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REVIEW ARTICLE

Recent advances in engineering propionyl-CoA metabolism for microbial production of value-added chemicals and biofuels

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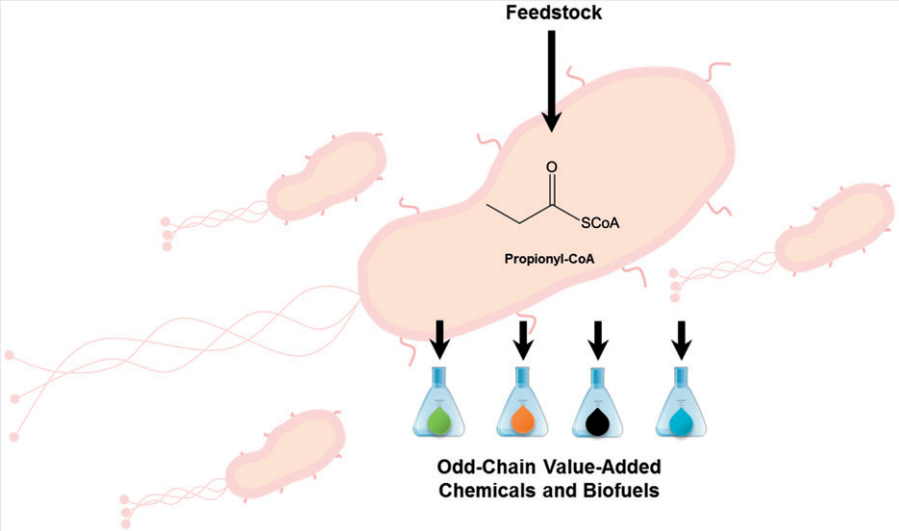
ABSTRACT

Diminishing fossil fuel reserves and mounting environmental concerns associated with petrochemical manufacturing practices have generated significant interests in developing whole-cell biocatalytic systems for the production of value-added chemicals and biofuels. Although acetyl-CoA is a common natural biogenic precursor for the biosynthesis of numerous metabolites, propionyl-CoA is unpopular and non-native to most organisms. Nevertheless, with its C3-acyl moiety as a discrete building block, propionyl-CoA can serve as another key biogenic precursor to several biological products of industrial importance. As a result, engineering propionyl-CoA metabolism, particularly in genetically tractable hosts with the use of inexpensive feedstocks, has paved an avenue for novel biomanufacturing. Herein, we present a systematic review on manipulation of propionyl-CoA metabolism as well as relevant genetic and metabolic engineering strategies for microbial production of value-added chemicals and biofuels, including odd-chain alcohols and organic acids, bio(co)polymers and polyketides.



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Introduction

Due to the prognosticated demise of fossil fuel reserves in the years to come and the increasing environmental concerns associated with petrochemical-based processes, there is an ever-growing need for the development of whole-cell biocatalysts for the production of platform chemicals and fuels.[1,2] In addition to alleviating global reliance on fossil fuels, whole-cell biocatalytic platforms offer several technological advantages, such as providing absolute enantio- and regio-control on the configuration of the

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target compound and the ability to catalyze reactions with multiple chemical steps under ambient conditions.[3] However, the applicability of natural biological systems is often limited due to inaccessible metabolic pathways. Accordingly, the present focus in metabolic engineering and systems biology is to expand the metabolic repertoire of genetically tractable host platforms.[1,4,5] To improve the catalytic scope and efficacy of microbial systems, it is often necessary to rewire host metabolism to ensure an adequate supply of endogenous precursors and intermediary metabolites of the target compound. To date, significant progress has been made through the development of novel genetic and metabolic engineering strategies for efficient conversion of carbonaceous feedstocks into acetyl coenzyme A (acetyl-CoA).[6,7] Given that acetyl-CoA is ubiquitous, as an acetyl transfer agent of the central metabolism in all living systems, it serves as a key intermediate for the production of numerous even-chain products. On the other hand, the endogenous production of propionyl-CoA, the three-carbon biogenic counterpart to acetyl-CoA, is considered a rare natural metabolite and is restricted to a limited number of phylogenetically diverse soil-dwelling microorganisms and commensals of the mammalian gut.[8] In these organisms, propionyl-CoA is an intermediary molecule derived from a variety of pathways, such as thioesterification of propionate and dissimilation of odd-chain fatty acids or α-amino acids (e.g. L-threonine).[9] Accordingly, metabolic engineering approaches for the implementation and overexpression of pathways for high-level propionyl-CoA biosynthesis in model microorganisms are still in their infancy. However, it is well-recognized that tapping and harnessing propionyl-CoA metabolism can greatly expand the capacity for the microbial synthesis of a wide variety of odd-chain compounds.

In prokaryotes, at least three metabolic pathways toward propionyl-CoA formation exist. In the first one, i.e. the acrylate pathway (Figure 1(a)), lactoyl-CoA (activated from lactate) is dehydrated by lactyl-CoA dehydratase to acryloyl-CoA, and subsequently reduced by the acryloyl-CoA reductase complex to propionyl-CoA. This pathway is endogenous to selected amino-acid utilizing Clostridia, such as *Clostridium propionicum*. The second route involves the methylmalonyl-CoA pathway (Figure 1(b)).[10] This cyclic pathway is generally confined to Gram-positive propionic acid bacteria (of the genus *Propionibacterium*) and relies on the biosynthesis of oxaloacetate by one of the two enzymes, i.e. pyruvate carboxylase and methylmalonyl-CoA carboxytransferase, which catalyze the transfer of the carboxyl moiety from (*S*)-methylmalonyl-CoA to pyruvate, concomitantly generating oxaloacetate and propionyl-CoA.[11] The third route (Figure 1(c)) involves the 3-hydroxypropionate pathway and is endogenous to thermophilic bacteria *Chloroflexus aurantiacus*.[12] In this bicyclic pathway, acetyl-CoA is first fused with bicarbonate to form 2-malonyl-CoA by an acetyl-CoA carboxylase. Next, 2-malonyl-CoA is reduced to propionyl-CoA by the action of two enzymatic activities, i.e. malonyl-CoA reductase and propionyl-CoA synthase. It is unclear as to why these metabolic pathways for propionyl-CoA biosynthesis are not evolutionarily conserved amongst microbial communities. However, as propionyl-CoA is toxic at high concentrations, presumably most organisms may have evolved to prevent intracellular accumulation of propionyl-CoA.[8,13] Furthermore, one of the key enzymes for the conversion of succinyl-CoA to propionyl-CoA is methylmalonyl-CoA mutase (Figure 1(b)), whose activity requires a vitamin B_{12}-derived prosthetic group, adenosylcobalamin, in order to function. Given that most microbial systems do not possess the ability for *de novo* biosynthesis of cobalamin, its exogenous provision as a vitamin precursor is required to utilize this pathway.[14]

Various strategies have been developed to increase the intracellular level of propionyl-CoA as a precursor for the biosynthesis of a range of chemicals and biofuels (Figure 2), particularly in genetically tractable hosts with propionyl-CoA as a non-native metabolite such as *E. coli*. As a biochemical approach, propionate or odd-chain fatty-acid-rich feedstocks have been exogenously supplemented in the culture medium for their direct conversion to propionyl-CoA.[15] However, the high costs associated with these feedstocks potentially limit practical application of this approach. Alternatively, at least two metabolic pathways based on catabolism of either 2-ketobutyrate (i.e. the L-threonine biosynthetic pathway [16]) or succinyl-CoA (i.e. the sleeping beauty mutase (Sbm) pathway [17]) have also been explored to enable biosynthesis of propionyl-CoA from unrelated carbon sources in *E. coli* (Figure 3). Such explorations have opened an avenue for novel biosynthesis. Herein, we intend to review novel approaches undertaken to engineer propionyl-CoA metabolism in native and engineered microbial cell factories for biomanufacturing purposes. These biotechnological illustrations are made using representative chemicals with propionyl-CoA as a key precursor for their biosynthesis (Figure 2), specifically including odd-chain alcohols (as fusel alcohols, solvents or potential biofuels), odd-chain organic acids (as food or animal feed additives or for pharmaceutical applications), bio(co)polymers (as biodegradable plastics or for medical applications)

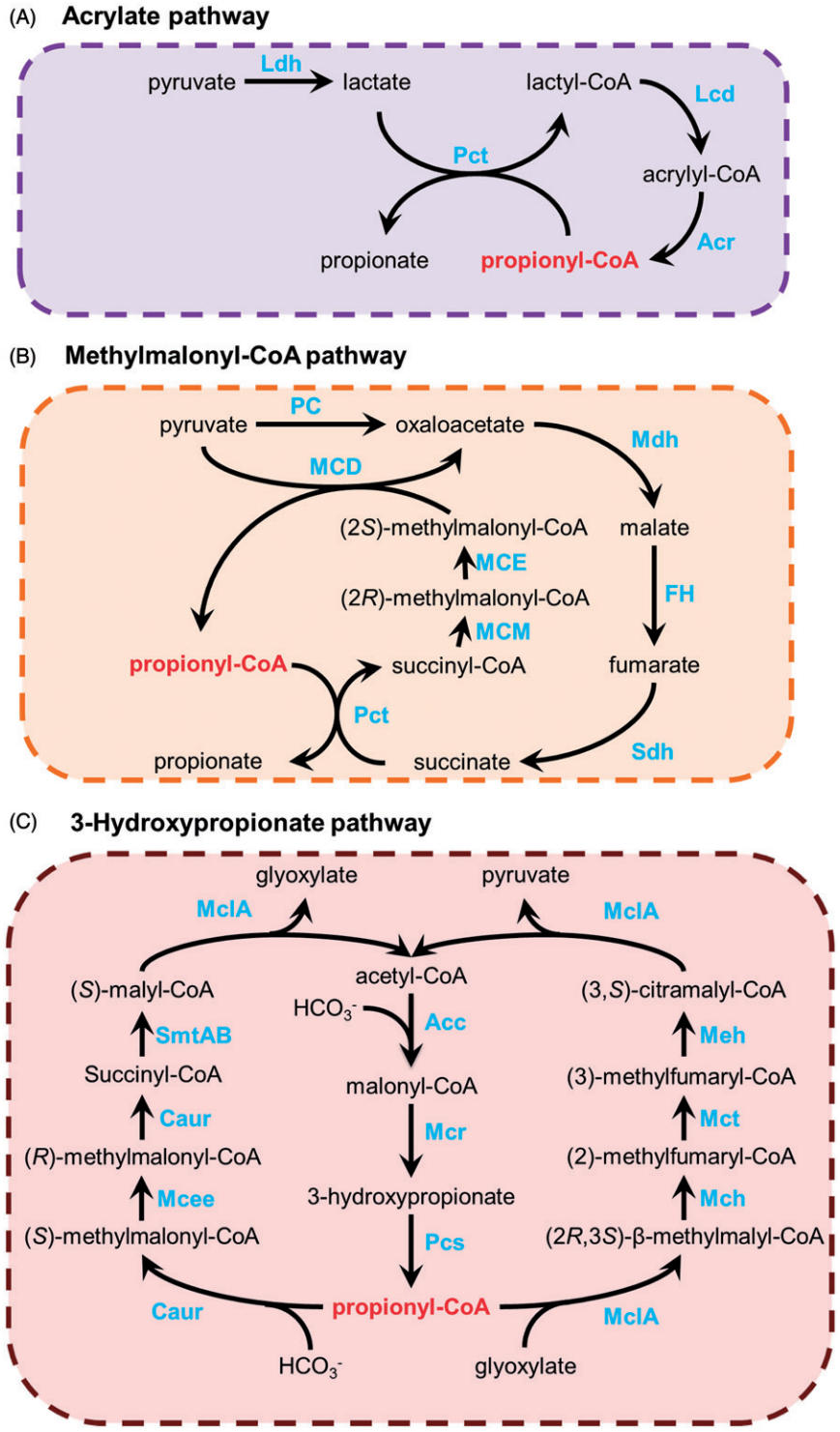

Figure 1. Natural pathways associated with propionyl-CoA metabolism found in microorganisms. (a) The acrylate pathway of *Clostridium propionicum*. (b) The methylmanoyl-CoA pathway of propionic bacteria. (c) The 3-hydroxypropionate pathway of *Chloroflexus aurantiacus*. Key enzymes in the pathways are: Ldh: lactate dehydrogenase; Pct: propionyl-CoA transferase; Lcd: lactyl-CoA dehydratase; Acr: acrylyl-CoA reductase; PC: pyruvate carboxylase; Mdh: malate dehydrogenase; FH: fumarate hydratase; Sdh: succinate dehydrogenase; MCM: methylmalonyl-CoA mutase; MCE: methylmalonyl-CoA epimerase/isomerase; MCT: methylmalonyl-CoA carboxytransferase, Acc: acetyl-CoA carboxylase; Mcr: malonyl-CoA reductase; Pcs: propionyl-CoA synthase; Caur: propionyl-CoA carboxylase; MclA: (*S*)-malyl-CoA/β-methylmalyl-CoA/(*S*)-citramalyl-CoA (MMC) lyase.

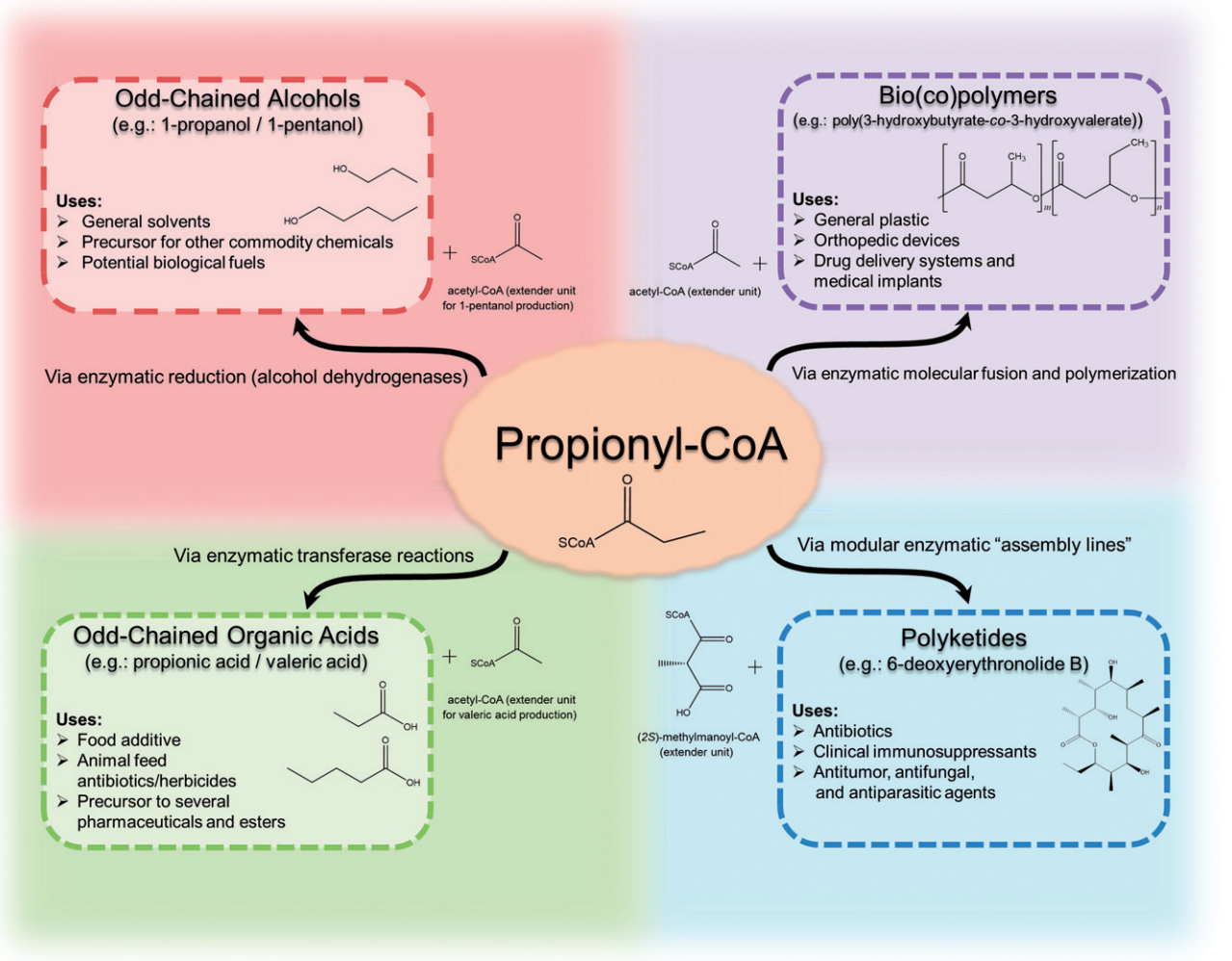

Figure 2. Propionyl-CoA acting as a precursor. Propionyl-CoA can act as a key biogenic precursor for the production of several value-added chemicals and biofuels of industrial importance.

and polyketides (for medical applications or as antifungal and insecticidal agents).

Production of odd-chain alcohols

1-Propanol production via non-fermentative pathways

Compared to the most popular biofuel ethanol, 1-propanol and other higher-chain alcohols are considered better energy carriers because of their more favorable physicochemical properties, including higher energy density, octane number and lower hygroscopicity. Additionally, 1-propanol has found its use as a multipurpose solvent for various industrial applications, including paints, cleaning products and cosmetics.[18] Because no native microbial producer has been identified so far, large-scale production of 1-propanol is conducted primarily through chemical synthesis. Nevertheless, rational synthetic biology and metabolic engineering strategies for 1-propanol production in microbial hosts, particularly genetically tractable *E. coli*, have been developed [17,19–21,22] (Table 1). To this end, recent studies aim at modulating the intracellular pool of propionyl-CoA and/or other key intermediates by engineering heterologous or endogenous metabolic pathways (Figure 3) to enhance 1-propanol production.

In addition to propionyl-CoA, 2-ketobutyrate can be another key precursor to 1-propanol. In many microorganisms, including *E. coli*, 2-ketobutyrate is an endogenous product associated with L-threonine degradation and a reductive pathway (such as alcohol production) can be used to dispose of electrons generated during amino acid metabolism. The production of 1-propanol via the non-fermentative pathway for L-threonine degradation has been previously detected in *Clostridium* sp. 17cr1.[37] Alternative to L-threonine degradation, the most direct route for forming 2-ketobutyrate is via

Table 1. Overview of key propionyl-CoA-derived metabolites produced in microbial platforms.

Pathway	Target metabolite	Titer(g/L)	Genetic approach	Carbon source(s)	Reference
Sleeping beauty mutase	1-propanol	~0.17	Expression of the endogenous sleeping beauty mutase and gene encoding for succinyl-CoA synthetase in *E. coli*; introducing aldehyde/alcohol dehydrogenases	20 g/L glucose	Srirangan et al. [17]
		~7.00	Expression of the endogenous sleeping beauty mutase operon in *E. coli*	30 g/L glycerol	Srirangan et al. [22]
		~0.48	Addition of bifunctional butyraldehyde/alcohol dehydrogenase to *T. fusca*; deletion of cellulase regulator gene, *CelR*	20 g/L switchgrass	Deng and Fong [23]
	propionic acid	~11.8	Expression of the endogenous sleeping mutase operon; inactivation or overexpression of glycerol dissimilation genes	30 g/L glycerol	Akawi et al. [24]
	butanone	~1.30	Expression of the endogenous sleeping beauty mutase operon; molecular fusion of propionyl-CoA and acetyl-CoA to produce 3-ketovaleryl-CoA	30 g/L glycerol	Srirangan et al. [25]
	P(3HB-*co*-3HV)	~34.2% DCW[a]	Development of expression system in *S. enterica* serovar Typhimurium, containing polyhydroxyalkanoate operon and sleeping beauty mutase genes	25 g/L glycerol and 0.6 g/L propionic acid	Aldor et al. [26]
1, 2-propandiol	1, 2-propandiol	~0.8	Expression of methylglyoxal synthase, alcohol dehydrogenase and methylglyoxal reductase obtained from *E. coli*	20 g/L glucose	Jain and Yan[27]
		~4.5	Co-expression of methylglyoxal synthase, glycerol dehydrogenase and either yeast alcohol dehydrogenase or 1,2-propandiol oxidoreductase	10 g/L glucose	Altaras and Cameron [28]
	1-propanol	~0.25	Co-expression of 1,2-propendiol dehydratase from *K. oxytoca* with methylglyoxal synthase, alcohol dehydrogenase and methylglyoxal reductase	20 g/L glucose	Jain and Yan [27]
L-threonine	1-propanol	~2.00	Introducing heterologous decarboxylase and dehydrogenase; deregulation of amino-acid synthesis and deletion of competing metabolic pathways	30 g/L glucose	Shen and Liao [20]
		~10.8	Threonine dehydratase introduced into *E. coli* host; competing pathways were eliminated; overexpressing citramalate synthase and acetate kinase A	20 g/L glucose	Choi et al. [16]
	propionic acid	~4.00	Threonine dehydratase introduced into *E. coli* host; competing pathways were eliminated; overexpressing citramalate synthase and acetate kinase A	20 g/L glucose	Choi et al. [16]
	P(3HB-*co*-3HV)	~11.1% DCW[a]	Overexpression of threonine deaminase; mutation and overexpression of *thrABC* operon; deletion of competing propionyl-CoA to 3-hydroxyvaleryl-CoA pathway	20 g/L xylose	Chen et al. [29]
	(*S*)-3HB	~0.31	Overexpression of *thrABC* operon; *T7lac* promoter substituted for native promoter; competing threonine formation and degradation pathways removed	20 g/L glucose	Tseng et al. [30]
	(*R*)-3HB	~0.96	Overexpression of *thrABC* operon; *T7lac* promoter substituted for native promoter; competing threonine formation and degradation pathways removed	20 g/L glycerol	Tseng et al. [30]

(*continued*)

Table 1. Continued

Pathway	Target metabolite	Titer(g/L)	Genetic approach	Carbon source(s)	Reference
citramalate	1-propanol	~2.50	Citramalate synthase directed evolution by error-prone PCR and *ilvI* knockouts	72 g/L glucose	Atsumi and Liao [31]
	1-butanol	~0.39	Citramalate synthase directed evolution by error-prone PCR and *ilvI* knockouts	72 g/L glucose	Atsumi and Liao [31]
	P(3HB-*co*-3HV)	~61.7% DCW[a]	Expressing *E. coli* pyruvate oxidase mutant (*poxB L253F V380A* gene) together with *R. eutropha* propionyl-CoA synthase and PHA biosynthesis genes	20 g/L glucose	Yang et al. [32]
methylcitric acid cycle	propionic acid	~17.0	Inactivation of acetate kinase (*ack* gene) in *P. acidipropionici* by gene disruption and integrational mutagenesis	30 g/L glucose	Suwannakham et al. [33]
		~13.2	Cloning phosphoenolpyruvate carboxylase (*ppc* gene) into *P. freudenreichii*	25 g/L glucose	Ammar et al. [34]
	P(3HB-*co*-3HV)	~68.6% DCW[a]	Genetic disruption of 2-methylcitrate synthase genes (*prpC1* and *prpC2*)	20 g/L glucose	Zhang et al. [35]
acrylate	propionic acid	~0.27	Extending the carbon chain of 2-ketoacids by engineering leucine biosynthesis	20 g/L glucose	Marcheschi et al. [36]

[a]DCW: dry cell weight.

citramalate biosynthesis in select microorganisms, including *Methanococcus jannaschii*.[38,20] These observations formed the basis for 1-propanol production in *E. coli* via non-fermentative pathways.[21,31,39] Specifically, the production of 1-propanol was mediated through promoting 2-ketobutyrate biosynthesis via the L-threonine or citramalate pathway [31] (Figure 3). To increase the level of L-threonine precursor, the concerted feedback inhibition exerted by L-threonine biosynthesis was overcome through directed mutagenesis of *thrA*, which encodes the bifunctional aspartokinase/homoserine dehydrogenase with a target site for allosteric feedback inhibition by L-threonine (Figure 3(a)). Alternatively, 2-ketobutyrate can be derived through the condensation of acetyl-CoA and pyruvate via the citramalate pathway (Figure 3(b)). By overexpression of the *cimA* gene from *M. jannaschii* to form citramalate and then the endogenous *leuABCD* operon to form 2-ketobutyrate along with inactivation of several competing pathways, ~0.5 g/L 1-propanol was produced. The 1-propanol titer can be significantly increased by overexpressing an evolved *cimA* (*cimA3.7*) derived through multiple rounds of error-prone PCR.[31] Note that a promiscuous 2-ketoacid decarboxylase (i.e. Kivd from *Lactococcus lactis*) and a broad-range alcohol dehydrogenase (i.e. ADH2 from *S. cerevisiae*) were used for direct conversion of 2-ketobutyrate to 1-propanol.[21,31] While 2-ketobutyrate can be converted endogenously to 1-propanol through the intermediates propionate and propionyl-CoA, the aforementioned direct conversion of 2-ketobutyrate to 1-propanol [21] can potentially circumvent the production of propionate as a byproduct. Furthermore, by synergistically combining the strategies of deregulated L-threonine biosynthetic pathway [21] and evolved *cimA*,[31] as well as more extensive inactivation of pathways competing for the essential precursor 2-ketobutyrate, the production of 1-propanol was significantly enhanced.[16,20] The L-threonine biosynthetic pathway activity was enhanced to increase the intracellular 2-ketobutyrate pool through directed-evolution of the *ilvA* gene encoding L-threonine dehydratase and overexpression of the endogenous L-threonine synthetic operon *thrABC*.[16] Alternative to direct conversion of 2-ketobutyrate to 1-propanol via promiscuous 2-ketoacid decarboxylase and alcohol dehydrogenase, various genes in the other 1-propanol-formation pathway, i.e. *ackA* encoding acetate/propionate kinase, *atoDA* encoding acetyl-CoA:acetoacetyl-CoA synthase and an aerobic-tolerant *adhE* encoding alcohol/aldehyde dehydrogenase were overexpressed.[16]

1-Propanol production via fermentative pathways

Recently, 1-propanol production via a novel C3-fermentative pathway by activation of the native, but physiologically inactive, Sbm operon in *E. coli* was reported.[17,22] In contrast to the non-fermentative L-threonine pathway, the key metabolite intermediate propionyl-CoA was derived based on extended dissimilation of succinate under anaerobic conditions [17] (Figure 3(c)). By episomally expressing key genes, such as the Sbm operon genes, *sucCD* encoding succinyl-CoA synthetase, and *adhE2* encoding NADH-dependent alcohol dehydrogenase (ADHE2) from *Clostridium acetobutylicum*, heterologous production of 1-propanol was

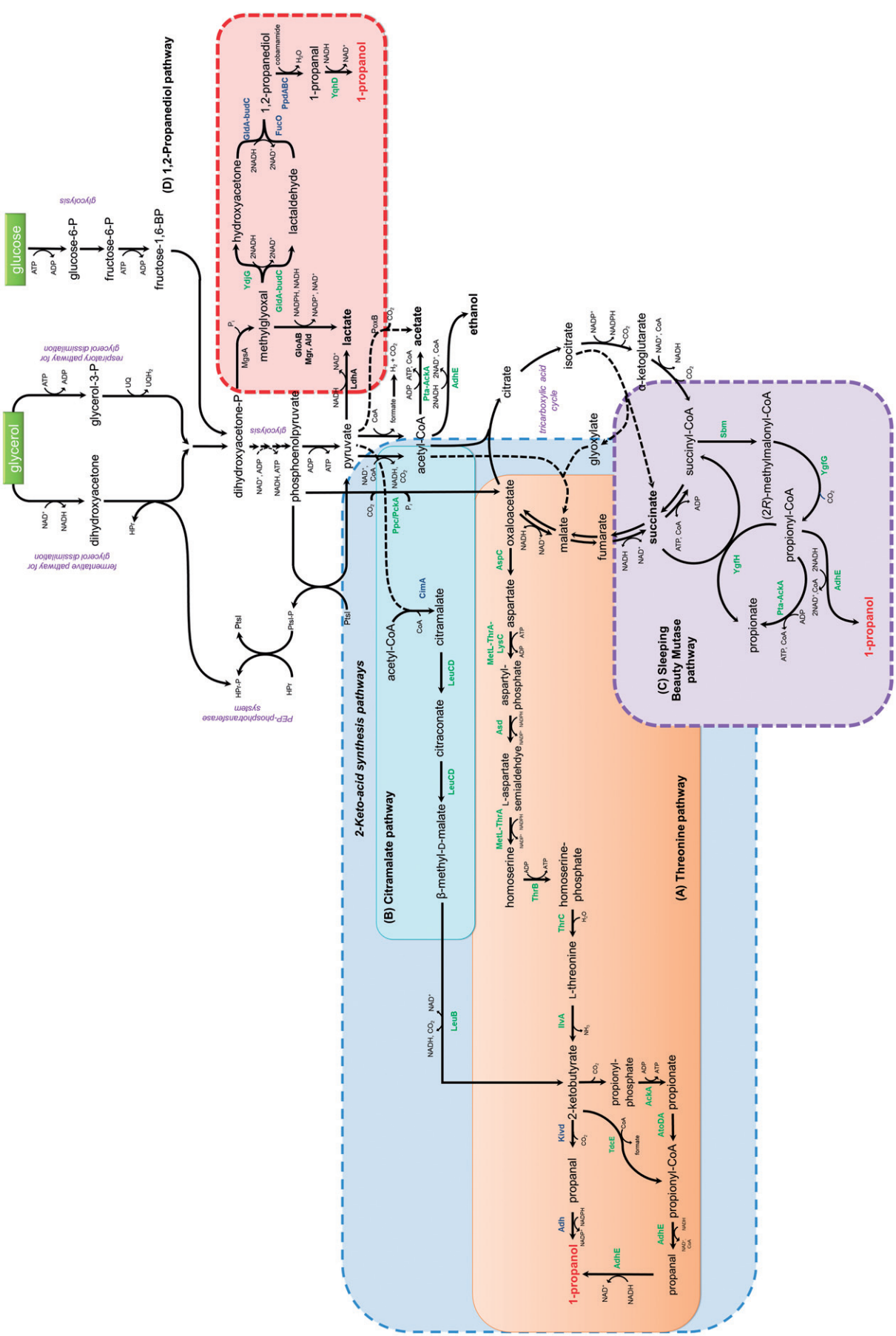

Figure 3. Metabolic pathways for 1-propanol production in engineered *E. coli*. Pathways include the non-fermentative pathways via (a) L-threonine and (b) citramalate biosynthesis, as well as the fermentative ones via (c) the Sbm pathway (d) synthetic extension of 1,2-propanediol. Key enzymes in the pathways are: AspC: aspartate aminotransferase; LeuB: 3-isopropylmalate dehydrogenase; LeuCD: 3-isopropylmalate isomerase A and B; ThrA: homoserine dehydrogenase; ThrB: homoserine kinase; ThrC: homoserine deaminase; IlvA: L-threonine deaminase; Sbm: sleeping beauty mutase; YgfG: methylmalonyl-CoA decarboxylase; YgfH: propionyl-CoA:succinate transferase; GldA: glycerol dehydrogenase; YqhD: alcohol dehydrogenase; AdhE: bifunctional alcohol/aldehyde dehydrogenase. Heterologous enzymes are represented in blue, whereas native *E. coli* enzymes are represented in green. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

demonstrated.[17] Subsequently, a plasmid-free propanogenic *E. coli* strain was derived by inserting a regulatable *trc*-promoter in front of the genomic Sbm operon for activating its expression as well as inactivating the endogenous *ldhA* gene.[22] Using this engineered strain for fed-batch cultivation with glycerol as the carbon source, high-level coproduction of 31 g/L ethanol and 7 g/L 1-propanol was demonstrated. A pathway similar to the Sbm pathway of *E. coli* potentially exists in *Thermobifida fusca*, a thermophilic, Gram-positive and cellulose-degrading microorganism. Because of the presence of native propionyl-CoA, 0.48 g/L 1-propanol was produced in an engineered cellulolytic *T. fusca* strain with heterologous expression of *adhE2* (encoding alcohol dehydrogenase) from *C. acetobutylicum*, demonstrating direct conversion of lignocellulosic biomass to 1-propanol.[23] It is noteworthy to highlight another fermentative pathway for 1-propanol production, albeit without involving propionyl-CoA, based on the expansion of the canonical 1,2-propanediol pathway in engineered *E. coli* (Figure 3(d)).[27] The conversion of 1,2-propanediol to 1-propanol was mediated by 1,2-propanediol dehydratase (encoded by *ppdABC* from *Klebsiella oxytoca*) and alcohol dehydrogenase (encoded by *yqhD* from *E. coli*). For more effective production of 1-propanol, a synthetic pathway was created to shunt carbon flux from glycolysis towards 1,2-propanediol synthesis via a methylglyoxal intermediate.[27]

Advanced odd-chain alcohols

Currently, microbial production of advanced (i.e. long) odd-chain alcohols continues to be challenging with limited achievement. Since propionyl-CoA and acetyl-CoA are typical starter and extending units, respectively, for biosynthesis of long-odd-chain alcohols, the intracellular availability of propionyl-CoA can be a major technical limitation. In a culminating study, heterologous production of 1-pentanol in *E. coli* was demonstrated through the introduction of key genetic and metabolic modules.[40] First, a feedback-resistant threonine biosynthetic pathway and a feedback-resistant mutant of *ilvA,* encoding threonine deaminase, were expressed to reinforce the production of 2-ketobutyrate, a precursor to propionyl-CoA. For 1-pentanol production, propionyl-CoA was first fused with endogenous acetyl-CoA to form 3-ketovaleryl-CoA (a C5 biogenic thioester) via a biosynthetic thiolase (i.e. BktB from *Cupriavidus necator*).[41] Next, the nascent 3-ketovaleryl-CoA was reduced to valeryl-CoA by expressing the butyryl-CoA synthesis (BCS) operon from *Clostridium acetobutylicum*, which contains Hbd (3-hydroxybutyryl-CoA dehydrogenase), Crt (crotonase) and Bcd-EtfAB (butyryl-CoA dehydrogenase with its two electron transfer proteins).[42] Lastly, the bifunctional alcohol dehydrogenase, encoded by *adhE2* from *C. acetobutylicum*, was used to catalyze the reduction of valeryl-CoA to 1-pentanol (Figure 4).[40] While the use of glucose as the sole carbon source enabled slight production of 1-pentanol at 19 mg/L, the 1-pentanol titer was elevated to 534 mg/L using a related carbon source of valerate, implying potentially limited availability of propionyl-CoA.[40] Accordingly, strain engineering strategies aimed at boosting the propionyl-CoA level should offer promises for biosynthesis of long-odd-chain alcohols. On the other hand, given the cyclic nature of the CoA-dependent chain elongation pathway, it should be feasible to further engineer this biosynthetic platform for scalable production of longer odd-chain bulk alcohols (e.g. 1-heptanol and 1-nonanol) with their fuel characteristics similar to those of diesel.[36]

Production of odd-chain organic acids

Propionic acid production in *Propionibacteria*

Due to the versatility and long history of applications in the food, pharmaceutical, cosmetic and chemical industries, organic acids are among the most industrially and commercially important chemicals. While economical production of organic acids has been primarily carried out by chemical synthesis, increased demands for environmentally friendly production platforms are promoting alternative bio-based synthesis,[43,44] particularly based on microbial production with renewable feedstocks.[45] Similar to odd-chain alcohols, propionyl-CoA acts as a key precursor for biosynthesis of odd-chain organic acids. Current biological production of propionic acid in native hosts is limited to Gram-positive anaerobes, i.e. *Propionibacteria* via the methylmalonyl-CoA mutase pathway and *C. propionicum* via the acrylate pathway (Figure 1). High-level production of propionic acid has been demonstrated in several native *Propionibacteria* hosts.[46–48] However, product inhibition and byproduct (e.g. succinic acid and acetic acid) formation often limits culture performance. Metabolic engineering of the production strains can provide a means to overcome these limitations. For example, inactivating the *ack* gene (encoding acetate kinase) in *P. acidipropionici* successfully eliminated acetate byproduct formation and increased propionic acid titer.[33] In contrast to targeting the competing fermentative pathways, reengineering central carbon metabolism of *Propionibacterium* hosts for more effective carbon

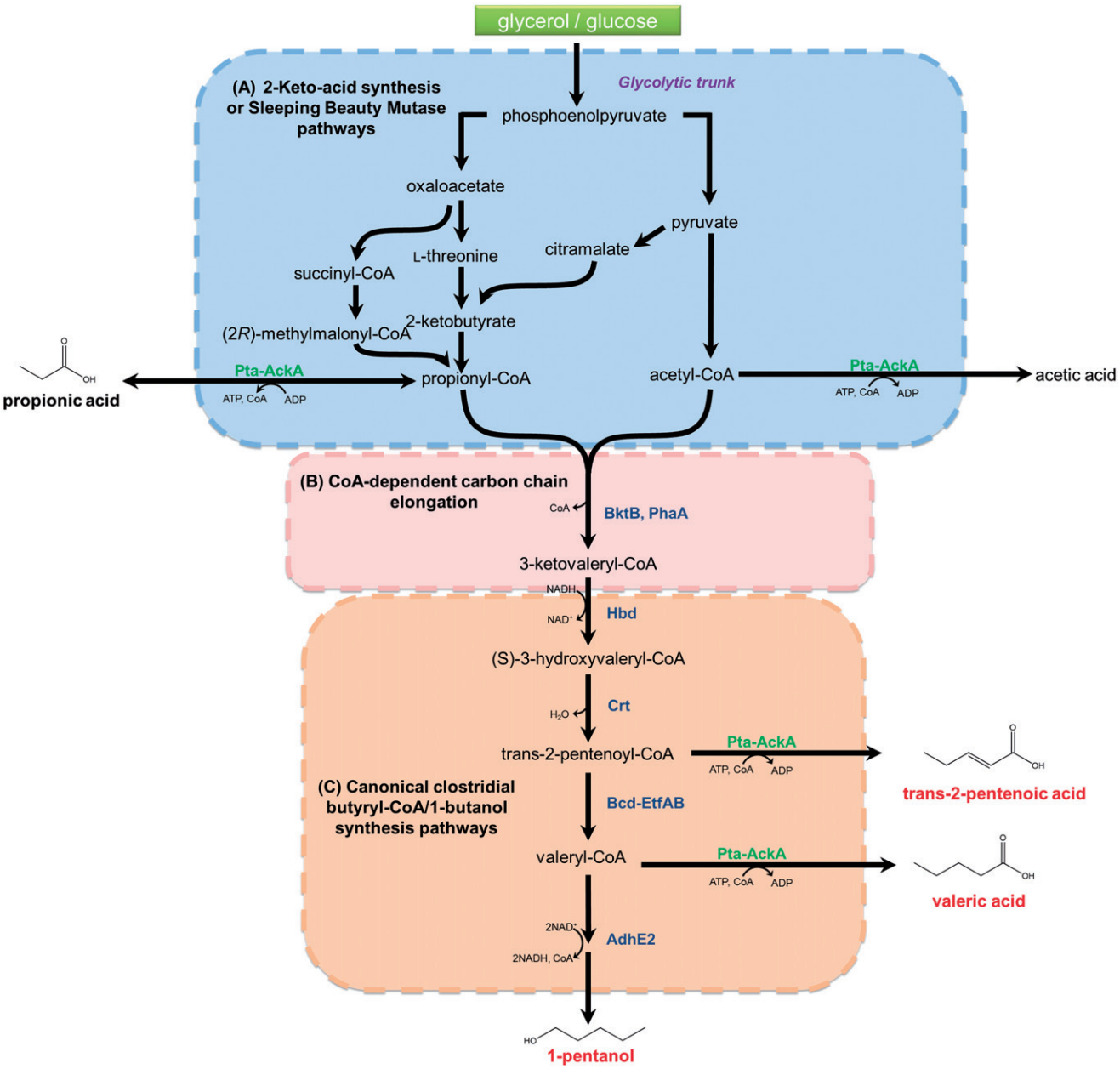

Figure 4. Metabolic pathways for 1-pentanol and valeric acid production in engineered *E. coli*. Propionyl-CoA is heterologously derived via either (a) 2-keto-acid synthesis or the Sbm pathway. Propionyl-CoA and acetyl-CoA are fused using (b) β-ketothiolases (i.e. BktB and PhaA from *C. necator*) to form 3-ketovaleryl-CoA, which is subsequently channeled into (c) the canonical clostridial butyryl-CoA and 1-butanol synthesis pathways (i.e. Hbd: (*S*)-3-hydroxybutyryl-CoA dehydrogenase; Crt: crotonase; Bcd-EtfAB, butyryl-CoA dehydrogenase and its electron-transfer flavoprotein complexes; AdhE2: bifunctional alcohol dehydrogenase from *C. acetobutylicum*) to generate 1-pentanol. The intermediary C5 CoA-thioesters trans-2-pentenoyl-CoA and valeryl-CoA can also be harnessed for biosynthesis of trans-2-pentenoic and valeric acids, respectively, using the native acid-formation pathway (i.e. Pta-AckA: phosphotransacetylase-acetate kinase). Heterologous enzymes are represented in blue, whereas native *E. coli* enzymes are represented in green. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

dissimilation can also enhance propionate production. For example, the *E. coli ppc* gene, which encodes phosphoenolpyruvate carboxylase for the conversion of phosphoenolpyruvate to oxaloacetate, was expressed in *P. freudenreichii* to enhance propionic acid production presumably by channeling more carbon flux through the methylmanoyl-CoA pathway and more effective incorporation of CO_2 into the methylmanoyl-CoA pathway.[34] Similarly, upon heterologous expression of the *Klebsiella pneumoniae gldA* gene encoding glycerol dehydrogenase, the recombinant strain showed more rapid glycerol consumption with enhanced propionic acid production and minimal byproduct formation.[49]

Propionic acid production in engineered E. coli

Although *Propionibacterium* species are native producers of propionic acid, they are not genetically amendable. Consequently, the use of *E. coli*, the most popular workhorse for biomanufacturing, for propionic acid production is worthy of exploration. Metabolic engineering of *E. coli* was conducted by episomally expressing three key genes of the acrylate pathway (i.e. *pct*, *lcd* and *acr* encoding propionyl CoA:lactyl-CoA transferase, lactyl-CoA dehydratase and acrylyl-CoA reductase, respectively) from *C. propionicum* (Figure 1(a)).[50] While heterologous production of propionic acid was demonstrated, the engineered *E. coli* had impaired cell growth with poor propionic acid productivity potentially due to limited functional expression of these acrylate pathway genes.[50] Because propionyl-CoA is a common precursor for 1-propanol and propionic acid, propanogenic *E. coli* strains can be used for potential propionic acid production. For example, the same L-threonine feedback inhibition strain used for 1-propanol production can produce propionic acid at relatively high levels.[16] Recently, the Sbm pathway in *E. coli* was also shown to be competent for synthesis of 1-propanol and propionic acid (Figure 3(c)). Using the propanogenic *E. coli* with its genomic the Sbm-operon being activated as well as tuning oxygenic conditions in the bioreactor, heterologous production of propionic acid was demonstrated.[24] Activation of the Sbm-operon also introduced a carbon flux competition between the C2-fermentative pathway (with ethanol and acetic acid as the major fermentative products) and the C3 one (with 1-propanol and propionic acid as the major fermentative products). The production of propionic acid can be significantly enhanced by manipulating various genes involved in the respiratory and fermentative branches of the pathway for glycerol dissimilation, implying significant carbon flux redirection toward the C3-fermentative pathway associated with these genetic manipulations.[24]

Production of long-odd-chain organic acids

Long-chain acids have extensive applications in the food, fragrance, cosmetics and personal care industries.[51] Being fermentative products derived from the same CoA thioesters, long-odd-chain acids and their corresponding long-odd-chain alcohols potentially share similar metabolic limitations in biosynthesis, with the availability of propionyl-CoA being a major one. Hence, microbial production of long-odd-chain alcohols and acids has been seldom explored. Coproduction of trans-2-pentenoic and valeric acids was observed upon heterologous production of 1-pentanol in *E. coli*.[40] As mentioned previously, the C5 biogenic precursor 3-ketovaleryl-CoA generated through hetero-fusion of propionyl-CoA and acetyl-CoA can be first reduced to trans-2-pentenoyl-CoA via either PhaJ1 from *C. necator* or Crt from *C. acetobutylicum*, and further reduced to valeryl-CoA via the clostridial two-enzyme system Bcd-EtfAB. These two C5 thioesters (i.e. trans-2-pentenoyl-CoA and valeryl-CoA) can be converted to trans-2-pentenoic acid and valeric acid, respectively, via the native phosphotransacetylase-acetate kinase (Pta-AckA) pathway of *E. coli* (Figure 4). Direct production of valeric acid was feasible with glycerol, but not glucose, as the sole carbon source. The valeric acid titer was further augmented by co-feeding the engineered strain with an odd-chain acid, such as propionate or trans-2-pentenoate. On the other hand, direct production of trans-2-pentenoic acid was feasible only when propionate was used as the sole carbon source.[40] Nevertheless, low titers and yields warrant major technological exploration, particularly in strain engineering, for more effective production of long-odd-chain acids. Because propionyl-CoA is a key precursor, the increased level of propionyl-CoA in engineered *E. coli* with the activated Sbm operon [22] can potentially offer a solution for direct synthesis of long-odd-chain alcohols and acids from unrelated carbon sources.

Production of bio(co)polymers and their monomers

Due to environmental concerns associated with worldwide accumulation of plastic wastes, substantial effort has been directed toward the production of degradable biopolymers using microbial biocatalytic platforms. Polyhydroxyalkanoates (PHAs) belong to one class of such biopolymers with a particular interest due to their promising thermostability, biocompatibility and versatility,[52] as evidenced by their implementation in numerous derived products, including packaging materials, cosmetic products, medical devices and pharmaceutical agents.[53,54] PHAs are produced naturally by several Gram-positive and Gram-negative bacteria, including those from the genera *Methylobacterium*, *Ralstonia*, *Azotobacter*, *Pseudomonas* and *Bacillus*,[55,56] and exist as insoluble granules in the cytoplasm of the cell as carbon and energy storage reserves. Being polyesters of (*R*)-hydroxyalkanoic acid monomers, PHAs can exist as pure homopolymers or copolymers containing a mixture of different monomers and, therefore, have diverse physicochemical properties based on the monomeric

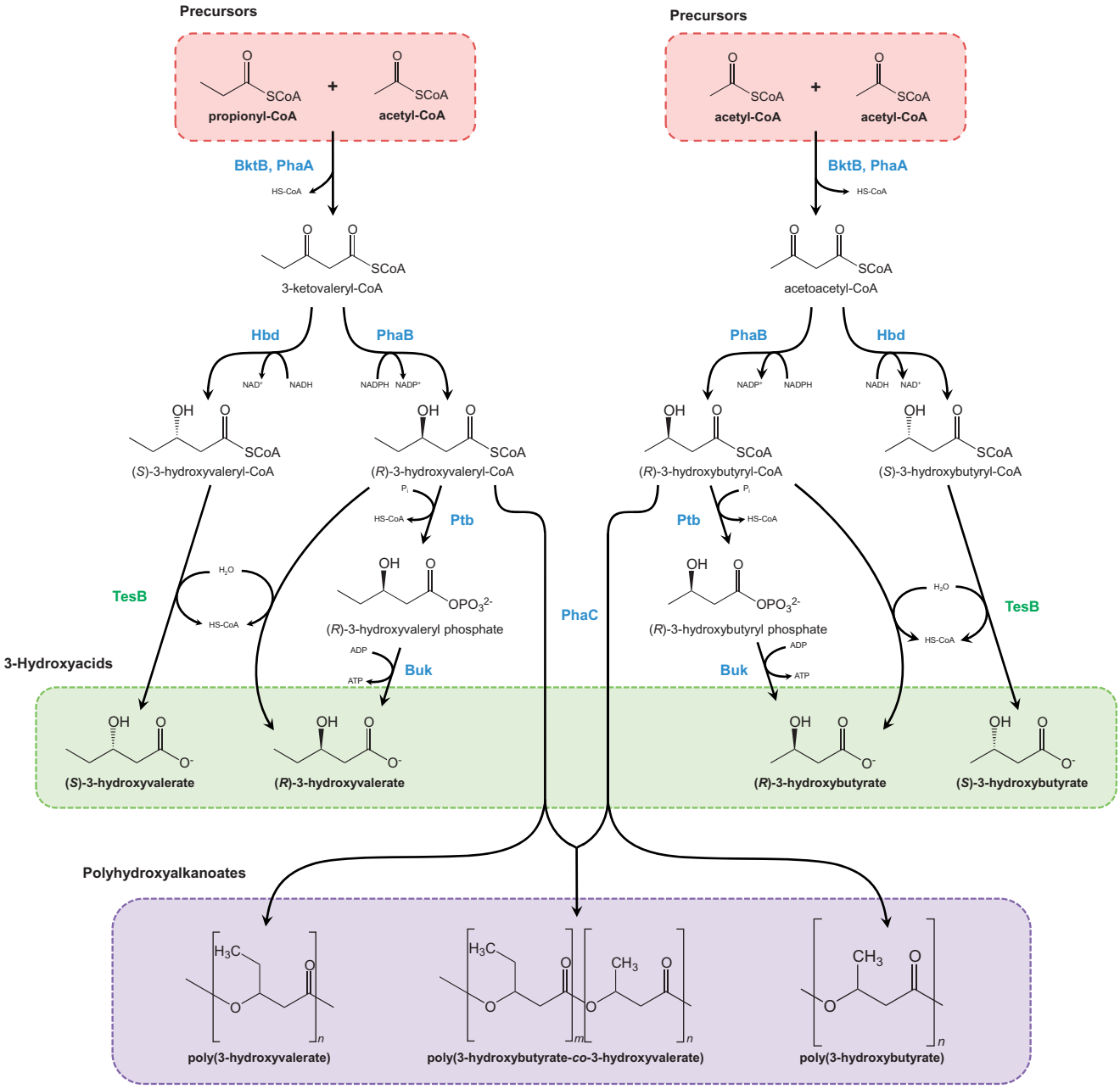

Figure 5. Metabolic pathways for polyhydroxyalkanoate and chiral 3-hydroxyacid production in engineered *E. coli*. Key enzymes in the pathways are: BktB and PhaA: β-ketothiolases from *C. necator*; Hbd: (*S*)-3-hydroxybutyryl-CoA dehydrogenase from *C. acetobutylicum*; PhaB: (*R*)-3-hydroxybutyryl-CoA dehydrogenase from *C. necator*; TesB: acyl-CoA thioesterase II from *E. coli*; Ptb: phosphotransbutyrylase from *C. acetobutylicum*; Buk: butyrate kinase from *C. acetobutylicum*; and PhaC: PHA synthase or polymerase from *C. necator*. Heterologous enzymes are represented in blue, whereas native *E. coli* enzymes are represented in green. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

constitution.[57] The most abundant form of PHAs is poly(3-hydroxybutyrate) (PHB) with its (*R*)-3-hydroxybutyryl monomer (3HB) being derived by homo-fusion of acetyl-CoA (Figure 5). Similar to petroleum-based synthetic polymers, bacterially synthesized PHB is often hard, brittle and dimensionally unstable due to its high crystallinity, thus limiting its application. On the other hand, a copolymer containing both 3HB and 3-hydroxyvalerate (3HV) monomers, known as poly(3-hydroxybutyate-*co*-3-hydroxyvalerate) (PHBV), displays more desirable mechanical properties, which can be modulated by the relative proportion of 3HB and 3HV monomers in the copolymer.[54,58] Acetyl-CoA and propionyl-CoA are two major precursors for the biosynthesis of microbial PHBV via three key enzymatic reactions (Figure 5): [1] homo-fusion of two acetyl-CoA

molecules (to form acetoacetyl-CoA) and hetero-fusion of propionyl-CoA and acetyl-CoA (to form 3-ketovaleryl-CoA), catalyzed by a set of promiscuous β-ketothiolases (e.g. BktB and PhaA from *C. necator*) with activities toward long-chain thioesters, [2] reduction of the nascent acetoacetyl-CoA and 3-ketovaleryl-CoA to form their respective (*R*)-3-hydroxyacyl-CoA monomers, catalyzed by an NAD(P)H-dependent acetoacetyl-CoA reductase (e.g. PhaB from *C. necator*) and [3] polymerization of (*R*)-3-hydroxyacyl-CoA monomers, catalyzed by a PHA polymerase or synthase (e.g. PhaC from *C. necator*).[59]

PHBV production in natural hosts

C. necator (formerly known as *Alcaligenes eutrophus*) first attracted industrial interest nearly 50 years ago based on its high capacity in producing PHB and other polyesters of biotechnological relevance.[60] Using *C. necator* strains, PHBV (under the trade name Biopol) was produced commercially by Imperial Chemical Industries and later by Monsanto. Circa 1998, Monsanto withdrew from the PHBV market primarily due to high production cost.[61,62] A major factor contributing to the high production cost was the necessity for exogenous supplementation of propionate in the cultivation media as a precursor to form propionyl-CoA in *C. necator*.[62] To alleviate this effect, strategies in strain engineering and cultivation have been developed to improve PHBV production. For instance, a mutant PHA producer, *Alcaligenes* sp. SH-69, which do not require propionate supplementation for PHBV production was isolated from sewage sludge.[63] Optimization of culture conditions resulted in PHBV accumulation of up to 57% dry cell weight (DCW) with the 3HV fraction of ~5 mol% of total PHBV.[64] On the other hand, metabolic engineering strategies can be applied toward producing PHBV from unrelated carbon sources in *C. necator*. For example, several PHBV-producing *C. necator* strains with a high 3-HV fraction were derived by manipulating the methylcitric acid cycle and methylmalonyl-CoA pathway genes, generating 68.6% DCW PHBV with 26 mol% 3-HV fraction using glucose as the sole carbon source.[35] In addition to overexpression of the *E. coli* Sbm operon genes, which mediate the conversion of succinyl-CoA to propionyl-CoA,[14,17] gene disruptions of one or both of *prpC1* and *prpC2* (i.e. 2-methylcitrate synthase variants) were employed to inactivate the methylcitric acid cycle that competes for propionyl-CoA. This study also demonstrated that genetically modulating propionyl-CoA metabolism can be potentially applied to tailor the monomer fraction of the PHBV co-polymer.[35]

PHBV production in recombinant hosts

Although natural biopolymer producers such as *C. necator* are employed for industrial production, genetic manipulation of these microorganisms is ineffective. *E. coli* can be an alternative producer for the following reasons. First, *E. coli* can inherently utilize multiple carbon sources, thus reducing high production costs associated with feedstock. Second, extraction of biopolymers from *E. coli*, which has a fragile outer membrane structure, is less tedious with high recovery yields. Third, being a non-native PHA producer, *E. coli* lacks PHA-degradation enzymes (e.g. PHA depolymerase; PhaZ from *C. necator*), which often reduce PHA accumulation in native producers.[65] In an engineered *E. coli* strain harboring the *C. necator* PHA-biosynthetic pathway, a null mutation of the *fadR* gene and a functional mutation of the *atoC* gene that results in constitutive activation of the expression of the *ato* operon greatly improved PHBV production.[66] The *fadR* gene encodes a repressor protein that represses transcription of the *fad* operon, whose gene products are involved in fatty acid biosynthesis. Likewise, A to C is a positive regulator for the expression of the *ato* operon, whose gene products are involved in the uptake and catabolism of short-to-medium chain fatty acids. Note that, in addition to fatty acids, activation of the *fad* operon in conjunction with *atoC*, ostensibly improves uptake and oxidation of exogenous propionate.[67] Similarly, genetically engineered *E. coli* harboring the PHA-biosynthetic genes from *Alcaligenes latus* was derived to produce PHBV.[68,69] While these studies demonstrate the feasibility of using engineered *E. coli* for PHBV production, exogenous supplementation of propionate is required due to the lack of pathways for propionyl-CoA biosynthesis, thus limiting their industrial application.

Genetic strategies have been applied to enhance PHBV production in recombinant hosts without propionate supplementation. For instance, metabolically engineered *Salmonella enterica* Serovar Typhimurium, episomally expressing the *Acinetobactor* sp. PHA synthesis genes, was derived to produce PHBV from single or mixed carbon sources.[26] Note that *S. enterica*, a close relative of *E. coli*, is an attractive host for PHBV production because of its well-studied propionate metabolism.[70] To avoid exogenous supplementation of propionate that potentially increases PHBV production costs, the *E. coli* Sbm operon genes were heterologously expressed for *de novo* biosynthesis of propionyl-CoA in *S. enterica*.[26] In another study,[32] an evolved *cimA* gene (encoding citramalate synthase) from *M. jannaschii* was applied together with episomal overexpression of *leuBCD* to promote 2-ketobutyrate synthesis in *E. coli*.

Competing pathways for propionyl-CoA utilization were inactivated through the deletion of two genes, i.e. *scpC* encoding a propionyl-CoA/succinate-CoA transferase and *prpC* encoding a 2-methylcitrate synthase involved in the 2-methylcitrate cycle. Additionally, the *E. coli* pyruvate oxidase (PoxB) mutant (with two amino acid substitutions) conferring a shift in binding affinity from pyruvate to 2-ketobutyrate [71] was overexpressed with the *C. necator prpE* gene encoding propionyl-CoA synthetase. While these strain engineering approaches [26,32] enabled direct biosynthesis of PHBV using glucose as the sole carbon source, the 3HV fraction was relatively low (3–10 mol%) presumably due to limited supply of propionyl-CoA.

A feedback-resistant L-threonine biosynthetic pathway was amplified to promote the production of 2-ketobutyrate as a precursor of propionyl-CoA in *E. coli*.[29] Specifically, the *thrABC* operon containing the mutated *thrA* gene (encoding an aspartate kinase mutant with relieved feedback inhibition by L-threonine) was coexpressed with the *ilvA* gene (encoding L-threonine deaminase) from *Corynebacterium glutamicum* to drive 2-ketobutyrate production. Pathways competing with subsequent conversion of 2-ketobutyrate to propionyl-CoA production were also inactivated. While overexpressing the PHA-synthesis operon from *C. necator* under this genetic background resulted in PHBV production using xylose as the sole carbon source, the PHBV content of 11% DCW was relatively low.[29] Note that xylose was used as the carbon source because it does not consume phosphoenolpyruvate (PEP), which is a key precursor to 2-ketobutyrate (Figure 3) upon its transport into the cell.[29] Furthermore, PHBV production was examined by combining the heterologous citramalate and endogenous L-threonine pathways.[72] Specifically, citramalate synthase, encoded by *cimA* from *Leptospira interrogans*, was introduced in *E. coli* for coexpression with the previously mentioned feedback-resistant *thrABC* operon to enhance 2-ketobutyrate production. After inactivating various competing pathways for consuming the essential precursors of acetyl-CoA and propionyl-CoA, the 3HV fraction in PHBV significantly increased up to 25 mol% using glucose as the sole carbon source. However, the total PHBV content was rather low at 14% DCW.[72]

Production of 3-hydroxyacids in native and heterologous hosts

3-Hydroxyacids, the monomeric units of PHAs, are valuable raw materials since the presence of two functional groups (i.e. hydroxyl and carboxylic groups) and the chiral β-carbon makes 3-hydroxyacids ideal for the synthesis of antibiotics, vitamins and perfumes.[73] Due to the highly stereospecific nature of synthesis enzymes, biological production of 3-hydroxyacids is far superior to chemical synthesis for economical production of enantiomerically pure products.[52,73] Among various studies on chiral 3-hydroxyacid production in *E. coli*, two general synthetic strategies are utilized: [1] depolymerization of PHAs to release 3-hydroxyacid monomers and [2] direct synthesis of 3-hydroxyacids. Depolymerization of PHAs via PHA depolymerases (e.g. PhaZ from *C. necator*) produces pure (*R*)-3-hydroxyacids. Intracellular PHA synthesis is kinetically driven by the formation of insoluble polymeric granules, thus enhancing 3-hydroxyacid production.[74] (*R*)-3-hydroxybutyrate (*R*-3HB) and (*R*)-3-hydroxyvalerate (*R*-3HV) were coproduced using an engineered *E. coli* with the *C. necator* PHA biosynthetic operon being integrated into the genome and the *C. necator* PHA depolymerase gene, i.e. *phaZ*, being expressed episomally.[75] However, *R*-3HV production still required supplementation of propionate as a precursor. On the other hand, direct synthesis of 3-hydroxyacids bypasses the limitation associated with PHA depolymerization, based on which only the (*R*)-enantiomer is produced. Both pure *R*-3HB and *S*-3HB were produced by utilizing reductases specific to the two enantiomers.[30] The pathway (Figure 5) first utilizes β-ketothiolases (e.g. BktB and PhaA from *C. necator*) for fusion of precursor molecules (i.e. acetyl-CoA or propionyl-CoA). The subsequent reduction is catalyzed by one of two reductases, i.e. NAD(P)H-dependent PhaB from *C. necator* for (*R*)-3-hydroxyacyl-CoA production, or NADH-dependent Hbd from *C. acetobutylicum* for (*S*)-3-hydroxyacyl-CoA production. The final CoA-removal to form free 3-hydroxyacids is catalyzed either by a two-enzyme system consisting of phosphotransbutyrylase and butyrate kinase (e.g. Ptb and Buk from *C. acetobutylicum*) or by an acyl-CoA thioesterase II (e.g. TesB from *E. coli*). The Ptb-Buk system is stereospecific for (*R*)-3-hydroxyacyl-CoA and thus limited to (*R*)-3-hydroxyacid production, whereas TesB can be used to produce both enantiomers. Furthermore, TesB produced high levels of both enantiomers of 3HB, thus being more effective than the Ptb-Buk system.[30]

The above pathways for chiral 3HB production are adaptable to chiral 3HV production if propionyl-CoA is available as a precursor.[76,77] Exogenous feeding of propionate and heterologous expression of a propionyl-CoA transferase (e.g. Pct from *Megasphaera elsdenii*) were employed to increase the intracellular propionyl-CoA level.[77] Furthermore, to increase the 3HV/3HB production ratio, the *C. necator* BktB was selected as the thiolase due to its higher enzyme specificity to C5-substrates, whereas other thiolases

(e.g. PhaA from *C. necator* and Thl from *C. acetobutylicum*) are specific to shorter carbon chains.[41] Using TesB for catalyzation, both enantiomeric *R*-3HV and *S*-3HV were produced with glucose and propionate as the carbon sources.[77] By further manipulating the carbon sources, other high-value chiral 3-hydroxyacids can be produced. For example, using the above-mentioned developed biocatalytic system with glucose and glycolate as the carbon sources, 3-hydroxy-γ-butyrolactone, whose production has been limited to chemical synthesis due to the lack of natural biosynthetic pathways, was synthesized.[77]

Similar to the approach for PHBV production, genetic manipulation of production hosts to increase the intracellular level of propionyl-CoA can be employed to produce 3HV without the need for propionate supplementation. For example, the native L-threonine biosynthesis pathway was manipulated to form 2-ketobutyrate, which was subsequently converted to propionyl-CoA by inactivating competing pathways and overexpressing key enzymes.[76] The engineered strains produced *R*-3HV and *S*-3HV under aerobic conditions using glucose or glycerol as the sole carbon source [76] (Table 1). However, the modest yields obtained in this study may result from an insufficient intracellular propionyl-CoA pool. Recent approaches based on genomic activation of the Sbm operon in *E. coli* appear to be effective in increasing the endogenous propionyl-CoA level [22,24] and such strain background should be appropriate for 3HV and PHBV production.

Production of polyketides

Propionyl-CoA is a key precursor for the production of polyketides, a group of secondary metabolites valuable for their use as antibiotic, anticancer and immunosuppressant agents.[78,79] Polyketides consist of poly-β-ketones that vary in the extent of reduction of the β-carbon. Due to the structural complexity, polyketides are primarily manufactured through biosynthesis. Nevertheless, natural producers of polyketides are found widely, including plant, fungal and bacterial species, predominantly Actinomycetes and Cyanobacteria. Because most of these organisms lack well-developed genetic tools and therefore are unsuitable for practical application, heterologous expression of the polyketide biosynthetic pathways in biotechnologically tractable organisms, such as *Streptomyces* or *E. coli*, becomes the major strategy for biological production.

Polyketide synthesis occurs through a series of condensation reactions using acyl thioester precursors, such as acetyl-CoA, malonyl-CoA and methylmalonyl-CoA, to construct a carbon backbone. Propionyl-CoA often acts as a starter unit to initiate this reaction sequence, which is mediated by a consortium of enzyme complexes known as polyketide synthases (PKSs).[80] PKSs are typically divided into Type I, Type II and Type III variants based on their enzyme architecture and functionality. Type I PKSs consist of several multifunctional domains and are further grouped into modular (mPKSs) and iterative (iPKSs) classifications. mPKS contain several distinct modules with various domains required for specific modification and chain extension of the growing polyketide (see Figure 6 for a schematic representation of a typical mPKS assembly platform). It should be noted that mPKSs are distinct in their frequent use of (*2S*)-methylmalonyl-CoA, which is a non-native metabolite in most heterologous production hosts, as a chain extender unit. On the other hand, iPKSs, similar to Type II and Type III PKSs, only have a single enzymatic module, thus repeatedly use the same active site for priming, iterative chain extension and cyclization to form polyketides.[78,81]

The modular organization of mPKSs enables specific tailoring and decorating of complex polyketide products from simple intracellular precursors. Accordingly, mPKS paradigms are paving the way as an alternative assembly route for the production of newer semi-synthetic antibiotics.[80,83–85] The biosynthesis of aglycon macrolide core of erythromycin, 6-deoxyerythronolide B (6-dEB), has long served as a model for structural and functional illustration of the mPKS enzymatic assembly lines [80,84,86] (Figure 6). 6-dEB is assembled by a macrostructure containing three large (∼350 kDa) domains, i.e. deoxyerythronolide B synthases (DEBS) 1, 2 and 3, and each domain contains a few modules with catalytic functionalities that closely resemble those of fatty acid synthases.[87] These catalytic domains are responsible for chain extension [by ketosynthase (KS), acyltransferase (AT) and acyl carrier protein (ACP)] and reduction at the β-carbon [by dehydratase (DH), enoyl-reductase (ER) and ketoreductase (KR)]. For 6-dEB biosynthesis, one starter unit of propionyl-CoA (approaching Loading Module) and six extender units of (*2S*)-methylmalonyl-CoA (approaching Module 1–6) are required. Post-PKS processing of 6-dEB functionalizes the macrolide to yield erythromycin.

Heterologous production of polyketides, such as 6-dEB,[88] erythromycin C,[89] 6-deoxyerythromycin D [90] and erythromycin A,[91] in genetically tractable host *E. coli* has long been explored though it faces a number of genetic and metabolic challenges, particularly post-translational modification of various synthetic enzymes and precursor availability.[92] In a typical study,[88] the three multi-modular DEBS domains from the soil bacterium *Saccharopolyspora*

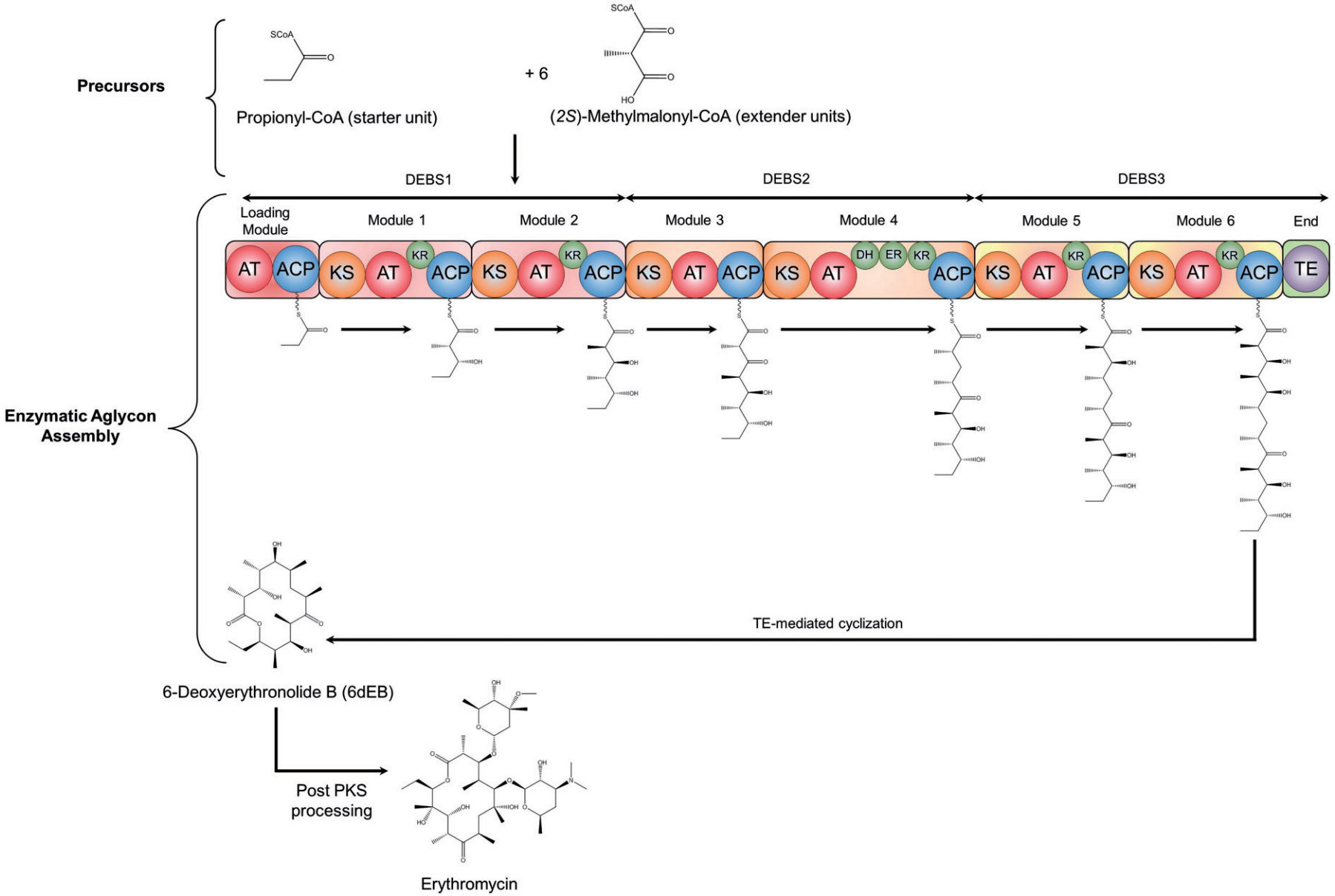

Figure 6. Assembly line for 6-deoxyerythronolide B biosynthesis via Type I mPKS. Propionyl-CoA is primed and loaded onto the loading module (i.e. AT and ACP). Iterative extension is done via modules 1–6, which consist of the core elements (i.e. KS, AT and ACP) and one or more reductase (i.e. DH, ER or KR). The carbon chain is terminated by the terminal module (TE) and cyclized to form 6-dEB. Post PKS processing is performed to generate erythromycin. The enzymes involved are: AT: acyltransferase; ACP: acyl carrier protein; KS: ketosynthase; DH: dehydratase; ER: enoyl reductase; KR: ketoreductase; TE: thioesterase. Figure was modified based on previous publication.[82]

erythraea were functionally expressed for heterologous production of 6-dEB in *E. coli*. The *E. coli* producing strain was further engineered to ensure proper post-translational processing of the PKS components and precursor supply for 6-dEB biosynthesis. First, the native *prp* operon gene (i.e. *prpRBCD*) associated with propionate catabolism was deleted to prevent undesirable conversion of propionyl-CoA precursor. Also, the remaining *prp* operon gene, i.e. *prpE* encoding propionyl-CoA synthetase, was overexpressed to promote intracellular propionyl-CoA accumulation. Next, the *sfp* gene from *Bacillus subtilis*, encoding a phosphopantetheinyl transferase potentially critical for post-translational modification of the heterologously expressed PKSs, was incorporated into the *E. coli* host genome. Lastly, given that (2*S*)-methylmalonyl-CoA is not a native metabolite to *E. coli*,[14] this C4 thioester was generated by expression of the *pcc* gene, encoding propionyl-CoA carboxylase (PCC), from *Streptomyces elicolor*.[88] PCC catalyzes the ATP-dependent carboxylation of propionyl-CoA into (2*S*)-methylmalonyl-CoA. Note that the covalent binding of biotin is required to form holo-PCC. Accordingly, a biotin ligase carrier protein (encoded by *E. coli birA*) was coexpressed to mediate the attachment of biotin to the apo-PCC to generate its holo-form.[88,93]

Because of the limited intracellular availability of propionyl-CoA and (2*S*)-methylmalonyl-CoA for polyketide production in *E. coli*, most approaches to date have relied on exogenous provision of propionate in the culture medium to generate these precursors.[89,90] Recently, the effect of the three *E. coli* Sbm operon enzymes (i.e. Sbm, YgfG and YgfH) for direct 6-dEB biosynthesis was investigated.[94] The Sbm pathway has been identified as an endogenous route to form propionyl-CoA in *E. coli*.[14,17] Nevertheless, the generated (2*R*)-methylmalonyl-CoA from the Sbm pathway cannot be incorporated into the mPKS pathway, in which (2*S*)-methylmalonyl-CoA is the building block, for 6-dEB synthesis.[95] This limitation can be potentially overcome by direct epimerization of (2*R*)-methylmalonyl-CoA to (2*S*)-methylmalonyl-CoA through heterologous expression of the propionyl-CoA-producing pathway from *Propionibacterium*, in which (2*S*)-methylmalonyl-CoA is an intermediary metabolite. On the other hand, two alternative propionyl-CoA-generating pathways have been identified to support 6-dEB production in *E. coli* in the presence of PCC.[96] For both pathways, propionyl-CoA was derived from 2-ketobutyrate through expression of either the citramalate or the L-threonine biosynthetic pathway genes (see Figure 3 for the two pathways relevant to 2-ketobutyrate formation). The resulting 6-dEB titers were comparable to those of the approaches based on exogenous propionate supplementation, successfully demonstrating direct synthesis of 6-dEB from unrelated basic carbon source. The availability of propionyl-CoA and (2*S*)-methylmalonyl-CoA precursors was also shown to be critical for biosynthesis of several other naturally-occurring polyketides, such as the immunosuppressive rapamycin [97] and the antibiotic spiramycin,[98] reaffirming the importance of the aforementioned metabolic engineering strategies associated with propionyl-CoA metabolism for polyketide production.

Future directions

Future research effort can be made toward extension of propionyl-CoA-formation pathways for biosynthesis of various value-added products of industrial relevance (Figure 7). For instance, small to medium volatile esters have an extensive use as flavor and fragrance products in the food, beverage and cosmetics industries [99] and make up a $17 billion dollar industry worldwide. Volatile esters are commonly produced through traditional chemical-based acid-catalyzed esterification of organic acids and alcohols. Previous studies have shown that the alcohol *O*-acyltransferase (ATF) class of enzymes can be used for fusion of acyl-CoA and alcohols to form small esters.[100,101] Accordingly, the presence of different acyl-CoA and alcohol formation pathways enables biosynthesis of a wide variety of short- and long-chain esters using whole-cell biocatalysts. Similar to acetyl-CoA,[102] propionyl-CoA and its biogenic C5 counterpart valeryl-CoA can be potentially used as the starter molecular for microbial production of propionate and valerate esters (Figure 7(a)).

Synthetic biology strategies have been applied for the production of natural gas and petroleum constituents, such as short-chain alk(a/e)nes. These short-chain alk(a/e)nes are attractive hydrocarbon fuels due to facile phase separation, which not only enables effective recovery of fuels from the bulk culture but also minimizes product toxicity and inhibition on producing cells.[103] Several cyanobacteria of the genera *Synechococcus* and *Synechocystis* can convert CoA-derived aldehydes into long- and short-chain (C1-C17) alk(a/e)nes via endogenous aldehyde decarbonylases.[104,105] Although the biological role of alk(a/e)ne production and the catalytic mechanism of the cryptic aldehyde decarbonylases in these cyanobacteria remain largely unknown, the production of gaseous olefins appears to result from cleavage of non-cognate substrates.[106] Such a method for alkane production was recently adopted for the biosynthesis of propane from acetyl-CoA-derived butyraldehyde in engineered *E. coli*.[107] Presumably, the same chemistry can be

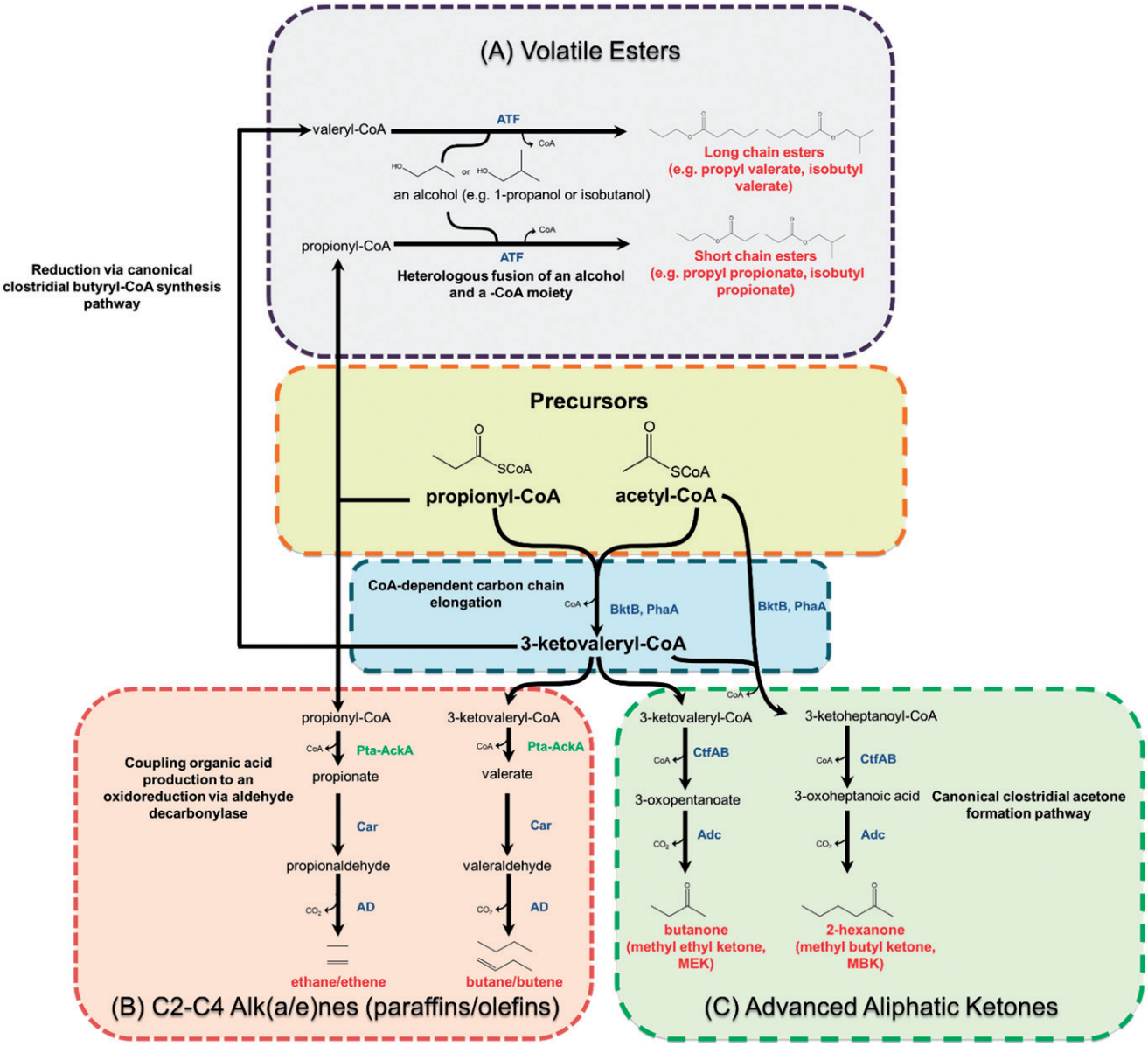

Figure 7. Putative metabolic pathways for the production of small chain volatile esters, C2–C4 alk(a/e)nes, and advanced ketones using propionyl-CoA as a key precursor. (a) Propionyl-CoA or its five-carbon biogenic counterpart, valery-CoA, can be fused with a linear or branched alcohol (e.g. 1-propanol or isobutanol) via ATF, an alcohol-O-acetyl transferase from *Saccharomyces cerevisiae* for the production of various volatile esters. (b) Similarly, using the acid-formation pathway (i.e. Pta-AckA, phosphotransacetylase-acetate kinase) of *E. coli*, propionyl-CoA or 3-ketovalery-CoA can also be converted into non-activated organic acids propionate or valerate, respectively. Next, through the action of a carboxylic acid reductase (CAR) from *Mycobacterium marinum* and a cryptic aldehyde reductase (AD) from *Synechocystis* sp, propionate and valerate can be reduced into their respective aldehydes and alk(a/n)es. (c) Lastly, propionyl-CoA derived C5 and C7 biogenic precursors 3-ketovaleryl-CoA and 3-ketoheptanoyl-CoA, respectively, can also be channeled into the canonical clostridial acetone-formation pathway (i.e. CtfAB-Adc, acetoacetyl-CoA: acetate/butyrate: CoA transferase and acetoacetate decarboxylase from *C. acetobutylicum*) for thioester hydrolysis and decarboxylation to form the advanced ketones. Heterologous enzymes are represented in blue, whereas native *E. coli* enzymes are represented in green. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

extended to larger CoA thioesters, such as propionyl-CoA and valeryl-CoA for the production of eth(a/e)ne and but(a/e)ne, respectively (Figure 7(b)).

Moreover, the production of advanced aliphatic commodity ketones in engineered microbial systems has gained significant attention recently.[108–110] Larger-chain ketones, such as butanone, are widely used as general solvents in the printing and textile industries and components of synthetic rubber.[111] In the canonical clostridial-acetone pathway, the C4 thioester

acetoacetyl-CoA (formed via the homo-fusion of two acetyl-CoA moieties) is converted to acetone via acetoacetyl-CoA:acetate/butyrate:CoA transferase (CtfAB) and acetoacetate decarboxylase (Adc).[112] *In vitro* studies suggest that CtfAB has a wide range of substrate specificities to various short- and long-chain CoA derivatives and organic acids.[113] Accordingly, this pathway was successfully applied for the production of butanone.[25] However, production of other advanced ketones (e.g. 2-hexanone) using long-chain acyl-CoA derived from propionyl-CoA (e.g. 3-ketoheptanoyl-CoA) is yet to be explored (Figure 7(c)).

Note, the implementation of auxiliary CoA-dependent chain elongation pathways for hetero-fusion of propionyl-CoA (or longer CoA moieties) and acetyl-CoA is critical in mediating the production of these long-chain products. Despite limited success in implementing these recursive elongation pathways for long-chain alcohol and organic acid production,[40,30,76] these applications have yet to be fully realized due to a major challenge of striking a flux balance between the C2 and C3 fermentative pathways with acetyl-CoA and propionyl-CoA, respectively, as the major metabolic hubs. Other potential challenges include utilization of unrelated carbon sources, ensuring cofactor balance and availability, effective supply of metabolic energy for biosynthetic pathways and eliminating potential intracellular flux bottlenecks. Some of these limitations can be overcome via combinatorial pathway optimization [114] and dynamic pathway optimization.[115]

Conclusions

Mounting concerns associated with the use of petrochemical manufacturing practices have rekindled the enthusiasm in microbial transformational platforms. While microorganisms are often limited in natural repertoire of metabolic pathways, advances in metabolic engineering can greatly expand nature's "green" strategies for producing a myriad of structurally diverse and industrially important non-native chemicals. As documented in this review, significant progress has been made to modulate propionyl-CoA metabolism in microbial platforms for novel biomanufacturing of value-added products, including odd-chained alcohols and organic acids, PHAs and their free acid monomers, medium to long-chain oleochemicals, advanced aliphatic commodity ketones and polyketides.

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Disclosure statement

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