aspartic acid family amino acids and their derivatives, such as L-threonine and cadaverine, have been reported (Lee et al., 2007; Qian et al., 2011), relatively few studies have sought to produce aspartic acid as an end-product of fermentation. Instead, aspartic acid has been commercially produced from fumaric acid through enzymatic amination, using either whole cells or highly active aspartase (Tsao et al., 1999; Fig. 5B). Although direct production by fermentation would be preferable, a single-step enzymatic conversion of the biologically produced fumaric acid would be worthwhile in terms of cost and ease of production compared to the current technologies.

C4 Alcohols: 1-Butanol and Isobutanol

1-Butanol and isobutanol are 4-carbon alcohols with applications in the paint industry and as fuel additives. The 1-butanol derivatives, butyl acrylate and methacrylate esters, can be used for the production of latex surface coatings, enamels, and lacquers (Kirschner, 2006; Lee et al., 2008), while isobutanol ester derivatives, such as diisobutyl phthalate, can be used as plasticizer agents (Atsumi et al., 2008b). In the early 20th century, 1-butanol was produced biologically using *Clostridial* species. More recently, however, production has been undertaken by chemical processes in which propylene is catalytically converted to 1-butanol (Jones and Woods, 1986).

Several species belonging to genus *Clostridium* naturally produce 1-butanol via the acetone–butanol–ethanol (ABE) fermentation pathway (Jang et al., 2012a; Fig. 5a). In this context, *Clostridial* species have advantages over other bacteria and yeasts in utilizing various carbon sources (Jang et al., 2012a), but they typically produce acetone and organic acids as byproducts. Thus, metabolic engineering of *Clostridium* has been undertaken to improve 1-butanol production by reducing byproduct formation. One notable example is the production of 16.7 g/L of 1-butanol with a productivity of 0.31 g/L/h in anaerobic batch fermentation of glucose, using a butyrate kinase-inactive mutant of *C. acetobutylicum* in which formation of the byproduct, butyrate, was blocked (Green and Bennett, 1998; Green et al., 1996; Harris et al., 2000). Initial attempts to transfer the *Clostridial* 1-butanol biosynthesis pathway into other host organisms have not been successful (Atsumi et al., 2008a; Inui et al., 2008; Nielsen et al., 2009; Steen et al., 2008). Recently, 1-butanol production of 14–15 g/L with a 0.33–0.36 g/g yield and 0.20–0.29 g/L/h productivity was achieved during glucose batch fermentations using engineered *E. coli* harboring a modified *Clostridial* pathway partially replaced with enzyme analogs from various organisms (Dellomonaco et al., 2011; Shen et al., 2011). However, although researchers have made significant progress in microbial 1-butanol production, the production efficiency still needs to be improved for it to become economically viable.

Metabolic engineering for isobutanol production was only recently demonstrated, when production of various alcohols from amino acid precursors was reported using the Kdc from *L. lactis* with the *S. cerevisiae* Adh2 (Atsumi et al., 2008b; Fig. 5A). In the engineered *E. coli* strain, more than 20 g/L of isobutanol was produced from glucose (Atsumi et al., 2008b). This strategy has also been implemented in several other organisms (Blombach et al., 2011a; Higashide et al., 2011; Li et al., 2011; Smith et al., 2010); among them, *C. glutamicum* might be an attractive host, since it efficiently produces several amino acids, including L-valine, which has 2-ketoisovalerate as a precursor. Recently, a *C. glutamicum* strain was successfully engineered to produce 13 g/L of isobutanol with a yield of 0.20 g/g on glucose (Blombach et al., 2011b). Since the efforts to produce isobutanol from

engineered *E. coli* and *C. glutamicum* are relatively new, further work on engineering these strains and exploring their tolerances may allow researchers to increase the product titers and yields to an industrial scale.

C4 Diols: 1,4-Butanediol and 2,3-Butanediol

The 1,4- and 2,3-butanediols (BDOs) are currently produced from fossil oil feed-stocks, such as acetylene, butane, propylene, and butadiene (Kroschwitz and Howe-Grant, 1993). Among the technologies for BDO production, Reppe chemistry using acetylene represents the largest portion of BDO production worldwide (Cukalovic and Stevens, 2008). Annually, about 1.3 million tons of 1,4-BDO are produced for use in plastics, elastic fibers, and solvents (Fig. 2), whereas 2,3-BDO has applications in the food, fine chemical, cosmetics, pharmaceutical, and agrochemical industries (Magee and Kosaric, 1987).

Genomatica recently reported the first direct biocatalytic route from renewable carbohydrate feed-stocks to 1,4-BDO (Yim et al., 2011; Fig. 5D). They effectively combined a system-wide metabolic engineering approach with rational metabolic and protein engineering strategies. In silico network modeling was used to generate and screen numerous synthetic pathways for 1,4-BDO production, and a metabolic pathway that appeared to be thermodynamically favorable while requiring minimal non-indigenous reaction steps was identified and implemented in *E. coli*. In addition, carbon flux toward 1,4-BDO biosynthesis was increased by blocking competing pathways (e.g., the formate-lyase and Adh pathways) and by enhancing the NAD(P)H driving force using engineered pyruvate dehydrogenase and citrate synthase. The engineered *E. coli* strain produced 18 g/L of 1,4-BDO from glucose with a productivity of 0.15 g/L/h in microaerobic fed-batch fermentation (Yim et al., 2011). This illustrates the synergistic advantages of combining systems biology with metabolic and protein engineering, and emphasizes the continuous expansion of metabolic engineering principles for the biological production of important non-natural chemical building blocks. Notably, Genomatica will begin producing 1,4-BDO in a commercial-scale plant in 2013, and 1 year later, larger plants will undertake 1,4-BDO production in USA, Europe, and Asia (<http://www.genomatica.com/products/bdo/>).

2,3-BDO is endogenously produced by bacteria, such as *Klebsiella* species, *Serratia marcescens*, *Enterobacter aerogenes*, and *Pseudomonas chlororaphis* (Celinska and Grajek, 2009; Ma et al., 2009; Zhang et al., 2010; Fig. 5D). Two engineered strains, *K. pneumoniae* SDM and *S. marcescens* ($\Delta swrW$), have demonstrated promising results for bio-based industrial-scale production of 2,3-BDO. The *K. pneumoniae* SDM strain, which was constructed by ion-beam mutation, was shown to produce 150 g/L of 2,3-BDO with a yield of 0.48 g/g and a productivity of 4.21 g/L/h in glucose fed-batch fermentation (Ma et al., 2009). In an

engineered *S. marcescens* strain, the *swrW* gene (encoding serrawettin W1) was deleted to decrease foam production and avoid the need to add excessive antifoam during 2,3-BDO production. The resulting strain produced 2,3-BDO at to 152 g/L with a yield of 0.46 g/g and productivity of 2.67 g/L/h in glucose fed-batch fermentation (Zhang et al., 2010). Based on the production cost advantage and continuing improvements of the biological processes (Zeng and Sabra, 2011), bio-based 2,3-BDO is expected to hit the industrial market in the foreseeable future.

C4 Diamine: Putrescine

Putrescine (1,4-diaminobutane) is a linear chain diamine that has attracted recent interest as a component of polymers, pharmaceuticals, agrochemicals, surfactants, and other additives (Qian et al., 2009). In particular, putrescine is used as a monomer in the production of nylon-4,6 by polycondensation with adipic acid (Scott et al., 2007; Fig. 2). *E. coli* has a biosynthesis pathway for putrescine; production has been increased through deletion of both degradation- and byproduct-formation pathways and overexpression of putrescine production pathway members, such as the *spec* and *argCDE* genes (encoding ornithine synthase and ornithine decarboxylase, respectively), which are responsible for the last two reaction steps in the putrescine pathway (Qian et al., 2009; Fig. 5C). Putrescine production was further improved by deleting the *argI* gene (encoding ornithine carbamoyltransferase chain I), to direct carbon flux toward the precursors available for putrescine formation, and the *rpoS* gene (encoding the stress-responsive RNA polymerase sigma factor). The engineered *E. coli* strain produced 24.2 g/L putrescine with a productivity of 0.75 g/L/h in glucose fed-batch culture (Qian et al., 2009). Similar engineering strategies have been applied to *C. glutamicum*, and the engineered strain produced 6 g/L putrescine with an overall yield of 0.12 g/g glucose and a productivity of 0.1 g/L/h in flask batch culture (Schneider and Wendisch, 2010).

C4 Diene: 1,3-Butadiene

1,3-Butadiene, a major commodity of the petrochemical industry, is used in the production of various polymers and copolymers. Copolymers with acrylonitrile and styrene are widely used for automobile tires, synthetic resins, latex, and engineering plastics (White, 2007). 1,3-Butadiene is primarily produced as a byproduct of ethylene cracking (White, 2007), and global demand was forecasted to be over 20 billion pounds (or approximately \$40 billion) in 2011 (www.genomatica.com). Biosynthetic routes for the direct production of 1,3-butadiene have recently been patented (Burk et al., 2011). Three potential pathways have been suggested for producing 1,3-butadiene from biomass-sugar: (1) from acetyl-CoA via crotonyl-CoA; (2) from

erythrose-4-phosphate; and (3) via a condensation reaction with malonyl-CoA and acetyl-CoA (Burk et al., 2011). However, no information on the production titers or yields of 1,3-butadiene obtained using these strategies have been reported to date. Since the current production process is highly dependent on the petrochemical industry, the development of biological processes for 1,3-butadiene production from renewable resources would have a substantial impact on the sustainable supply of 1,3-butadiene.

Production of C5 Chemicals

In this section, we review the bio-based production of representative C5 platform chemicals, including dicarboxylic acids (itaconic, glutaconic, and glutaric acids), hydroxyl acid (3-hydroxyvaleric acid), alcohols (2-methyl-1-butanol, 3-methyl-1-butanol, and 1-pentanol), polyol (xylitol), and diamine (cadaverine). We also review recent trends and visions, and discuss patents related to the production of C5 platform chemicals.

C5 Dicarboxylic Acids: Itaconic, Glutaconic, and Glutaric acids

Itaconic acid (2-methylidenebutanedioic acid) is used as a building block for acrylic plastics, acrylate latexes, super-absorbents, and anti-scaling agents (Okabe et al., 2009). Glutaconic ((E)-pentene-1,5-dioic acid) and glutaric (pentane-1,5-dioic acid) acids have potential applications in the polyurethane, lubricant, biodegradable polymer, and pharmaceutical industries.

Itaconic acid arises naturally from the decarboxylation of *cis*-aconitate, and is industrially produced by fermentation using the natural-producer, *Aspergillus terreus* (Fig. 6A). However, the costs of this biological process are relatively high. The industrial producer strain, TN-484, was isolated through random chemical mutagenesis of *A. terreus* IFO-6365 followed by selection for resistance to high itaconic acid concentrations (Yahiro et al., 1995). The TN-484 strain produced 82.3 g/L of itaconic acid with a yield of 0.54 g/g glucose in a 6-day flask culture, and produced more than 85 g/L of itaconic acid in a 100-kL scale fermentor from glucose and corn steep (Okabe et al., 2009; Yahiro et al., 1995). A recent patent describes the development of engineered *E. coli* (Liao et al., 2010). The engineered strain, PCI 519, produced 4.2 g/L itaconic acid with a yield of 0.52 g/g glucose in 72 h, in a flask batch culture. Although the titer, yield, and productivity of itaconic acid production by the engineered *E. coli* are limited, this marks a crucial step in the development of alternative microorganisms for enhanced production (especially since *A. terreus* grows slowly and its production is stage-dependent; Liao et al., 2010).

Studies on the biological production of glutaconic and glutaric acids are still in their early stages. A proof of concept of the biological production of glutaconic acid was demonstrated by Buckel and colleagues, who overexpressed in *E. coli* six enzymes found in glutamate-fermenting bacteria (Djurdjevic et al., 2011) to create a new anaerobic pathway that starts from 2-ketoglutarate and proceeds via 2-hydroxyglutarate, 2-hydroxyglutaryl-CoA, and glutaconyl-CoA to glutaconic acid. A minimal amount of glutaconic acid (351 mg/L in 20 h) was detected in the medium, but a higher intracellular concentration (2.1 g/L) was noted (Djurdjevic et al., 2011), suggesting the existence of a transport problem. Although the reduction of glutaconic acid would theoretically yield glutaric acid, this has not yet been demonstrated experimentally. However, glutaric acid is also naturally found as a metabolic intermediate in the L-lysine catabolic pathway (Fig. 6B). Lysine is degraded to δ -aminovaleric acid, which is in turn oxidized to form glutaric acid. In *Pseudomonads*, the enzymatic conversion of δ -aminovaleric acid to glutaric acid could be induced in a δ -aminovalerate-dependent manner, suggesting that there is a functional metabolic pathway that could be engineered for high-titer biological production of glutaric acid (Revelles et al., 2004, 2005). Higher titer and productivity could also possibly be achieved through the common metabolic engineering strategy of increasing the precursor pool, that is, δ -aminovalerate (Revelles et al., 2004, 2005; Fig. 6B). Although the biological productions of these compounds have not been yet realized at significant levels, the existing reports suggest potential routes for the biological production of glutaconic and glutaric acids by engineered microorganisms.

C5 Hydroxy Acid: 3-Hydroxyvaleric Acid

3-Hydroxyvaleric acid (3-HV), which contains one hydroxyl group and one carboxyl group (Fig. 2), is a potential building block for a diverse range of products, including biodegradable polyhydroxyalkanoates (PHAs), pharmaceuticals, and food additives (Fig. 2).

Biological production of 3-HV has been demonstrated several times, although each strategy required the growing strains to be supplemented with precursor molecules (e.g., valeric acid, propionate, levulinic acid, valine, and threonine) that are often toxic to cells at high concentrations. High-titer production of 3-HV has been reported from engineered *P. putida* (Martin and Prather, 2009; Tseng et al., 2010). For example, *P. putida* KT2440 expressing the *E. coli* thioesterase II (TesB) produced 5.3 g/L 3-HV in a flask culture containing M9 minimal medium supplemented with glucose and levulinate, an inexpensive 3-HV precursor (Martin and Prather, 2009). Furthermore, *E. coli* expressing a feedback-resistant threonine deaminase (IlvA) from *C. glutamicum* produced 1.2 g/L 3-HV in a flask culture containing glucose and threonine (Tseng et al., 2010; Fig. 6C). In this case, the engineered pathway begins with the

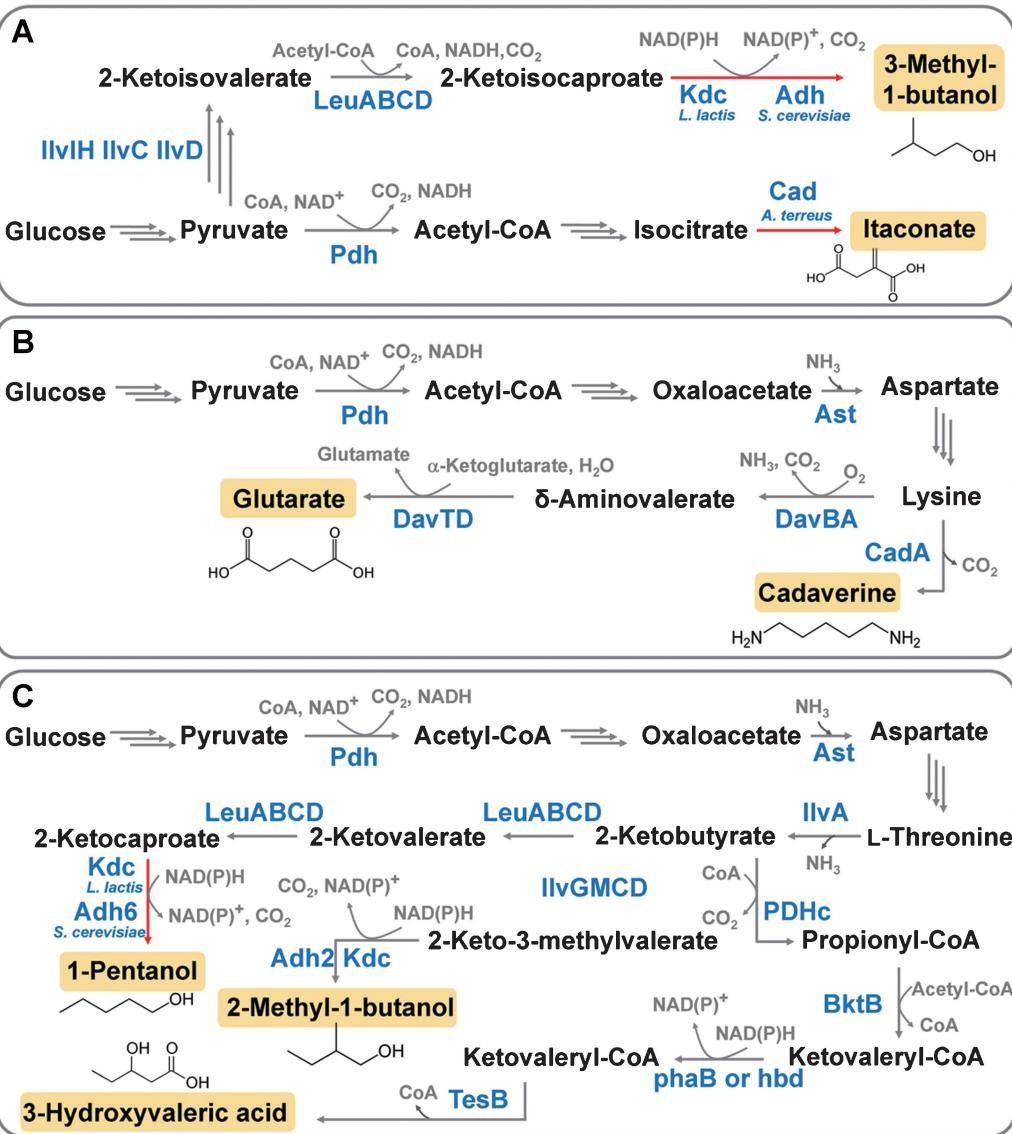


Figure 6. Representative pathways for the production of C5 platform chemicals. **A:** Metabolic pathways that produce 3-methyl-1-butanol and itaconic acid. **B:** Metabolic pathways that produce glutarate and cadaverine. **C:** Metabolic pathways that produce 1-pentanol, 2-methyl-1-butanol, and 3-hydroxyvaleric acid through the threonine biosynthesis pathway. Major metabolic intermediates and enzymes with their common sources are shown in black and blue, respectively, next to the corresponding reactions. Multiple arrows indicate multistep reactions. Reactions mediated by heterologous enzymes and chemical catalysis are shown in red and purple, respectively. Final products are highlighted in orange boxes with their chemical structures. Enzymes: Adh, alcohol dehydrogenase; Adh6, alcohol dehydrogenase VI; Ast, aspartate aminotransferase; BktB, β-ketothiolase; Pdh, pyruvate dehydrogenase; Cad, *cis*-aconitate decarboxylase; CadA, lysine decarboxylase; DavBA, oxidative decarboxylase and hydrolase. Enzymes: DavTD, those responsible for catalyzing the reaction converting glutarate from δ-aminovalerate via glutarate semialdehyde; IlvA, threonine deaminase; IlvC, ketol-acid reductoisomerase; IlvD, dihydroxyacid dehydratase; IlvIH, acetolactate synthase; Kdc, ketoacid decarboxylase; LeuABCD, 2-isopropylmalate synthase, 3-isopropylmalate dehydrogenase, and 3-isopropylmalate dehydratase; PfID, formate C-acetyltransferase; TesB, thioesterase II.

condensation of acetyl-CoA and propionyl-CoA mediated by H16 β-ketothiolase (*bktB*). This produces ketovaleryl-CoA, which is converted to hydroxyvaleryl-CoA by reductases. Finally, thioesterase II (*tesB*) from *E. coli* converts hydroxyvaleryl-CoA to 3-hydroxyvalerate. Notably, the chirality of the 3-hydroxyvalerate could be controlled by using stereospecific reductases. Tseng et al. (2010) eliminated the need for toxic precursor molecules by enhancing

the threonine biosynthetic pathways. They demonstrated formation of 3-HV entirely from glucose as a single carbon source, which was a major step in this biopolymer production process. The *E. coli* strain was further engineered by overexpression of the threonine operon (*thrABC*) and deletion of competing pathways, yielding a titer of 0.81 g/L from glucose in flask culture (Tseng et al., 2010). These studies demonstrate the conversion of 3-HV from glucose

with and without supplements, such as levulinic acid and threonine as precursors. For future industrial applications, additional work is needed to support the use of inexpensive precursors and develop technologies for the direct conversion of 3-HV from sugars.

C5 Alcohols: 2-Methyl-1-Butanol, 3-Methyl-1-Butanol, and 1-Pentanol

C5 pentanols are used as solvents and for forming esters. Liao and colleagues demonstrated branched C5 alcohol production from engineered *E. coli* strains by genetically modifying the amino acid metabolic pathways (Atsumi et al., 2008b). Their system used the broad-substrate-range Kdc from *Lactococcus lactis* and the Adh2 from *S. cerevisiae* to convert various 2-ketoacids into higher alcohols, including C5 alcohols, such as 2-methyl-1-butanol and 3-methyl-1-butanol. They subsequently improved this system for higher-titer alcohol production. The main strategy for improving 2-methyl-1-butanol production consisted of engineering the native isoleucine biosynthesis pathway in *E. coli* (Cann and Liao, 2008). Since threonine biosynthesis is the direct upstream pathway of isoleucine biosynthesis, the *thrABC* operon was also overexpressed to increase the threonine pool. These strategies yielded a strain that produced 1.25 g/L 2-methyl-1-butanol with a yield of 0.17 g/g glucose. The strategy for improving 3-methyl-1-butanol production included engineering of the native valine and leucine biosynthesis pathways (Connor and Liao, 2008). The valine biosynthesis genes, *ilvCD* and *Bacillus subtilis* aceto-lactate synthase (*alsS*), were overexpressed to increase 2-ketoisovalerate, the precursor for leucine biosynthesis. A feedback-resistant *leuA* and a synthetic ribosomal binding site (for better expression) were also included. The resulting strain produced 1.28 g/L 3-methyl-1-butanol with a yield of 0.11 g/g glucose. In another study, a non-natural *leuABCD* was used to convert 2-ketobutyrate into 2-ketovalerate, and the latter into 2-ketocaproate. This pathway using engineered LeuA and Kdc produced 750 mg/L of 1-pentanol from 20 g/L of glucose in 40 h (Zhang et al., 2008; Fig. 6C) and demonstrated a novel approach for the production of non-natural metabolites by building artificial metabolic pathways.

C5 Polyol: Xylitol

Xylitol is a rare five carbon sugar alcohol that has been used as a sweetener and food additive, and has potential as a building block for biodegradable polymers, such as poly(xylitol-co-citrate) and poly(xylitol-co-sebacate) (Bruggeman et al., 2008; Granstrom et al., 2007). At the industrial level, xylitol is manufactured by the chemical hydrogenation of D-xylose. The increasing xylitol market is estimated to be \$340 million per year (Prakasham et al., 2009).

Production by *Candida tropicalis*, a well-known natural xylitol producer, was reportedly 182 g/L xylitol with a yield of 0.85 g/g and a productivity of 12.0 g/L/h in oxygen-limited culture with cell recycling (Granstrom et al., 2007). However, the productivity without cell recycling was in the range of 0.5–3.9 g/L/h, and the opportunistic pathogenic *Candida* strains are not suitable for industrial-scale production (Granstrom et al., 2007). The biological conversion of xylose to xylitol using engineered *E. coli* expressing the xylose reductase of *Candida boidinii* has gained attention as an alternative for industrial xylitol production (Cirino et al., 2006). Cirino et al. (2006) developed an engineered *E. coli* strain, PC09, which produced 38 g/L of xylitol with a productivity of 0.8 g/L/h from 27 g/L glucose and 46 g/L xylose in fed-batch fermentation.

C5 Diamine: Cadaverine

Cadaverine is a 1,5-diaminopentane that can be used as a monomer for the synthesis of polyamide (Fig. 2). The annual production volume of polyamide is estimated to be 3,500,000 metric tons, and its building block, diamine, is produced via a petrochemical route from fossil resources (Kind et al., 2010).

Biologically, cadaverine can be obtained as a catabolic intermediate of the lysine degradation pathways found in microbes. Wittmann's group took a systematic approach to redesigning the *C. glutamicum* metabolic circuit, and improved the yield up to 0.17 g/g with supplementation of pyridoxal (Kind et al., 2010). A systems metabolic engineering approach on the cadaverine biosynthesis, degradation, and utilization pathways of *E. coli* yielded a strain that produced 9.6 g/L cadaverine from glucose with a yield of 0.12 g/g (Qian et al., 2011). Additional efforts have been directed toward producing cadaverine from biomasses (xylose or soluble starch) by introducing the L-lysine decarboxylase and xylose utilization genes (*E. coli* xylose isomerase, XylA; and xylulokinase, XylB) or *Streptococcus bovis* α -amylase (AmyA), respectively, into *C. glutamicum* (Buschke et al., 2011; Tateno et al., 2009; Fig. 6B).

Production of C6 Chemicals

In this section, we review the bio-based production of representative C6 platform chemicals, including dicarboxylic acids (adipic and glucaric acids) and aromatics (anthranilic acid, catechol, phenol, *p*-hydroxybenzoic acid, *p*-hydroxystyrene, and styrene). Due to rapid advances in omics and systems approaches, the target platform chemicals for biological production have been expanded to various C6 platform chemicals, most of which were previously considered to be incompatible with biological systems. Here, we briefly discuss the recent trends and

vision, the limitations related to product toxicity, and ongoing efforts to alleviate the remaining issues.

C6 Dicarboxylic Acids: Adipic Acid and Its Derivative, ϵ -Caprolactam, and Glucaric Acids

The six-carbon adipic acid (1,6-hexanedioic acid) and its derivative, ϵ -caprolactam, are mainly used as building blocks for polyamides (e.g., Nylon-6,6 and Nylon-6, respectively; Fig. 2). The global annual productions of adipic acid and ϵ -caprolactam are 2.2 and 4 million metric tons, respectively (Lee et al., 2011a). In industry, adipic acid is produced from oxidation of a cyclohexanone and cyclohexanol (ketone-alcohol oil) mixture, while ϵ -

caprolactam is manufactured by cyclization of ϵ -aminocaproic acid. Another C6 dicarboxylic acid, glucaric acid, is naturally found in fruits, vegetables, and mammals, and has been investigated for therapeutic applications in cholesterol- and cancer-related diseases (Moon et al., 2009). Glucaric acid is currently produced by oxidation of glucose, using nitric acid as a catalyst (Moon et al., 2009).

The bio-based production of adipic acid, particularly from renewable resources, would be an attractive alternative that would address environmental concerns regarding the harsh chemicals used in the chemical production route. In this context, researchers explored a combined biological and chemical synthesis route for producing adipic acid from glucose (Fig. 7A). In *E. coli*, glucose was converted to *cis,cis*-muconic acid by engineering of the native shikimate

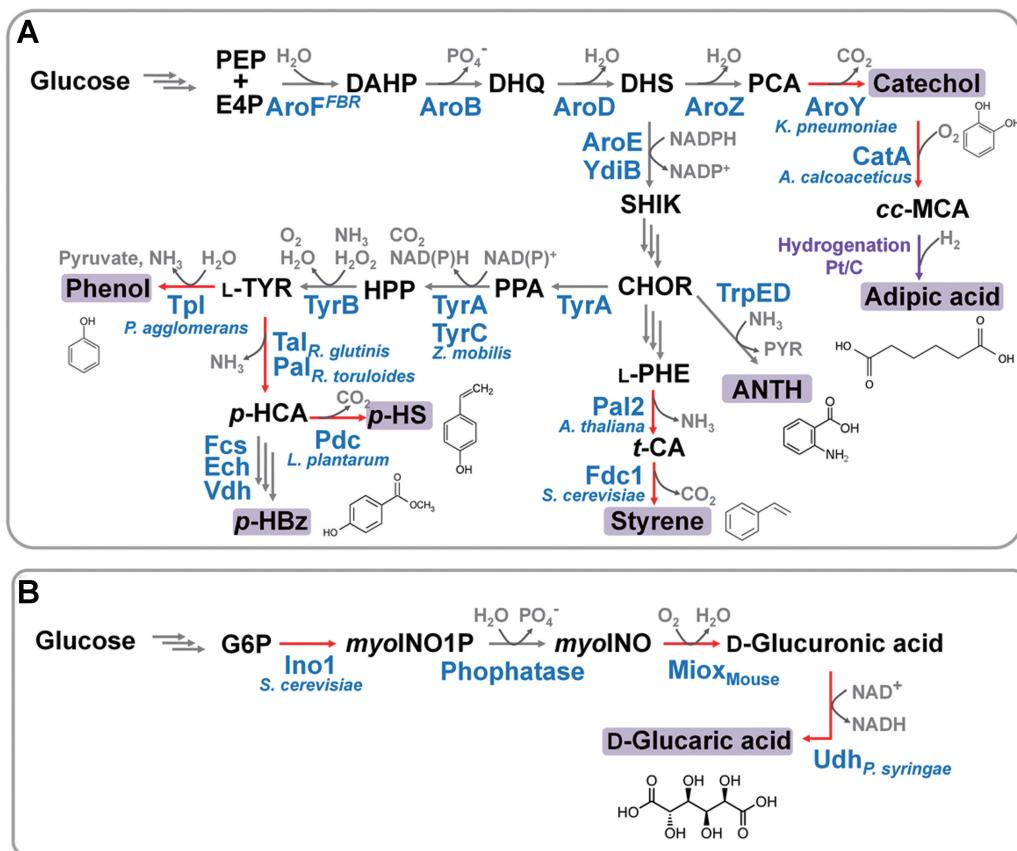


Figure 7. Representative pathways for the production of C6 platform chemicals. **A:** Metabolic pathways that produce phenol, benzene, and *p*-hydroxystyrene (*p*-HS), tyrosine biosynthesis, styrene (phenylalanine biosynthesis), anthranilic acid (chorismate biosynthesis), catechol, and adipic acid. ANTH, anthranilic acid; *t*-CA, trans-cinnamic acid; CHOR, chorismate; DAHP, 3-deoxy-D-arabinoheptulose-7-phosphate; DHQ, dehydroquinate; DHS, dehydroshikimate; E4P, erythro-4-phosphate; *p*-HBz, *p*-hydroxybenzoic acid; *p*-HCA, *p*-hydroxycinnamic acid; HPP, *p*-hydroxyphenylpyruvate; *p*-HS, *p*-hydroxystyrene; *cc*-MCA, *cis,cis*-muconic acid; PCA, protocatechuic acid; PEP, phosphoenolpyruvate; L-PHE, L-phenylalanine; PPA, prephenate; PYR, pyruvate; SHIK, shikimate; L-TYR, L-tyrosine; AroB, dehydroquinate synthase; AroD, dehydroquinate dehydratase; AroE and YdiB, shikimate dehydrogenase isozyme; AroF^{FBR}, feedback inhibition-resistant DAHP synthase; AroY, protocatechuic acid decarboxylase; AroZ, 3-dehydroshikimate dehydratase; CatA, catechol 1,2-dioxygenase; Ech, *p*-coumaroyl-CoA hydratase/lyase; Fcs, *p*-coumaroyl-CoA synthetase; Fdc1, ferulate decarboxylase; Tal, tyrosine ammonia-lyase; TrpED, anthranilate synthase–phosphoribosyl transferase complex; PDC, *p*-hydroxycinnamic acid decarboxylase; Tpl, tyrosine phenol-lyase; TyrA, prephenate dehydrogenase; TyrB, tyrosine aminotransferase; TyrC, prephenate decarboxylase; Vdh, *p*-hydroxybenzaldehyde dehydrogenase. **B:** A metabolic pathway that produces D-glucaric acid from glucose. Major metabolic intermediates and enzymes with their common sources are shown in black and blue, respectively, next to the corresponding reactions. Multiple arrows indicate multistep reactions. Reactions mediated by heterologous enzymes and chemical catalysis are shown in red and purple, respectively. Final products are highlighted in purple boxes with their chemical structures. G6P, glucose-6-phosphate; myoINO1P, myo-inositol-1-phosphate; myoINO, myo-inositol; Ino1, myo-inositol-1-phosphate synthase; Miox, myo-inositol oxygenase; Udh, uronate dehydrogenase.

biosynthetic pathway, and the secreted *cis,cis*-muconic acid was converted to adipic acid by one-step chemical catalysis (Fig. 7A; Niu et al., 2002). The engineered strain produced 36.8 g/L of *cis,cis*-muconic acid with a yield of 0.17 g/g glucose within 48 h in oxygen-limited (10% air saturation) fed-batch culture; thereafter a 97% catalytic conversion of *cis,cis*-muconic acid into adipic acid was achieved (Niu et al., 2002). A recent Genomatica patent describes various biochemical routes leading to the biosynthesis of adipic acid and ϵ -caprolactam (Burgard et al., 2010). One such route utilizes a reverse adipic acid degradation pathway present in organisms such as *Penicillium chrysogenum*; it begins with the condensation of succinyl-CoA and acetyl-CoA and proceeds via 3-oxoadipyl-CoA, 3-hydroxyladipyl-CoA, 5-carboxyl-2-penenoyle-CoA and adipyl-CoA to adipic acid. Another approach suggested by Verdezyne combines the β - and ω -oxidation routes of fatty acid metabolism (www.verdezyne.com; Picataggio et al., 2012). Although details on the metabolic capabilities of the engineered organisms have not yet been reported, these examples illustrate the ongoing efforts and vast opportunities for the bio-based production of adipic acid and ϵ -caprolactam as precursors of bio-based nylons (Boussie et al., 2010).

Biologically, glucaric acid is produced by the mammalian glucuronic acid pathway (Fig. 7B). A synthetic pathway comprising part of the glucuronic acid pathway together with additional enzymes from various organisms was constructed in *E. coli*. The recombinant *E. coli* strain, which expressed *S. cerevisiae* *myo*-inositol-1-phosphate synthase (Ino1), mouse *myo*-inositol oxygenase (MIOX), and *Pseudomonas syringae* uronate dehydrogenase (Udh), produced 1.1 g/L glucaric acid from 10 g/L glucose (Moon et al., 2009; Fig. 7B). The concept of an “enzyme scaffold” was thereafter used to increase the substrate availability for each enzyme in the pathway, and the resulting *E. coli* strain produced about 2.5 g/L glucaric acid from 10 g/L glucose in flask culture (Dueber et al., 2009; Moon et al., 2010). In the business sector, Rivertop Renewables recently contracted DTI to scale up the production of glucaric acid, and Rennovia is also aiming at the bio-based production of adipic acid from glucaric acid (Boussie et al., 2010).

C6 Platform Aromatics: Anthranilic acid, Catechol, Phenol, *p*-Hydroxybenzoic Acid, *p*-Hydroxystyrene, and Styrene

Anthranilic acid (2-aminobenzoic acid), catechol (1,2-dihydroxybenzene), and phenol are a group of monoaromatic hydrocarbons derived from petrochemicals. These aromatics have been produced around the world as starting materials, and are extensively used in industry for plastic, detergents, pesticides production, and as the major constituents of many petroleum and fine chemical products (Balderas-Hernandez et al., 2009; Schmidt, 2005; Yanofsky et al., 1971).

The bio-based aromatic hydrocarbons are derived from the aromatic amino biosynthetic pathway (Fig. 7A). Anthranilic acid is a metabolic intermediate derived from chorismate in the tryptophan biosynthetic pathway. Amplified production of anthranilic acid was achieved by engineering the natural tryptophan biosynthetic pathway (Fig. 7A) to redirect the carbon flux toward common precursors and mutate enzymes that are sensitive to feedback inhibition (Balderas-Hernandez et al., 2009; Yanofsky et al., 1971). The engineered *E. coli* strain produced 14 g/L of anthranilic acid with a yield of 0.20 g/g glucose and a productivity of 0.41 g/L/h in fed-batch culture (Balderas-Hernandez et al., 2009).

Catechol can be converted from microbes capable of degrading aromatic hydrocarbons such as benzene and benzoate (Axcell and Geary, 1973; Wang et al., 2001). More favorably, catechol can also be produced from glucose by engineering the shikimate biosynthesis pathway (Fig. 7A). An *E. coli* strain engineered to produce catechol from 3-dehydroshikimate (DHS) produced 4.2 g/L catechol over 36 h in a glucose-rich medium (>30 g/L; Draths and Frost, 1995; Li et al., 2005). To overcome the yield limitations arising from the toxicity of catechol (5–7%), researchers investigated microbial production of non-toxic intermediates such as 3-hydroquinone, followed by chemical conversion to catechol (Li et al., 2005). The overall yield from glucose to catechol improved to 26–49% depending on the methods used to extract the intermediates and the catalytic conditions. However, the complicated extraction, purification, and chemical catalysis steps remain as challenges in this alternative approach to the bio-production of catechol.

The bioconversion of glucose to phenol using tyrosine as a precursor was demonstrated in the solvent-tolerant strain, *P. putida* S12 (Wierckx et al., 2005; Fig. 7A). The *tpl* gene from *Pantoea agglomerans* (encoding tyrosine–phenol lyase) was introduced into *P. putida* S12 to enable phenol production, and the engineered strain produced 0.14 g/L of phenol with a yield of 3.5 g/g glucose in 24-h flask culture using mineral medium with glucose (MMG) supplemented with salicylate. In fed-batch culture, the highest titer was limited to 0.47 g/L due to product toxicity. When octanol was used as the phenol extractant in a biphasic medium-octanol system, the phenol concentration in the octanol phase was increased to 5.5 g/L (Wierckx et al., 2005).

In addition to phenol, the microbial productions of *p*-hydroxybenzoic (1.8 g/L) acid, *p*-hydroxystyrene (0.4 g/L), and styrene (0.26 g/L) have been demonstrated using engineered *P. putida* S12 and *E. coli* (McKenna and Nielsen, 2011; Verhoef et al., 2007, 2008; Fig. 7A). The titers of these aromatic hydrocarbons are already close to the toxicity thresholds (e.g., 0.3 mg/L for styrene), limiting cell growth, and continuous production (McKenna and Nielsen, 2011). Thus, processes development for prolonged cell growth and production is critical, as is improving metabolic pathway efficiency. Organisms such as *P. putida* show high tolerance to toxic chemicals (Verhoef et al., 2007; Wierckx et al., 2005; Wijte et al., 2011). Gaining new understanding

of the biological and biochemical mechanisms underlying this tolerance may help us develop improved strains with the desired properties (Bandounas et al., 2011; Ramos et al., 2002; Wijte et al., 2011).

Summary and Future Directions

Almost all organic chemical products are currently produced from fossil oil, but the petroleum-based system is currently facing global crises, such as climate change and fossil resource depletion. Thus, there is an increasing demand for sustainable production of bio-based platform chemicals. In particular, microorganisms are considered attractive hosts for the production of platform chemicals from biomass-sugars. Chemical products with a broad range of carbon lengths, including C2–C6 carboxylic acids, dicarboxylic acids, hydroxy acids, amino carboxylic acids, alcohols, diols, and diamines, can be produced by the sugar fermentations of microorganisms. At present, lactic acid, ethanol, and 1,3-propanediol have already been commercialized for economical bio-based production, and succinic acid, 1,4-butanediol, isobutanol, acetic acid, and isoprene are nearing large-scale commercialization as bio-based chemicals.

However, intensive metabolic engineering is needed to enhance performance for the production of other chemicals, including the C2–C6 platform chemicals reviewed in this work. Common aspects to be considered in strain development are: (1) selecting and optimizing metabolic pathways to produce the desired products, (2) utilizing a variety of carbon sources, (3) improving end-product tolerance, (4) minimizing nitrogen sources, and (5) expanding metabolic pathways to produce platform chemicals by one-step reactions. Advances in various systems metabolic engineering fields, including traditional metabolic engineering, -omics, computational technologies, and synthetic biology, will allow us to accelerate the development of strains for the production of platform chemicals. We can perform rational reconstruction of metabolic pathways and gene regulatory networks, and use them to produce innate and non-innate platform chemicals. Systems metabolic engineering approaches have succeeded in producing many biofuels, biomaterials, and pharmaceuticals in microorganisms, and hold enormous promise in transitioning us from conventional petro-based industries to bio-based industries.

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