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Microbial production of building block chemicals and polymers

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Owing to our increasing concerns on the environment, climate change, and limited natural resources, there has recently been considerable effort exerted to produce chemicals and materials from renewable biomass. Polymers we use everyday can also be produced either by direct fermentation or by polymerization of monomers that are produced by fermentation. Recent advances in metabolic engineering combined with systems biology and synthetic biology are allowing us to more systematically develop superior strains and bioprocesses for the efficient production of polymers and monomers. Here, we review recent trends in microbial production of building block chemicals that can be subsequently used for the synthesis of polymers. Also, recent successful cases of direct one-step production of polymers are reviewed. General strategies for the production of natural and unnatural platform chemicals are described together with representative examples.

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Introduction

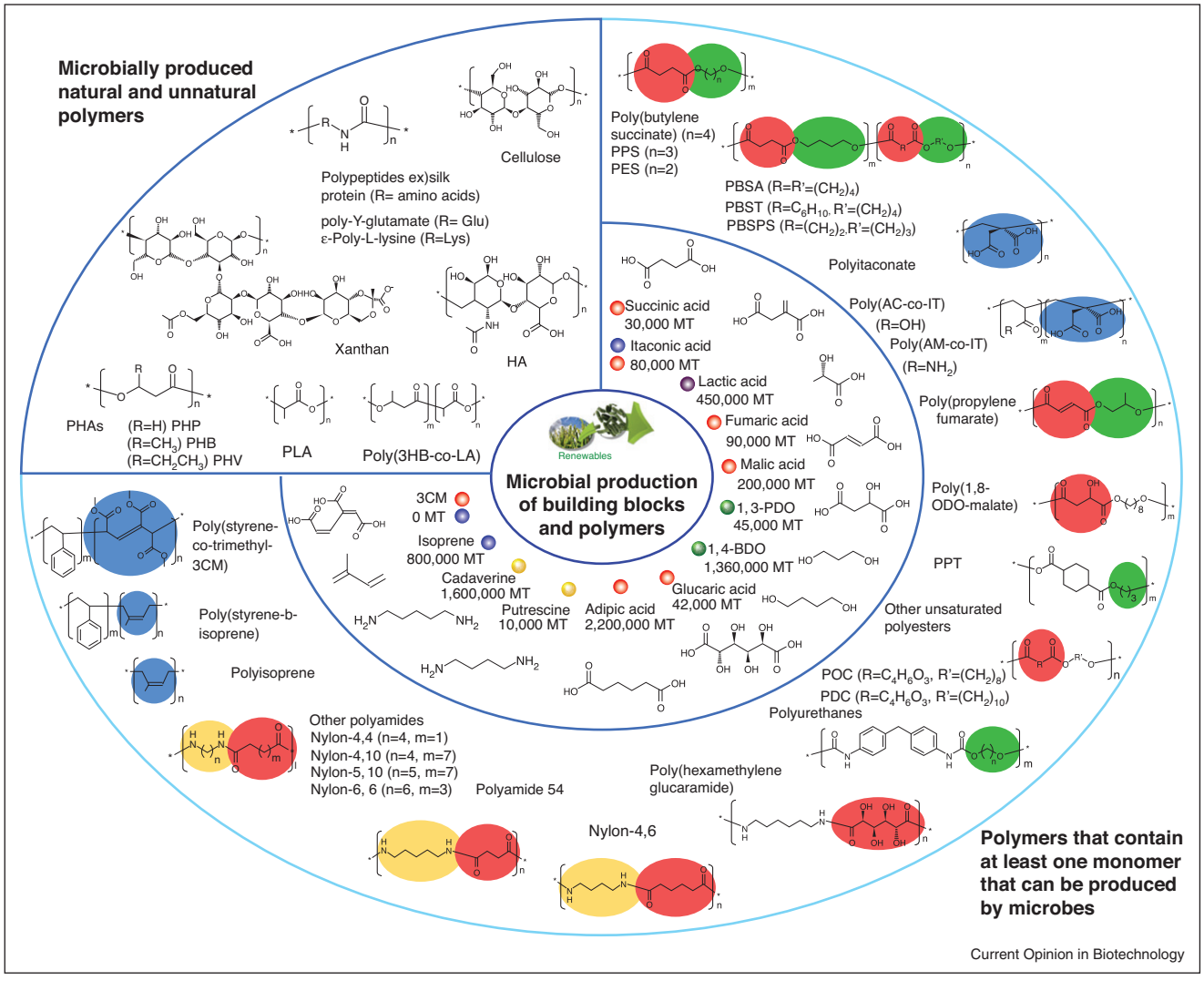
Microorganisms are endowed with capabilities to produce various chemicals and materials, many of which are important to our daily life. Early studies on microbial production of these chemicals were mainly carried out by identifying appropriate microorganisms that naturally overproduce target products, improving their performance by random mutagenesis, and by optimizing the fermentation and downstream processes. The advent of metabolic engineering contributed significantly to enhancing the performance of microbes in producing chemicals by many different means, including optimal induction of enzymes in the target pathway, elimination of competing pathways, redirection of central metabolism towards the target pathway, supplementation of necessary cofactors, and modulation of redox potential [1,2]. More recently, metabolic engineering has been integrated with systems biology for understanding of global-scale metabolic and gene regulatory characteristics, followed by more system-wide identification of target genes to be manipulated [1,3]. Furthermore, emergence of synthetic biology has broadened the spectrum of target products, such that even unnatural chemicals can be produced to satisfactory levels [4,5]. Such systems metabolic engineering approaches are becoming increasingly powerful in developing microbial strains for the production of chemicals and materials.

Here, we review the strategies employed for the production of platform chemicals for subsequent polymer synthesis and direct *in vivo* production of polymers together with recent representative examples. Some challenges remaining to realize efficient production of chemicals and polymers are described together with possible strategies to overcome.

Microbial products used in polymer industry

Polymers refer to macromolecules that are composed of a series of low molecular weight monomers. They can be generally classified into *condensation* and *addition* polymers based on their polymer structures [6]. Condensation polymers are synthesized by eliminating small molecules such as water during polymerization, and/or by joining the repeating units through their functional groups to make ester, amide, urethane, sulfide, and ether bonds. Polyamides synthesized from diamines and dicarboxylic acids are the most common examples of condensation polymers. Polymers that do not satisfy any of the aforementioned criteria for the condensation polymers can be categorized as addition polymers. For instance, building blocks that have carbon-carbon double bonds can be used as a monomer for addition polymers [6]. Thus, microbially produced chemicals for polymer should satisfy these criteria (Figure 1). Dicarboxylic acids (adipic, fumaric, glucaric, malic, and succinic acids), diamines (cadaverine and putrescine), and diols (propanediols and butanediols) are the most common monomers used in condensation polymerization reactions. On the contrary, itaconic acid and isoprene containing one and two carbon-to-carbon double bond(s), respectively, are typical

Figure 1



Microbially produced natural or unnatural building block chemicals used for polymer synthesis as well as polymers that can be directly produced *in vivo*. Numbers below each chemical name in the inner circle designate the amount of total annual production where MT represents metric ton. Information on annual production and applications of each building block chemical in polymer industry was obtained from the following references: 1,3-propanediol [57], 1,4-butanediol [58], 3-carboxymuconic acid [38], adipic acid [30], fumaric acid [59], glucaric acid [60], succinic acid [61], isoprene [36••,62,63], itaconic acid [18,21], lactic acid [7,8], and putrescine [33•]. Annual production of cadaverine and malic acid was estimated from previous publications [7,34•]. Structure of poly [styrene-*co*-trimethyl-3CM] was deduced from the reference [38]. Colored balls across layers indicate specific functional group(s) within chemical structures, which are specified by: red for dicarboxylic acids, yellow for diamines, blue for alkenes or dienes, purple for carboxylic acids, and green for diols. It should be noted that colored regions of each polymer in the outer layer specifically indicate building block chemicals having specific functional groups indicated by the aforementioned colors. Abbreviations are: 3CM, 3-carboxymuconic acid; 3HB, 3-hydroxybutyrate; AC, acrylate; AM, acrylamide; BDO, butanediol; HA, hyaluronic acid; IT, itaconate, LA, lactate; ODO, octanediol; PBSA, poly(butylene succinate-*co*-butylene adipate); PBSPS, poly(butylene succinate-*co*-propylene succinate); PBST, poly(butylene succinate-*co*-butylene terephthalate); PDC, poly(1,10-decanediol citrate); PDO, propanediol; PES, poly(ethylene succinate); PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate; PHP, polyhydroxypropionate; PHV, polyhydroxyvalerate; PLA, polylactate; POC, poly(1,8-octanediol citrate); PPS, poly(propylene succinate); PPT, poly(propylene terephthalate).

example monomers for addition polymerization. The strategies employed for their production by microbial fermentation are described below. Despite their great importance, diols are not covered in this paper as they are covered by another article in this issue.

Production of building block chemicals by natural microbes

When we consider microbial production of building block chemicals for polymers, the most preferable way is to explore microorganisms in the nature that can efficiently

Table 1

Microbial production of building block chemicals and polymers.

Chemicals and polymers	Organisms	Carbon source	Strategy used	Titer (g/L)	Productivity (g/L/h)	Yield (g/g)[a]	References
Succinic acid	*Actinobacillus succinogenes*	Glucose	Adaptive evolution and screening	105.8	1.36	0.82	[13]
	Anaerobiosprillum succiniciproducens	Glucose	Integrated fermentation and separation process combining cell-recycled continuous bioreactor and electrodialysis	83	10.4	0.88	[11]
	Mannheimia succiniciproducens	Glucose	Metabolic engineering for eliminating competing pathways	52.4	1.80	0.76	[10]
	Corynebacterium glutamicum	Glucose	Metabolic engineering for reducing byproducts and fed-batch cultivation with high cell density inoculum	146	3.20	0.90	[12]
	Escherichia coli	Glucose	Repetitive metabolic and evolutionary engineering to eliminate byproducts	86.6	0.90	0.92	[22]
	Escherichia coli	Glucose	Repetitive metabolic and evolutionary engineering to eliminate byproducts	71.6	0.75	1.00	[23]
	Yarrowia lipolytica	Glycerol	Random mutagenesis and screening after metabolic engineering	45.5	0.27	0.36	[26]
Itaconic acid	*Aspergillus terreus*	Glucose	Random mutagenesis and screening	82.3	0.57	0.54	[19]
	Escherichia coli	Glucose	Metabolic engineering and synthetic biology for constructing itaconic acid pathway	4.16	0.057	0.52	[25]
Lactic acid	*Lactobacillus plantarum*	Corn starch	Metabolic engineering for producing optically pure D-lactic acid by using raw corn starch as a carbon source	86	4.51	0.89	[17]
	Lactococcus lactis	Glucose	Bioprocess development by using cell recycling continuous fermentation	30.1	33.1	0.91	[16]
	Lactobacillus delbrueckii	Hydrolyzed cane sugar	Random mutagenesis	135	3.4	0.90	[15]
	Lactobacillus casei	Glucose	Cell recycling continuous fermentation process with near infrared spectroscopy-aided cell culture monitoring	75	138	0.98	[14]
	Escherichia coli	Glucose	Metabolic engineering for eliminating competing pathways and byproduct-forming pathway	138	3.54	0.99	[24]
Glucaric acid	*Escherichia coli*	Glucose	Synthetic biology and metabolic engineering to construct new pathways by adopting eukaryotic enzymes	1.13	0.016	0.153	[27••]
	Escherichia coli	Glucose	Synthetic biology and metabolic engineering by using synthetic protein scaffolds for more efficient biochemical conversion of metabolites	2.37	0.049	-	[29]
Isoprene	*Escherichia coli*	Glucose	Synthetic biology and metabolic engineering to construct a novel pathway having enzymes from diverse organisms	60	2	0.11	[37]
Putrescine	*Escherichia coli*	Glucose	Metabolic engineering to eliminate competing pathways and enhance fluxes towards putrescine pathway	24.2	0.75	0.168	[33•]
Cadaverine	*Escherichia coli*	Glucose	Metabolic engineering to eliminate competing pathways and enhance fluxes towards cadaverine pathway	9.61	0.32	0.131	[34•]

3CM	*Escherichia coli*	Vanillin	Synthetic biology to introduce biosynthetic pathway from other bacterial species.	0.183	0.0046	1.00	[38]
PLA	*Escherichia coli*	Glucose	Systems metabolic engineering performing knockout and overexpression of genes based on expert knowledge and genome-scale metabolic simulation	–	–	0.11	[48••]
Poly(3HB-*co*-LA)	*Escherichia coli*	Glucose and 3HB	Systems metabolic engineering performing knockout and overexpression of genes based on expert knowledge and genome-scale metabolic simulation	–	–	0.56	[48••]
Poly(3HB-*co*-LA)	*Escherichia coli*	Glucose	Metabolic engineering based on expert knowledge and subsequent fed-batch cultivation without addition of inducer and succinic acid.	–	–	0.46	[51]

[a] Yield of building block chemicals represents g product/g carbon source while the yield of biopolymers represents g product/g dry cell weight.

produce them. Fumaric, itaconic, lactic, malic, and succinic acids are the typical chemicals and are endogenously produced by naturally isolated microorganisms: fumaric acid by *Rhizopus* species, itaconic and malic acids by *Aspergilli*, lactic acid by *Lactobacilli*, and succinic acid by rumen bacteria. These acids can be used as monomers for the synthesis of different polymers such as poly(propylene fumarate), poly(acrylate-*co*-itaconate), polylactate, polymalate, and poly(butylene succinate), respectively. Other diverse polymers that can be synthesized from these platform chemicals or their derivatives as monomers are displayed in Figure 1 with their chemical structures. In particular, as succinic, lactic, and itaconic acids have more diverse uses in polymer industry and are produced at relatively high titers with industrially acceptable productivities and yields (Table 1), much recent effort has been concentrated on their commercialization by microbial fermentation [7–9].

Several well-known native succinic acid producers are *Actinobacillus succinogenes*, *Anaerobiosprillum succiniciproducens*, *Mannheimia succiniciproducens*, and their various derivatives, which can produce 52–106 g/L of succinic acid with productivities and yields of 1.36–10.4 g/L/h and 0.76–0.88 g/g glucose, respectively, while fixing CO_2 through the reaction catalyzed by phosphoenolpyruvate carboxykinase (Table 1) [10–13]. In the case of lactic acid production, *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus delbrueckii*, and *Lactobacillus casei* have been reported to produce 30–135 g/L of lactic acid with productivities and yields of 3.4–138 g/L/h and 0.89–0.98 g/g from diverse carbon substrates, respectively [14–17]. Recently, *L. plantarum* producing optically pure 86 g/L D-lactic acid has also been reported (Table 1), which demonstrates an advantage of microbial production of platform chemicals composed of only one optically specific stereoisomer. Meanwhile, *Aspergillus terreus* is the most widely used fungus for the commercial production of itaconic acid [18,19] using its *cis*-aconitic acid decarboxylase as a key enzyme [20]. In fact, as fungal fermentation process is considered economically more competitive than chemical process for industrial level-production of itaconic acid, their chemical process has not been practiced commercially [21]. On the contrary, fumaric and malic acids are produced by chemical processes owing to their cost competitiveness even though there are various natural producers [9]. It is expected that microbial production of these chemicals can be improved to the economically competitive level by adopting recent metabolic engineering strategies described in next section and Box 1, and more bioprocesses will be commercialized.

Production of building block chemicals by engineered microorganisms

Some microbial metabolites that constitute important industrial polymers are not naturally produced because cells do not have respective biosynthetic pathways or

Box 1 General strategies for microbial production of building block chemicals and polymers

Analysis of recent successful examples of chemicals and polymers produced by microbial fermentation suggests important rational steps to consider for their efficient production. The first possible option is direct *in vivo* production of polymers by microorganisms, as in the case of PHAs [41], PLAs [48••] and polyamides [40], if the produced biopolymers are qualitatively and quantitatively competitive against existing chemical processes. If this option is not applicable, then microorganisms that can efficiently produce building block chemicals constituting polymers need to be sought. It is often the case that cells need to be metabolically engineered for the enhanced production of these building block chemicals [10–13,17,19,22–24,26]. For instance, there exist many microorganisms that have metabolic pathways for the biosynthesis of building block chemicals, but cannot excrete them into the medium; in this case, gene manipulations can be performed to redirect fluxes towards the target products and introduce respective exporters, as demonstrated by putrescine and cadaverine production in *E. coli* [33•,34•]. Alternatively, another production host that is amenable to gene manipulation can be employed by introducing heterologous genes encoding the biosynthetic pathways for the target chemicals, as demonstrated for the microbial production of glucaric acid [27••], isoprene [37], and 3CM [38]. This option holds true for chemicals biosynthesized in mammalian or plant cells, and this particular step requires delicate synthetic biological tools that can control the proper expression of heterologous genes in microorganisms [29]. Once the production strains are developed by combined engineering approaches described above, then they can be further subjected to metabolic engineering aided with systems biological tools [48••], metabolic evolution [22,23,56•] and/or bioprocess development [11,37]. These steps can be repeated until satisfactory performance is achieved.

exporters. Thus, cells need to be metabolically engineered for the production of such metabolites. For instance, glucaric and adipic acids, isoprene, and 3-carboxymuconic acid (3CM) are monomeric chemicals for polymers that are not synthesized by natural microbial producers owing to the absence of their respective biosynthetic pathways. They can be produced by engineered production hosts, mostly *Escherichia coli*, that is amenable to metabolic engineering by introducing the corresponding genes from diverse genetic sources (Figure 2). Several important developments for (di)carboxylic acids, diamines, and dienes are summarized below.

(Di)carboxylic acids

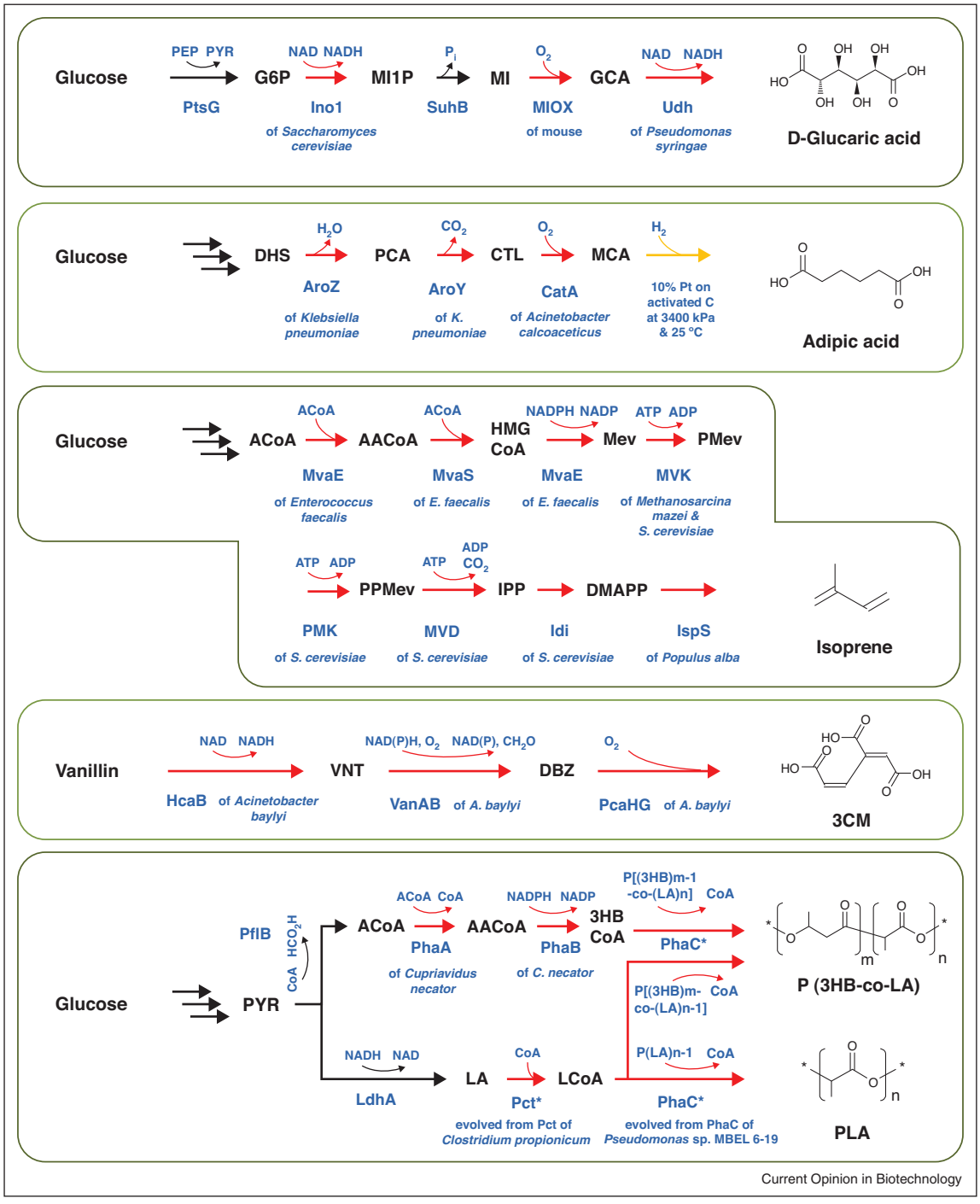
Naturally occurring products, including succinic, lactic, and itaconic acids, have also been produced by metabolically engineered *E. coli* strains by redirecting metabolic fluxes towards the desired products (Table 1). One notable example is the production of 87 g/L of succinic acid by *E. coli* through so called metabolic evolution that combines metabolic engineering and adaptive evolution strategy [22,23]. Lactic acid could also be successfully produced by *E. coli* knockout mutant strain, and one of the best examples showed production of 138 g/L of lactic acid with a yield of 0.99 g lactic acid/g glucose and an overall productivity of 3.54 g/L/h [24]. On the contrary, production of itaconic acid by an engineered *E. coli* strain was not as competitive as native fungal producers in terms of production capability, attaining only 4.16 g/L of itaconic acid in 72 h [25].

From an industrial perspective on the production of these acids, cultivation of cells at low pH is important because free acid form can be obtained without additional steps to remove cationic counter ions during separation and purification process. This bioprocess was successfully demonstrated by engineered acid-tolerant and osmo-tolerant *Yarrowia lipolytica* strain whose genes encoding succinate dehydrogenase was deleted and growth was enhanced via random mutation. Consequently, 45.5 g/L succinic acid was produced under low pH condition (Table 1) [26]. This example suggests that whole bioprocesses from upstream to downstream processes should be considered in an integrated way for the industrial level production of building block chemicals.

Glucaric and adipic acids are starting materials for several polymers: glucaric acid for poly(hexamethylene glucaramide) and adipic acid for nylon-4,6 and nylon-6,6, respectively (Figure 1). Since they are not produced by naturally isolated microorganisms, biosynthetic pathways for these chemicals need to be constructed in an appropriate host organism for their production. For glucaric acid, a direct synthetic pathway was recently constructed in *E. coli* by introducing *myo*-inositol-1-phosphate synthase from *Saccharomyces cerevisiae*, *myo*-inositol oxygenase from mouse, and urinate dehydrogenase from *Pseudomonas syringae* (Figure 2) [27••], which contrasts well against the already known complicated biosynthetic route of glucaric acid via pentose phosphate pathway in mammalian cells [28]. To enhance the production of glucaric acid, a polypeptide scaffold composed of protein domains that interact with one another was constructed to keep three key enzymes together and facilitate the conversion of metabolites through an engineered protein complex. Cells equipped with this scaffold subsequently increased glucaric acid titers up to 2.37 g/L, approximately 5-fold higher than that achieved without using the scaffold system [29].

For adipic acid, Frost and colleagues developed a process composed of both metabolic engineering and chemical conversion. They first developed an *E. coli* strain that produces 36.8 g/L *cis,cis*-muconic acid [30]. Subsequent chemical reaction of hydrogenating *cis,cis*-muconic acid led to adipic acid with a 0.97 g/g conversion yield at room temperature [30]. Current research efforts are geared towards designing biosynthetic pathways for adipic acid, which bypasses the last step of chemical reaction, as suggested in recent patents [31,32]. In these patents, systems biological tools, such as software packages for modeling and simulation of metabolic models and various gene targeting algorithms, contributed to predicting

Figure 2



Synthetic pathways constructed to produce various platform chemicals and polymers including glucaric acid, adipic acid, isoprene, 3CM, PLA, and P(3HB-*co*-LA), all of which are not naturally produced in wild-type *E. coli*. Synthetic pathways constructed by introducing heterologous enzymes or evolved enzymes are colored red. Various genetic sources from mammalian (mouse) to archaea (*Methanosarcina mazei*) are indicated below the respective enzyme names. Abbreviations for metabolites are: 3CM, 3-carboxymuconic acid; 3HB, 3-hydroxybutyrate; ACoA, acetyl-coenzyme A; AACoA, acetoacetyl-CoA; CTL, catechol; DBZ, 3,4-dihydroxybenzoate; DHS, 3-dehydroshikimic acid; DMAPP, dimethylallyl pyrophosphate; G6P, glucose 6-phosphate; GCA, D-glucuronic acid; HMGCoA, 3-hydroxy-3-methyl-glutaryl-CoA; IPP, isopentenyl diphosphate; LA, lactate; LCoA, lactyl-CoA; MCA, *cis,cis*-muconic acid; Mev, mevalonate; MI, *myo*-inositol; MI1P, MI 1-phosphate; PCA, protocatechuic acid; PEP, phosphoenolpyruvate; PLA, polylactate; PMev, phosphomevalonate; PPMev, diphosphomevalonate; PYR, pyruvate; VNT, vanillate. Abbreviations for enzymes are: AroY, PCA decarboxylase; AroZ, DHS dehydratase; CatA, CTL 1,2-dioxygenase; HcaB, 4-hydroxy benzaldehyde dehydrogenase; Idi, IPP isomerase; Ino1, MI1P synthase; IspS, isoprene synthase; LdhA, lactate dehydrogenase; MIOX, MI oxygenase; MvaE, AACoA thiolase/HMGCoA reductase; MvaS, Mev synthase; MVK, Mev kinase; MVD, PPMev decarboxylase; PcaHG, protocatechuate 3,4-dioxygenase; Pct, propionate CoA-transferase; PflB, pyruvate formate-lyase; PhaA, β-ketothiolase; PhaB, AACoA reductase; PhaC, PHA synthase; PMK, PMev kinase; PtsG, PEP-dependent glucose phosphotransferase; SuhB, phosphatase; Udh, uronate dehydrogenase; VanAB, vanillate monooxygenase.

possible combinations of gene knockout targets [32] and their resulting theoretical maximum yield of adipic acid [31]. These studies show important roles of systems and synthetic biology in metabolic engineering by fully utilizing the wealth of genomic information in expanding the spectrum of microbially producible chemicals [4].

Diamines

Putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) can be combined with diverse diacids to make polyamide 4 and 5 series, respectively: for example, nylon-4,6 by polymerizing putrescine and adipic acid and polyamide 54 by polymerizing cadaverine and succinic acid (Figure 1) [33•,34•]. Recently, metabolic engineering strategy was successfully employed to construct engineered *E. coli* strains that efficiently produce putrescine and cadaverine. Through system-wide metabolic engineering that included deleting degradation and utilization pathways, enhancing fluxes towards target products, and amplifying the key enzymes (ornithine decarboxylase for putrescine and lysine decarboxylase for cadaverine), the engineered *E. coli* strains were able to produce 24.2 g/L and 9.61 g/L of putrescine and cadaverine, respectively (Table 1) [33•,34•]. In another study, cadaverine was successfully produced by engineered *Corynebacterium* by similar metabolic engineering approaches [35]. Although production titers of these two building blocks need to be further improved for industrialization, these exemplary cases demonstrate the possibility of metabolic engineering that could replace the petrochemical synthetic process of building block chemicals.

Dienes

Isoprene, also known as 2-methyl-1,3-butadiene, is a five-carbon diene, and its polymer called poly(isoprene) has mainly been extracted from rubber tree [36••]. Recently, Genencor and Goodyear have developed the microbial process for isoprene production [36••]. They synthetically constructed a pathway towards isoprene production in *E. coli* by adopting genes from *Enterococcus faecalis*, *Methanosarcina mazei*, *S. cerevisiae*, and *Populus alba* (Figure 2) [37]. This work resulted in the production of more than 60 g/L of isoprene with high purity by collecting it from the off-gas emitted during microbial fermentation [37]. Synthetic biological strategy also enabled creation of unnatural 3CM, tricarboxylic acid with a diene structure. Using *E. coli* as a biocatalyst, 4-hydroxy benzaldehyde dehydrogenase, vanillate monooxygenase and protocatechuate 3,4-dioxygenase from *Acinetobacter baylyi* converted vanillin into 3CM with a yield of 1 g/g (Figure 2 and Table 1). The produced 3CM was chemically converted to trimethyl-3CM to make poly(styrene-*co*-trimethyl-3CM), which can be applied in the field of tissue engineering [38]. Although the productivity and titer are low at the moment, this example suggests another possibility and capacity of microorganisms engineered to produce novel building blocks that do not exist in nature, but are industrially in demand.

One-step direct microbial production of polymers

In contrast to building block chemicals so far discussed, microorganisms sometimes naturally produce, or can be engineered to produce polymers directly by fermentation without subsequent chemical processes. Microbial production of polysaccharides has been well established (see Figure 1) and thus is not covered in this paper. One step microbial production of polymers is a preferred approach because it allows delicate control of polymer composition by combining metabolic intermediates of monomers at varying ratios in one step biosynthetic process from renewable resources, and does not require additional costly processes involving environmentally harmful chemical catalysts and intermediates.

Microbial production of polyesters and polyamides

Polyhydroxyalkanoates (PHAs) are microbial polyesters, which accumulate inside cells under the growth limiting conditions in the presence of excessive carbon source [39,40]. PHAs have attracted great metabolic engineering efforts in order to replace some of petroleum-based plastics for sustainable development owing to their favorable features, including biodegradability, biocompatibility, and composition versatility with more than 150 known monomers [41,42]. Some of recent metabolic engineering efforts include control of PHA composition by genetically attenuating fatty acid β-oxidation pathway for efficient incorporation of externally supplied fatty acids [43,44], production of PHAs from engineered methanol-utilizing bacterium [45], and development of stress-induced system for automatic induction of PHA production [46]. The ultimate goal is to develop a cost-effective bioprocess for PHAs with various industry-favorable properties, and for this, Metabolix and ADM teamed up to commercially produce PHAs.

Polylactic acid (PLA) is also an important polyester, which is becoming increasingly popular in many industries. Microbial production of PLA and its copolymers had not been possible until recently owing to the lack of natural enzymes and pathways leading to the formation of PLA. PLA is currently produced in two step processes: microbial production of lactic acid followed by chemical polymerization process. For the one-step fermentative production of PLA and its copolymers (Figure 2), the artificially evolved heterologous propionate CoA transferase and PHA synthase were introduced into *E. coli* [47–50]. Central carbon metabolism of *E. coli* was subsequently engineered to redirect metabolic fluxes towards precursors of these biopolymers based on genome-scale metabolic simulation. The resulting strains were able to produce PLA homopolymer from glucose up to 11 wt% of dry cell weight and poly[3-hydroxybu-

tyrate(3HB)-*co*-LA] containing 55–86 mol% lactic acid up to 56 wt% of dry cell weight from glucose and 3HB. By introducing β-ketothiolase and acetoacetyl-CoA reductase from *Cupriavidus necator* (Figure 2), P(3HB-*co*-LA) containing 70 mol% lactic acid was successfully produced up to 46 wt% of dry cell weight [48••]. In addition, a more efficient bioprocess that does not require inducer and succinic acid in the culture medium was developed for the production of PLA and its copolymers [51]. The approach of combining systems biology and synthetic biology with metabolic engineering as demonstrated in these studies should serve as a platform technology for production of other polymers.

Microbial production of polyamides was recently demonstrated by the production of ultra-high MW (285 kDa) spider silk protein, with potential applications in protective clothing and biomedical industries owing to its exceptional mechanical strength, biodegradability and biocompatibility [52•]. In this work, *E. coli* was metabolically engineered to increase the pool of glycyl-tRNA because the spider silk is enriched in glycine (ca. 43% of total protein). Additional engineering and analysis of *E. coli* strains using comparative proteomic analyses provided further insight into their production performance and targets to be manipulated. Other notable polyamides include poly-γ-glutamate and ε-poly-L-lysine, used for drug delivery/cosmetics and feed preservatives, respectively, whose microbial production needs to be enhanced by metabolic engineering approaches described above (Box 1) [40].

Conclusion

A range of microbially producible building blocks is constantly expanding thanks to the recent advances in metabolic engineering, systems biology, synthetic biology, and bioprocess engineering. Constructing synthetic pathways by using genes from various organisms (and from nature through metagenome) and by creating (or evolving at least) enzymes facilitates production of building block chemicals and polymers that cannot otherwise be produced in nature. Recently, multiplexed approaches including metabolic evolution, global transcription machinery engineering, trackable multiplex recombineering, and multiplex automated genome engineering that intersect borders among metabolic engineering, genome engineering, systems biology, synthetic biology, and evolutionary engineering have been developed [53–55,56•]. Integrated use of these emerging strategies will not only contribute to simultaneous engineering of multi-gene targets for enhanced production of chemicals, but also systematic optimization of new metabolic and gene regulatory networks established in the production host. With these advances, it is expected that more chemicals and materials will be produced through microbial fermentation from renewable resources, which will consequently contribute to establishing bio-based economy and achieving low carbon green growth.

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- •• of outstanding interest

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