Towards renewable flavors, fragrances, and beyond

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Highlights

- Microbial cell factories offer sustainable and renewable production of natural esters.
- Modular cell design enables rapid construction of optimal ester-producing strains with minimal strain optimization cycles.
- Repurposed chloramphenicol acetyltransferase enables thermophilic microbial production of esters.
- Thermophilic consolidated bioprocessing microbial platform offers economical production of renewable esters.

Abstract

Esters constitute a large space of unique molecules with broad range of applications as flavors, fragrances, pharmaceuticals, cosmetics, green solvents, and advanced biofuels. Global demand of natural esters in food, household cleaner, personal care, and perfume industries is increasing while the ester supply from natural sources has been limited. Development of novel microbial cell factories for ester production from renewable feedstocks can potentially provide an alternative and sustainable source of natural esters and hence help fulfill growing demand. Here, we highlight recent advances in microbial production of esters and provide perspectives for improving its economic feasibility. As the field matures, microbial ester production platforms will enable renewable and sustainable production of flavors and fragrances, and open new market opportunities beyond what nature can offer.

Introduction

Esters are ubiquitous in nature and often responsible for the characteristic fragrances of fruits and flowers [1]. Esters comprise of carboxylic acid and alcohol moieties, that can be linear, branched, saturated, unsaturated, aromatic or any features with carbon chain lengths up to C18 and higher [2••]. With a diversity of chemical moieties, esters can make up a large space of unique molecules that have broad applications as flavors, fragrances, cosmetics, pharmaceuticals, green solvents, and advanced biofuels [2-5••].

According to the BCC research's recent report, the global market for flavors and fragrances was valued at US$26 billion in 2015 and is expected to reach US$37 billion by 2021 [6]. Cosmetics takes an even larger share of global market valued at over US$500 billion in 2017, and its market is expected to achieve over US$805 billion by 2023 [7]. The increasing preference for using natural and sustainable products pushes large fragrance and ingredient firms to find alternative sources for production of natural ingredient [8]. Since microbes have long been exploited for production of foods and beverages [9] and biochemicals [10], harnessing microbial cell factories for production of natural esters from renewable feedstocks is primed to be a promising sustainable alternative.

Here, we highlight i) recent advances in microbial production of esters, ii) challenges and opportunities in microbial production of esters, iii) modular cell design for efficient combinatorial biosynthesis of esters, and iv) thermophilic consolidated bioprocessing (CBP) microbial platform for sustainable production of esters. In addition, we provide perspectives for improving economic feasibility of microbial production of esters from lignocellulosic biomass.

Recent advances in microbial production of esters

In nature, microbes and in particular plants possess diverse metabolic pathways to provide numerous precursor metabolites for ester biosynthesis (Figure 1). For instance, to make linear (carbon) chain esters, linear short-to-long fatty acyl-CoAs and alcohols can be derived from the fatty acid biosynthesis and reversed β-oxidation pathways [11]. For branched chain esters, enzymatic conversion of α-keto acids by branched-chain keto acid dehydrogenase complex (KDHC) and α-keto acid pathways can be exploited to generate branched acyl-CoAs [12] and alcohols [13], respectively. For aromatic esters, aryl-CoAs and alcohols can be synthesized from the shikimate and phenylpropanoid pathways [14]. For terpene esters, methylerythritol phosphate (MEP) and mevalonate (MVA) pathways can supply various chain length of terpenols [15-18]. Like the fatty acid biosynthesis cycles, the polyketide biosynthesis pathways can also be employed to generate a large and diverse library of linear, branched, and cyclic acyl-CoAs and alcohols [19]. By harnessing these naturally existing biosynthesis pathways, novel microbial cell factories have been developed to produce various natural esters (Table 1). The physical and organoleptic properties of these esters are also summarized in Table 2. To date, microbial biosynthesis of the linear, branched, and short-to-medium chain esters is better studied than the underexplored novel biosynthesis of the aromatic and terpene esters. Below are a few representative studies that present the recent advances in microbial production of esters.

Acetate esters, derived from acetyl-CoA and alcohols, constitute a large space of molecules with unique odor and taste (Table 2). Since acetyl-CoA is an important precursor metabolite for cell synthesis, acetate esters are often found in nature. Acetate esters such as ethyl acetate (sweet, pear drop-like), isobutyl acetate (floral smell often found in raspberries), isoamyl acetate (fruity smell

commonly found in banana or pear) and 2-phenylethyl acetate (rose and honey scent found in fruits) are often used as food additives or fragrances to generate the flavors or odors of interest [20]. Among the microbial cell factories that have been engineered to produce these esters, isobutyl acetate production has been reported with the highest titer of 36 g/L in an engineered *E. coli* harboring an isobutanol biosynthesis pathway and an alcohol acyltransferase (ATF1) [21••]. Since isobutyl acetate is toxic to the cell [22•], high production level was achieved by implementing *in situ* fermentation and ester stripping.

Lactate esters, derived from lactyl-CoA and alcohols, also represent a large library of molecules with unique odor and taste (Table 2). Unlike acetate esters, lactate esters are less commonly found in nature due to scarcity of lactyl-CoA. Lactate esters are generally considered as green solvents due to their favorable taxological and environmental profiles [23]. Industrially, lactate esters such as ethyl lactate exhibit the same or better properties and performances as compared to traditional solvents in many applications [23]. Recently, Lee and Trinh has demonstrated the direct fermentative production of lactate esters from glucose in *E. coli* [4•]. To achieve the *de novo* biosynthesis of ethyl- and isobutyl lactate directly from glucose, both the pyruvate-to-lactate esters and alcohols pathways were designed, constructed, and co-expressed in an engineered modular *E. coli* chassis.

Retinyl acetate (Vitamin A acetate) is an acetate ester of retinol with potential antineoplastic and chemopreventive functions [24]. This ester is widely used in cosmetic products because the ester form of retinol (Vitamin A) is more stable than retinol [25]. Recently, Jang *et al.* has demonstrated microbial production of retinyl acetate in *E. coli* expressing the β-carotene biosynthesis pathway

together with a β-carotene-15,15'-monooxygenase (BCMO) [18]. Interestingly, the authors also observed that an endogenous dehydrogenase (*ybbO*) and a chloramphenicol acetyltransferase (*cat*) on a plasmid partially contributed to the biosynthesis of retinol and retinyl acetate, respectively.

Caffeic acid phenethyl ester (CAPE) is one of the most therapeutically bioactive polyphenol components derived from honeybee propolis [26]. Due to its diverse bioactive activities, CAPE has recently been recognized as a potential drug candidate to prevent Alzheimer's and Parkinson's diseases [27]. By introducing the biosynthesis pathway of 2-phenylethanol and a BAHD transferase (*PMT*), Song *et al* demonstrated CAPE biosynthesis in *E. coli* [3].

Terpene esters such as geranyl acetate are gaining interest as energy-dense advanced biofuels [28,29]. Recently, microbial production of geranyl acetate have been demonstrated in two model organisms: *S. cerevisiae* [15] and *E. coli* [5]. In *S. cerevisiae*, introduction of the biosynthesis pathway of geraniol, and an alcohol acyltransferase (*SAAT*) into the yeast genome enabled production of 22.5 mg/L geranyl acetate. In *E. coli*, co-expression of the biosynthesis pathway of geraniol and alcohol acyltransferase (*RhAAT*) using a plasmid achieved efficient and selective production of geranyl acetate at a final titer of 4.8 g/L [5].

Opportunities and challenges in microbial production of esters

Current production of natural products relies on chemical extraction of natural sources such as plants. This traditional technology faces several challenges. The low content and purity of the ingredients of interest derived from the natural sources limit large-scale production and quality control, and hence cause increasing concerns for their environmental impact [30]. Furthermore,

chemical extraction method has an inherent disadvantage in production of volatile compounds due to products loss resulting in a low recovery rate [31]. Harnessing microbial cell factories for natural ester production can potentially overcome many challenges present in the traditional approach. First, ester microbial biosynthesis enables industrial-scale production of pure compounds [32]. Second, the microbial conversion route can save time and cost due to higher production, faster growth of microbes than plants, and ease of ester recovery from fermentation [5,21]. Third, ester microbial production can utilize abundant, renewable and/or sustainable feedstocks from biological wastes such as carboxylic acids [33•,34] to lignocellulosic biomass [35]. Lastly, the well-established pathways for generating acyl-CoAs and alcohols [36] as the precursors for ester biosynthesis can be leveraged to quickly develop microbial cell factories for ester production.

In contrast to the traditional approach, the high volatility of esters become advantageous in the microbial conversion route [37]. Esters can be readily secreted outside of cells and easily removed from the fermentation broth by gas stripping or dual-phase separation [38]. *In situ* extraction and fermentation help overcome the product toxicity and hence improve final product titer, rate, and yield [38]. For instance, while cell growth was inhibited by 3 g/L of isobutyl acetate [2], gas stripping and dual-phase separation approaches enabled the isobutyl acetate microbial production to reach final titers of ~36 g/L (42% of theoretical maximum yield) [21] and ~17.2 g/L (80% of theoretical maximum yield) [2], respectively. Likewise, ineffective production of geraniol caused by its anti-microbial activity [39] has recently been overcome using the ‘detoxification via esterification’ strategy [5] where instead of geraniol, its esterified derivative geranyl acetate was produced with better properties [28] and lower toxicity [40]. The strategy comprises of two processes including conversion of toxic geraniol into less toxic geranyl acetate by AAT and

simultaneous removal of geranyl acetate from the medium with dual-phase separation. This strategy enables to achieve high production of geraniol acetate (~4.8 g/L) with high purity without any additional strain engineering such as expression of efflux pumps, changes in membrane properties, and activation of stress response genes. Taken together, microbial production of esters coupled with *in situ* product removal (ISPR) approach such as gas stripping or dual-phase separation offers an efficient ester production platform.

Despite of these benefits, the development of microbial cell factories for efficient ester biosynthesis is currently limited by the availability of enzymes responsible for the condensation of precursors into esters. To address this issue, efforts have been made by bioprocessing and engineering various ester-producing enzymes [38,41,42]. The most-studied family of enzymes for ester synthesis is BAHD acyltransferase [42••]. In plants, the members of this family play an important role in the formation of a wide range of secondary metabolites [43]. The other well-characterized member of this family is alcohol acyltransferase (AAT, EC 2.3.1.84), for instance, ATF1 of *S. cerevisiae* [20]. AATs function by catalyzing the transfer of acyl chains from an acyl-CoA donor to an acceptor alcohol (Figure 2A). Thus, the final ester products can be diverse, depending on the substrate specificity of AAT towards acyl-CoAs and alcohols [44]. Although ATF1 has been widely used in microbial production of various acetate esters [2,33,34], it has some drawbacks. For example, ATF1 cannot catalyze other acyl-CoAs more efficiently than acetyl-CoA [20] and showed very poor solubility in prokaryotes such as *E. coli* [45]. Its high K_M values for alcohol substrates (i.e., ~20.2 mM for isobutanol and ~26.0 mM for isoamyl alcohol) can lead to inefficient ester production, likely due to alcohol toxicity [21]. Moreover, no available 3D crystal structure of AATs makes it difficult for rational protein design to enhance AAT activities.

Most recently, chloramphenicol acetyltransferase (CAT, EC 2.3.1.28) has emerged as a promising ester-producing enzyme. For decades, CAT has been used as a selectable marker in various organisms due to its ability to detoxify the antibiotic chloramphenicol via acetylation [46] (Figure 2B). However, since the first discovery of the unexpected activity of CAT toward terpenols [47•], it can now be repurposed as a potential novel esters-producing enzyme [2,48,49]. Unlike ATF1, the 3D crystal structures of CATs are available (PDB:3U9B|3CLA|2XAT). CATs exist as a ternary complex with three binding pockets (Figure 2C), each of which contains two catalytic residues, histidine and asparagine, that form a highly conserved H-X-X-X-D motif in AATs [48••] (Figure 2D). From literature, harnessing CATs for microbial production of esters exhibit many advantages such as i) high solubility in prokaryotes [48], ii) broad substrate range [48], iii) high thermostability [48], iv) high evolvability [50,51•], and v) great potential for improved aromatic and terpene esters production [48]. However, CATs need to be reprogramed to exploit its versatility in microbial production of designer esters due to its high preference toward chloramphenicol than other alcohols [48] (Figure 2E).

Modular cell design for efficient combinatorial biosynthesis of esters

As the chemical properties of esters are determined by the types and compositions of fatty acid or alcohol moieties, a large space of esters can be synthesized. The conventional strain engineering approach is not effective to explore this space because it involves optimization of only one production strain to produce a single molecule at a time and the strain optimization cycles need to be repeated to make a new molecule. To address this limitation, the concept of modular cell deign, inspired by modern engineering disciplines and natural systems, has recently been proposed to

enable rapid generation of production strains to effectively produce a large space of desirable molecules (e.g., alcohols and esters) with minimal strain optimization cycles [52••]. Each optimal production strain is obtained to effectively produce a desirable molecule by assembling a modular (chassis) cell with an exchangeable production module(s) in a plug-and-play fashion [53,54]. A modular cell is designed to contain core metabolic pathways that are necessary but insufficient for cell growth and production of a desirable molecule. To function, it must couple with a production module that is an auxiliary metabolic pathway designed to make a desirable molecule. The coupling or interface between a modular cell and production modules is metabolically and genetically constrained. Based on the modular cell design principles, the best production strains or modules can be screened or selected based on the growth coupled to product formation phenotypes (Figure 3) [55•].

The concept of modular cell design for combinatorial biosynthesis of esters has been demonstrated for the production of butyrate esters [56••]. In the study, Layton and Trinh first laid out a general design of fermentative ester biosynthesis pathways as production modules for *de novo* biosynthesis of esters from fermentable sugars. Each module comprises of i) acyl-CoA synthesis submodule, ii) alcohol synthesis submodule, and iii) ester condensation submodule. By introducing a combination of the butyl-CoA plus AAT submodules and various alcohol production submodules in a modular *E. coli* cell, the *de novo* production platform of butyrate esters was successfully established. As compared to the wildtype, the engineered modular production strains achieved 27, 24, 48-fold improved production of ethyl butyrate, isopropyl butyrate, and isobutyl butyrate, respectively and exhibited the growth-coupled ester production phenotypes.

Thermophilic consolidated bioprocessing (CBP) microbial platform for sustainable production of esters

To improve economic feasibility of microbial biotransformation, a CBP configuration has been proposed to produce biochemicals directly from lignocellulosic biomass [57••]. In CBP, three biological processes, including production of saccharification enzymes, hydrolysis of pretreated biomass, and sugar fermentation, are combined in one reactor for conversion of biomass feedstocks to a desirable product and hence lower the process cost. *Clostridium thermocellum* is considered as an ideal biocatalyst for a thermophilic CBP microbial platform due to its optimal high temperature (55-65^{o}C) growth under anaerobic conditions and robust metabolic capability to solubilize cellulose effectively and make fermentable chemicals [58,59]. Thermophilic CBP microbial platforms have a great potential to achieve renewable, sustainable, and economic production of biochemicals from lignocellulosic biomass [60••].

Despite of the recent advances in engineering these platforms to produce alcohols [61-68], no cases have been reported for biosynthesis of esters until very recently when direct production of isobutyl acetate from cellulose by engineered *C. thermocellum* at the elevated temperatures (55^{o}C) was demonstrated [48]. One significant challenge in developing a thermophilic CBP microbial platform is to identify an efficient thermostable AATs, that has not yet been found in nature. To tackle this challenge, Seo *et al.* identified and repurposed a thermostable CAT_{Sa} F97W from *Staphylococcus aureus* to enhance its specificity towards isobutanol instead of the native substrate chloramphenicol. The engineered strain achieved ~3.5-fold improved isobutyl acetate production from cellulose. Although the titer of produced isobutyl acetate was low, this study demonstrated

the feasibility of engineering the thermophilic CBP microbial platform for ester biosynthesis as well as the potential of harnessing CATs to produce designer esters from renewable resources.

Conclusion and perspectives

Due to the increasing customer interest in all-natural products, microbial ester production is a promising alternative to the traditional ester production approach. In recent years, remarkable advances have been made in microbial production of esters such as i) success in scale-up ester production, ii) overcoming the product toxicity, iii) production of novel value-added ester molecules, iv) discovery of novel ester-producing enzymes, and v) development of a thermophilic, CBP microbial ester production platform utilizing lignocellulosic biomass. Further pushing the boundary of these advances would help to meet the increasing market demand of both natural and novel synthetic esters in a renewable, sustainable, and economic way (Figure 4) while contributing to the growth of biomass-based chemical economy.

Conflict of interest statement

Nothing declared.

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301 Table 1. Microbial production of esters by BAHD acyltransferases.

Products	Uses	Host strains	AATs	Titer (mg/L)	Alcohol supply	Notes	Ref.
Short-to-medium chain esters							
Ethyl acetate	Fuels, Solvents, Flavors, Fragrances	*E. coli*	ATF1	~116	2 g/L Ethanol	High-cell density culture	[4]
Ethyl acetate		*S. cerevisiae*	ATF1	~610	Endogenous	Co-expression with CAB2 and ACS2	[69]
Ethyl acetate		*S. cerevisiae*	Eat1	~130	Endogenous	Batch fermentation	[70]
Ethyl acetate		*E. coli*	Eat1	~4870	15 g/L Ethanol	Batch fermentation	[70]
Propyl acetate		*E. coli*	ATF1	~802	2 g/L Propanol	High-cell density culture	[4]
Butyl acetate		*E. coli*	CAT	~10	2 g/L Butanol	High-cell density culture	[48]
Butyl acetate		*E. coli*	ATF1	~1018	2 g/L Butanol	High-cell density culture	[4]
Isobutyl acetate		*C. thermocellum*	CAT	~2	Endogenous	First demonstration of thermophilic ester CBP platform with an engineered CAT	[48]
Isobutyl acetate		*E. coli*	CAT	~17	2 g/L Isobutanol	High-cell density culture	[48]
Isobutyl acetate		*E. coli*	ATF1	~1210	2 g/L Isobutanol	High-cell density culture	[4]
Isobutyl acetate		*E. coli*	ATF1	~17200	Endogenous	Batch culture with solvent overlay	[2]
Isobutyl acetate		*E. coli*	ATF1	~19700	Endogenous	Batch culture with solvent overlay, acetate was fed to improve carbon yield	[71]
Isobutyl acetate		*E. coli*	ATF1	~36000	Endogenous	Fed-batch fermentation, air stripping was used for *in situ* product removal	[21]
Isobutyl acetate		*S. cerevisiae*	ATF1	~260	Endogenous	Investigated the profile changes in distribution of branched-chain esters based on the expression location of ATF1	[72]
2-methyl-1-butyl acetate		*S. cerevisiae*	ATF1	~290	Endogenous		
Amyl acetate		*E. coli*	ATF1	~28	Endogenous	2 g/L pentanoate was added with co-expression of pct	[34]
Isoamyl acetate		*S. cerevisiae*	ATF1	~296	Endogenous	Investigated the profile changes in distribution of branched-chain esters based on the expression location of ATF1	[72]
Isoamyl acetate		*E. coli*	CAT	~300	3 g/L Isoamyl alcohol	3 g/L 2-ketovalerate was added	[2]
Isoamyl acetate		*E. coli*	ATF1	~693	2 g/L Isoamyl alcohol	High-cell density culture	[4]
Isoamyl acetate		*E. coli*	ATF1	~780	Endogenous	Batch culture	[21]
Hexyl acetate		*E. coli*	ATF1	~8.3	Endogenous	2 g/L hexanoate was added with co-expression of pct	[34]
Ethyl propionate		*E. coli*	VAAT	~67	Endogenous	2 g/L propionate was added with co-expression of pct	[37]
Propyl propionate		*E. coli*	SAAT	~4.7	Endogenous		[34]
Isobutyl propionate		*E. coli*	VAAT	~2.7	Endogenous		[33]
Ethyl lactate		*E. coli*	VAAT	~11	Endogenous	High-cell density culture	[4]
Propyl lactate		*E. coli*	VAAT	~5	2 g/L propanol	High-cell density culture	[4]
Butyl lactate		*E. coli*	VAAT	~12	2 g/L Butanol	High-cell density culture	[4]
Isobutyl lactate		*E. coli*	VAAT	~10	2 g/L Isobutanol	High-cell density culture	[4]
Isoamyl lactate		*E. coli*	VAAT	~25	2 g/L Isoamyl alcohol	High-cell density culture	[4]
Ethyl butyrate		*E. coli*	SAAT	~134	Endogenous	Batch culture with solvent overlay	[56]
Butyl butyrate		*E. coli*	SAAT	~37	Endogenous	Batch culture with solvent overlay	[56]

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303 Table 1. (Continued)

Products	Uses	Host strains	AATs	Titer (mg/L)	Alcohol supply	Notes	Ref.
Short-to-medium chain esters							
Butyl butyrate	Fuels, Solvents, Flavors, Fragrances	*C. acetobutylicum*	SAAT	~50	Endogenous	Batch culture	[73]
Butyl butyrate		*E. coli*	SAAT	~48	Endogenous	2 g/L butyrate was added with co-expression of pct	[34]
Butyl octanoate		*E. coli*	AAT16-S99G	~3.3	10 mM butanol	Engineered AAT for improved octanoyl-CoA substrate specificity	[41]
Isobutyl butyrate		*E. coli*	SAAT	~41	Endogenous	2 g/L butyrate was added with co-expression of pct	[33]
Isobutyl butyrate		*E. coli*	SAAT	~12.6	Endogenous	Batch culture with solvent overlay	[56]
Isobutyl isobutyrate		*E. coli*	CAT	~27	Endogenous	Batch culture	[2]
Isoamyl isobutyrate		*E. coli*	CAT	~5	3 g/L Isoamyl alcohol	3 g/L 2-ketovalerate was added	[2]
Ethyl valerate		*E. coli*	SAAT	~103	Endogenous	2 g/L pentanoate was added with co-expression of pct	[34]
Isobutyl valerate		*E. coli*	SAAT	~65	Endogenous		[33]
Amyl valerate		*E. coli*	SAAT	~40.3	Endogenous		[34]
Ethyl hexanoate		*E. coli*	SAAT	~5.2	Endogenous	2 g/L hexanoate was added with co-expression of pct	[34]
Isobutyl hexanoate		*E. coli*	SAAT	~3.2	Endogenous		[33]
Aromatic esters							
Benzyl acetate	Fuels, Solvents, Flavors, Fragrances	*E. coli*	CAT	~152	2 g/L Benzyl alcohol	High-cell density culture	[48]
Benzyl acetate		*E. coli*	ATF1	~1178	2 g/L Benzyl alcohol	High-cell density culture	[4]
Benzyl lactate		*E. coli*	VAAT	~52	2 g/L Benzyl alcohol	High-cell density culture	[4]
2-Phenylethyl acetate		*E. coli*	CAT	~300	3 g/L 2-Phenylehanol	3 g/L 2-ketovalerate was added	[2]
2-Phenylethyl acetate		*E. coli*	CAT	~955	2 g/L 2-Phenylehanol	High-cell density culture	[48]
2-Phenylethyl acetate		*E. coli*	ATF1	~687	Endogenous	Batch culture	[14]
2-Phenylethyl isobutyrate		*E. coli*	CAT	~0.5	3 g/L 2-Phenylehanol	3 g/L 2-ketovalerate was added	[2]
Methyl anthranilate	Flavors, Fragrances	*E. coli*	AAMT1	4470		Fed-batch fermentation with solvent overlay	[74●]
Methyl anthranilate		*C. glutamicum*	AAMT1	5740		Fed-batch fermentation with solvent overlay	[74]
Ethyl benzoate	Fuels, Solvents, Flavors, Fragrances	*S. cerevisiae*	BPBT	~0.2	Ethanol	Benzoic acid was added	[75]
Butyl benzoate		*S. cerevisiae*	BPBT	~0.5	Butanol	Benzoic acid was added	[75]
Isoamyl benzoate		*S. cerevisiae*	BPBT	~0.5	Isoamyl alcohol	Benzoic acid was added	[75]
2-Phenylethyl benzoate		*S. cerevisiae*	BPBT	~1.8	2-Phenylethanol	Benzoic acid was added	[75]
Caffeic acid phenethyl ester (CAPE)	Drug candidates	*S. cerevisiae*	BPBT	~0.0005	2-Phenylethanol	Caffeate was added	[75]
		E. coli	PMT	23.8	Endogenous	Batch culture	[3]
Terpene esters							
Geranyl acetate	Jet fuels, Favors, Fragrances, Pharmaceuticals, Biopesticides, Precursor chemicals	*S. cerevisiae*	SAAT	~22.5	Endogenous	Batch culture	[15]
Geranyl acetate		*E. coli*	RhAAT	~4800	Endogenous	Fed-batch fermentation with solvent overlay	[5]
Geranylgeranyl acetate		*E. coli*	ATF1	~119	Endogenous	Batch culture	[17]
Perillyl acetate		*E. coli*	CAT	~30	Endogenous	Discovered CAT activity toward terpenols	[47]
Farnesyl acetate		*E. coli*	ATF1	~201	Endogenous	Batch culture	[16]
Retinyl acetate	Drugs, Cosmetics, Food additives.	*E. coli*	CAT	~38	Endogenous	Batch culture with solvent overlay	[18]

Table 2. The physical and organoleptic properties of various esters. Information was obtained from http://www.thegoodscentscompany.com. Abbreviations: M.W., molecular weight; *n.a.*, not available.

Esters	Formula	M.W.	logP (o/w)	Odor Type	Odor Description	Flavor Type	Taste Description
Short-to-medium chain esters							
Ethyl acetate	$C_4H_8O_2$	88.11	0.730	Ethereal	Etherial, fruity, sweet, grape, and rum-like	Ethereal	Etherial, fruity, sweet, with a grape, and cherry nuance
Propyl acetate	$C_5H_{10}O_2$	102.13	1.240	Fruity	Solvent-like pungency, lifting, fusel, amyl alcohol, sweet, and fruity	Estery	Estry, fruity, etherial, tutti-frutti, banana, and honey
Butyl acetate	$C_6H_{12}O_2$	116.16	1.780	Ethereal	Sharp, etherial, diffusive, fruity, banana	Ethereal	Sweet, ripe banana, tutti frutti, tropical, and candy-like with green nuances
Isobutyl acetate	$C_6H_{12}O_2$	116.16	1.780	Fruity	Sweet, fruity, etherial with an apple banana nuance	Fruity	Sweet fruity with a banana tutti frutti note
Amyl acetate	$C_7H_{14}O_2$	130.19	2.300	Fruity	Ethereal, fruity, banana, pear, apple	Fruity	Sweet, fruity, pear overripe banana
Isoamyl acetate	$C_7H_{14}O_2$	130.19	2.260	Fruity	Sweet, banana, fruity with a ripe estry nuance	Fruity	Sweet fruity, banana-like with a green ripe nuance
Hexyl acetate	$C_8H_{16}O_2$	144.21	2.870	Fruity	Green, fruity, sweet, fatty, fresh, apple and pear	Fruity	Fruity, green, fresh, sweet, banana peel, apple, and pear
Ethyl propionate	$C_5H_{10}O_2$	102.13	1.210	Fruity	Sweet, fruity, rummy, juicy, fruity, grape, pineapple	Fruity	Etherial, fruity, sweet, winey, bubble gum, apple, and grape nuances
Propyl propionate	$C_6H_{12}O_2$	116.16	1.804	Chemical	Sharp, chemical, pungent with sweet fruity lift notes	Tropical	Sweet, lift, tropical green fruity notes
Isobutyl propionate	$C_7H_{14}O_2$	130.19	2.158	Fruity	Fruity, sweet, rummy, pungent, bubblegum estry with a tropical nuance	Fruity	Sweet, fruity, banana, tutti frutti, with rummy nuances
Ethyl lactate	$C_5H_{10}O_3$	118.13	-0.039	Fruity	Sweet, fruity, acidic, etherial with a brown nuance	Fruity	Sweet, fruity, creamy, pineapple-like with a caramellic brown nuance
Propyl lactate	$C_6H_{12}O_3$	132.16	0.470	Winey	Winey, yogurt, milky	*n.a.*	*n.a.*
Butyl lactate	$C_7H_{14}O_3$	146.19	0.980	Creamy	Creamy, dairy, milky, earthy, ketonic, waxy, lactonic, vanilla, and cheesy	Dairy	Dairy, creamy, milky, coconut, and nutty
Isobutyl lactate	$C_7H_{14}O_3$	146.19	0.824	Buttery	Faint, buttery, fruity, caramellic	Buttery	Buttery, caramellic, fruity
Isoamyl lactate	$C_8H_{16}O_3$	160.21	1.333	Fruity	Fruity, creamy, nutty	*n.a.*	*n.a.*
Ethyl butyrate	$C_6H_{12}O_2$	116.16	1.804	Fruity	Sweet, fruity, tutti frutti, lifting, and diffusive	Fruity	Fruity, sweet, tutti frutti, apple, fresh, and lifting, ethereal
Butyl butyrate	$C_8H_{16}O_2$	144.21	2.823	Fruity	Sweet, fruity, fresh, diffusive and ripe	Fruity	Sweet, fresh, fruity, slightly fatty
Butyl octanoate	$C_{12}H_{24}O_2$	200.32	4.861	Buttery	Butter, ether, herbal, dank	*n.a.*	*n.a.*
Isobutyl butyrate	$C_8H_{16}O_2$	144.21	2.760	Fruity	Sweet, fruity, candy, berry, cherry, tutti frutti, over ripe, and bubble gum-like	Fruity	Sweet, fruity, pineapple, apple, bubble gum, and tutti frutti
Isobutyl isobutyrate	$C_8H_{16}O_2$	144.21	2.511	Fruity	Ethereal, fruity, tropical, fruit, pineapple, grape skin, banana	Fruity	Fruity, pineapple, tropical fruit, ripe fruit
Isoamyl isobutyrate	$C_9H_{18}O_2$	158.24	3.021	Fruity	Sweet, fruity, estry, and green with a waxy nuance	Fruity	Sweet, fruity, green, and fatty with a berry nuance
Ethyl valerate	$C_7H_{14}O_2$	130.19	2.314	Fruity	Sweet, fruity, acidic, pineapple, apple, green, berry, and tropical	Fruity	Fruity, strawberry, sweet, estry, fruity, pineapple, and tropical fruit

Table 2. (Continued)

Esters	Formula	M.W.	logP (o/w)	Odor Type	Odor Description	Flavor Type	Taste Description
Short-to-medium chain esters							
Isobutyl valerate	$C_9H_{18}O_2$	158.24	3.177	Fruity	Ethereal, fruity	Fruity	Sweet, fruity, apple, strawberry
Amyl valerate	$C_{10}H_{20}O_2$	172.27	3.810	Fruity	Ripe fruity apple	*n.a.*	*n.a.*
Ethyl hexanoate	$C_8H_{16}O_2$	144.21	2.823	Fruity	Sweet, fruity, pineapple, waxy, fatty, and estry with a green banana nuance	Fruity	Sweet, pineapple, fruity, waxy, and banana with a green, estry nuance
Isobutyl hexanoate	$C_{10}H_{20}O_2$	172.27	3.686	Fruity	Sweet, estry, fruity pineapple, green apple, peach, and tropical	Fruity	Sweet, fruity, pineapple, green, tropical, estry
Aromatic esters							
Benzyl acetate	$C_9H_{10}O_2$	150.18	1.960	Floral	Sweet, fruity, and floral	Fruity	Fruity, sweet, with balsamic, and jasmin floral undernotes
Benzyl lactate	$C_{10}H_{12}O_3$	180.20	1.153	Floral	Floral, fatty, butter, fruity	*n.a.*	*n.a.*
2-Phenylethyl acetate	$C_{10}H_{12}O_2$	164.20	2.300	Floral	Sweet, honey, floral rosy, with a slight yeasty honey note with a cocoa, and balsamic nuance	Honey	Sweet, honey, floral, rosy with a slight green nectar fruity body, and mouth feel
2-Phenylethyl isobutyrate	$C_{12}H_{16}O_2$	192.26	3.161	Floral	Heavy fruity, honey, and yeasty with balsamic nuances, and waxy rosy floral notes on dry out	Honey	Heavy, honey, floral, aldehydic with floral nuances
Methyl anthranilate	$C_8H_9NO_2$	151.17	1.880	Fruity	Fruity, concord grape, musty with a floral powdery nuance	Fruity	Sweet, fruity, concord grape, with a musty and berry nuance
Ethyl benzoate	$C_9H_{10}O_2$	150.18	2.640	Minty	Sweet, wintergreen, fruity, medicinal, cherry, grape	Medicinal	Sweet, medicinal, green, minty, fruity, birch beer, and wintergreen-like
Butyl benzoate	$C_{11}H_{14}O_2$	178.23	3.840	Balsamic	Mild, amber, balsam, fruity	*n.a.*	*n.a.*
Isoamyl benzoate	$C_{12}H_{16}O_2$	192.26	4.150	Balsamic	Sweet, fruity, green, and waxy	Fruity	Sweet, fruity with a green tropical nuance
2-Phenylethyl benzoate	$C_{15}H_{14}O_2$	226.27	4.010	Floral	Soft, rose, balsam, honey, floral	Floral	Floral, green, rose, plastic, honey, balsamic
Caffeic acid phenethyl ester (CAPE)	$C_{17}H_{16}O_4$	284.31	3.734	*n.a.*	*n.a.*	*n.a.*	*n.a.*
Terpene esters							
Geranyl acetate	$C_{12}H_{20}O_2$	196.29	4.040	Floral	Floral, rosy, waxy, herbal, and green with a slight cooling nuance	Green	Waxy, green, floral, oily, and soapy with citrus and winey, rum nuances
Perillyl acetate	$C_{12}H_{18}O$	178.27	3.610	Fruity	Fruity, woody, raspberry	Berry	Ionone, raspberry
Farnesyl acetate	$C_{17}H_{28}O_2$	264.41	5.790	Floral	Green, floral, rose	*n.a.*	*n.a.*
Retinyl acetate	$C_{22}H_{32}O_2$	328.50	*n.a.*	*n.a.*	*n.a.*	*n.a.*	*n.a.*

Figure legends

Figure 1. Overview of biosynthetic pathways of esters. Precursor pathways include α-keto acid pathway (blue arrows), reversed β-oxidation pathway (yellow arrows), fatty acid biosynthesis pathway (green arrows), aromatic alcohols biosynthesis pathway (brown arrows), shikimate pathway (orange arrows), phenylpropanoid pathway (red arrows), terpenoids biosynthesis pathway (purple arrows), polyketide biosynthesis pathway (pink arrows). Abbreviations: *G3P*, glyceraldehyde-3-phosphate; *PEP*, phosphoenolpyruvate; *PYR*, pyruvate; *E4P*, erythrose-4-phosphate; *PPP*, pentose phosphate; *MEP*, methylerythritol phosphate; *MVA*, mevalonate; *DMAPP*, dimethylallyl diphosphate; *IPP*, isopentenyl diphosphate; *GPP*, geranyl pyrophosphate, *FPP*, farnesyl diphosphate; *GGPP*, geranylgeranyl pyrophosphate, *CM*, chorismate, *SA*, salicylate, *ANT*, anthranilate; *PP*, prephenate, *Tyr*, tyrosine; *Phe*, phenylalanine; *CN*, cinnamate; *p-CMA*, *p*-coumarate; *Ac-CoA*, acetyl-CoA; *AA-CoA*, acetoacetyl-CoA; *Ma-CoA*, malonyl-CoA; *ACP*, acyl carrier protein; *BAHD*, BAHD acyltransferase

Figure 2. Catalytic reaction of **(A)** alcohol acyltransferases (AATs) and **(B)** chloramphenicol acetyltransferases (CATs). Chemicals: acetate moiety (in red); chloramphenicol (in blue). The purple arrows indicate the sequence of electron transfer. (**C)** 3D structure of chloramphenicol acetyltransferase (CAT). **(D)** Magnified binding pocket of CAT. Black arrows indicate the substrate routes to binding pocket. His, and Asp are known as catalytic residues. **(E)** Proposed rational engineering strategy for AATs. Arrows indicate the direction of protein evolution. Abbreviations: *His*, histidine; *Asp*, asparagine; *CoA*, coenzyme A.

Figure 3. Schematic of the modular cell design principles for efficient combinatorial production of esters. TRY stands for titer, rate, and yield.

Figure 4. Schematic of a thermophilic CBP microbial platform for production of esters from renewable resources.

Figure 1.

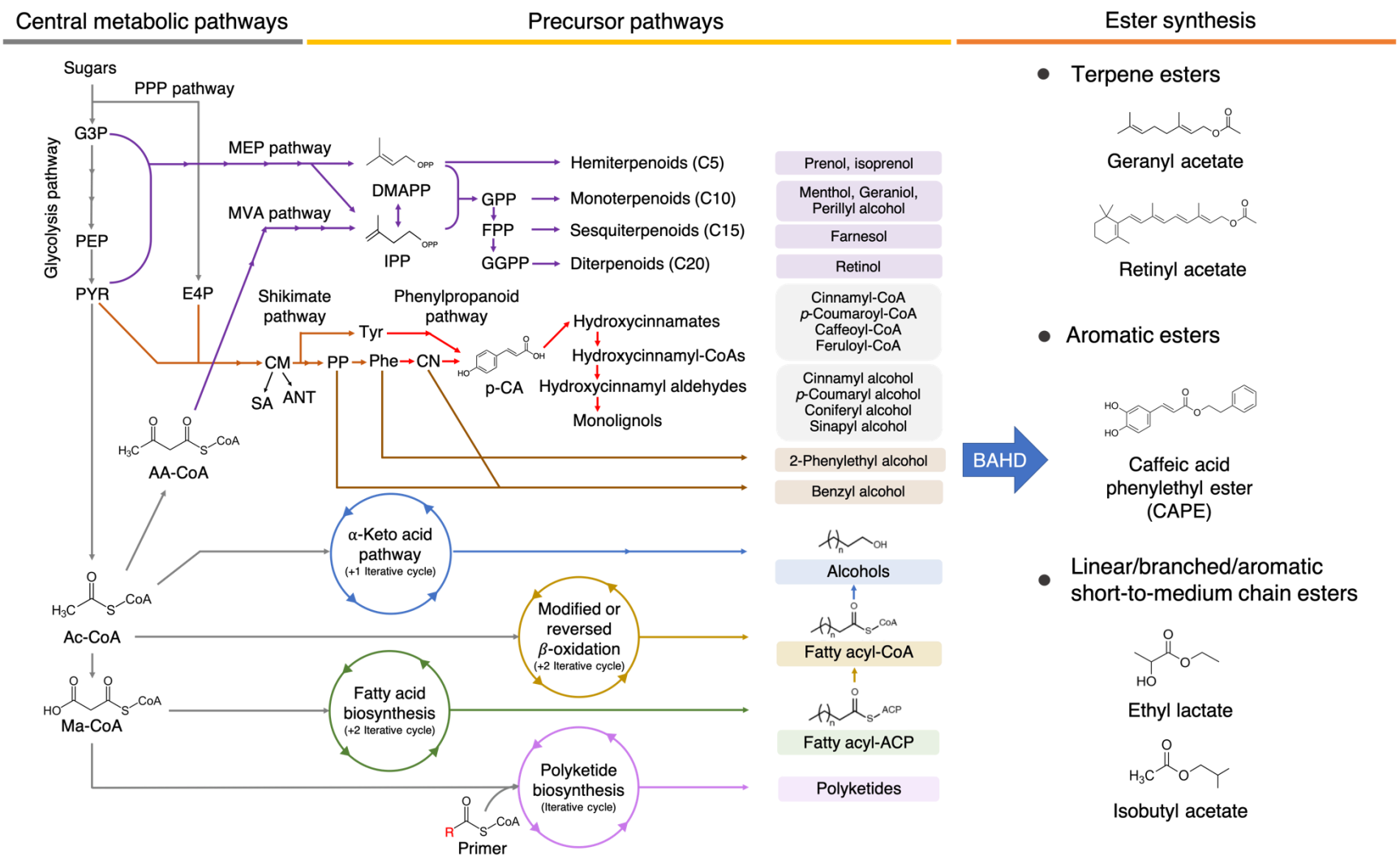

Central metabolic pathways
Precursor pathways
Ester synthesis
Sugars
PPP pathway
G3P
PEP
PYR
Glycolysis pathway
E4P
MEP pathway
MVA pathway
DMAPP
IPP
GPP
FPP
GGPP
Hemiterpenoids (C5)
Monoterpenoids (C10)
Sesquiterpenoids (C15)
Diterpenoids (C20)
Shikimate pathway
Phenylpropanoid pathway
Tyr
CM
PP
Phe
CN
SA
ANT
p-CA
Hydroxycinnamates
Hydroxycinnamyl-CoAs
Hydroxycinnamyl aldehydes
Monolignols
AA-CoA
Ac-CoA
Ma-CoA
α-Keto acid pathway
(+1 Iterative cycle)
Modified or reversed β-oxidation
(+2 Iterative cycle)
Fatty acid biosynthesis
(+2 Iterative cycle)
Polyketide biosynthesis
(Iterative cycle)
Primer
Prenol, isoprenol
Menthol, Geraniol, Perillyl alcohol
Farnesol
Retinol
Cinnamyl-CoA
p-Coumaroyl-CoA
Caffeoyl-CoA
Feruloyl-CoA
Cinnamyl alcohol
p-Coumaryl alcohol
Coniferyl alcohol
Sinapyl alcohol
2-Phenylethyl alcohol
Benzyl alcohol
Alcohols
Fatty acyl-CoA
Fatty acyl-ACP
Polyketides
BAHD
Terpene esters
Geranyl acetate
Retinyl acetate
Aromatic esters
Caffeic acid phenylethyl ester (CAPE)
Linear/branched/aromatic short-to-medium chain esters
Ethyl lactate
Isobutyl acetate

Figure 2.

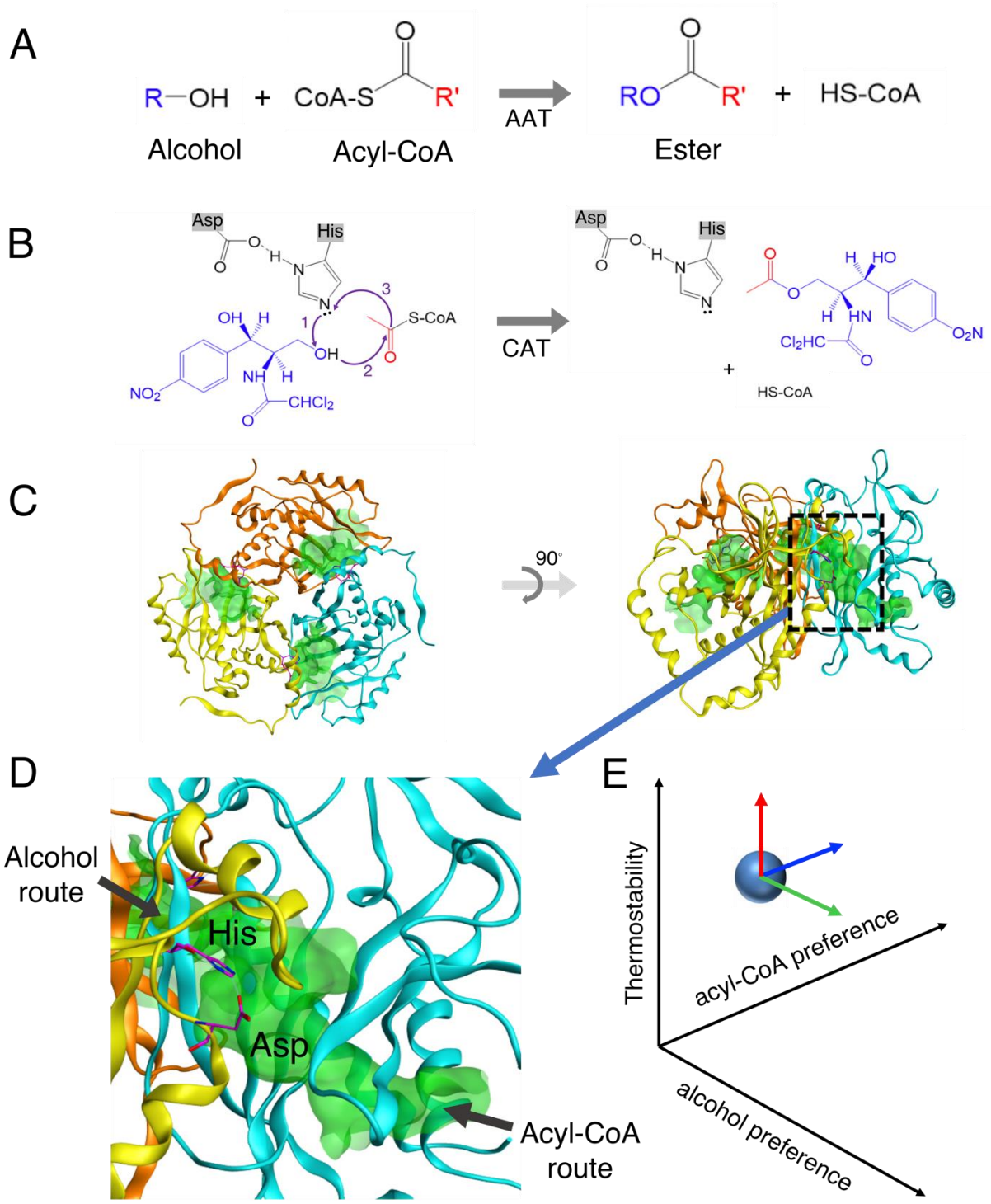

A
R—OH
+
CoA-S
R'
Alcohol
Acyl-CoA
AAT
RO
R'
+
HS-CoA
Ester
B
Asp
His
S-CoA
CAT
HS-CoA
C
90°
D
Alcohol route
His
Asp
Acyl-CoA route
E
Thermostability
acyl-CoA preference
alcohol preference

Figure 3.

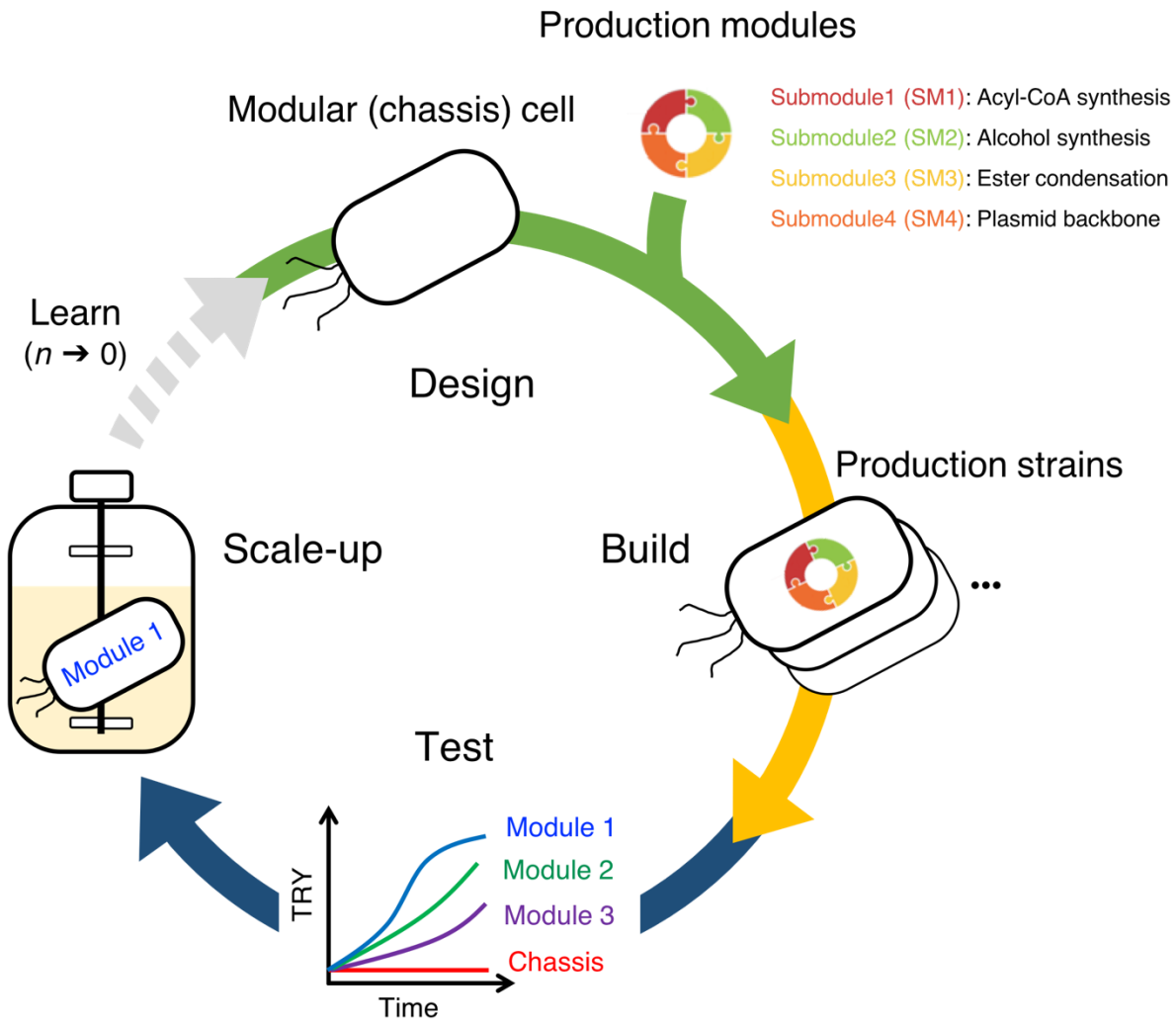
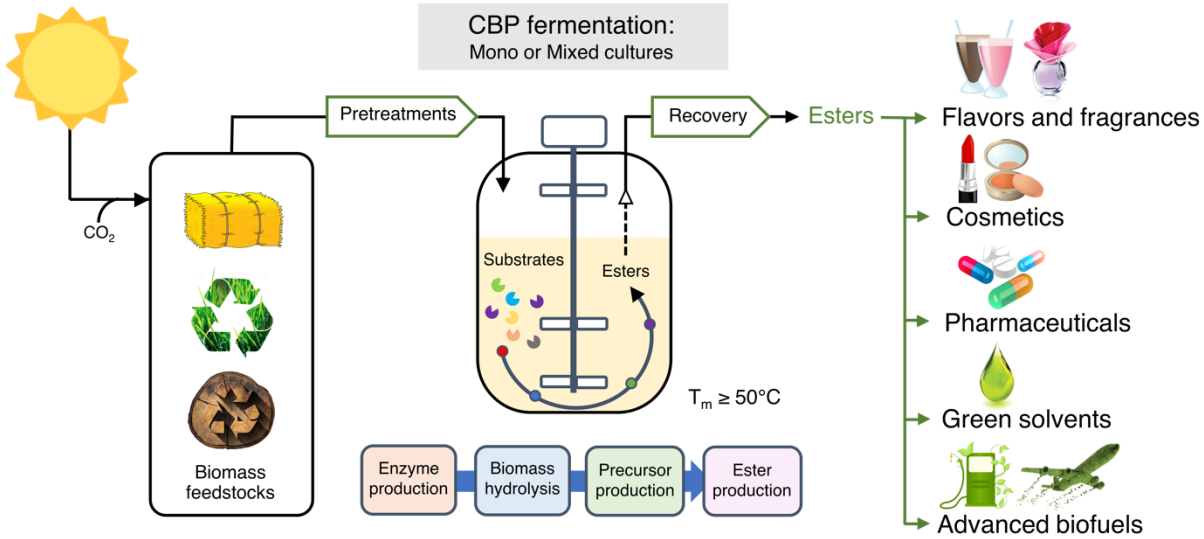
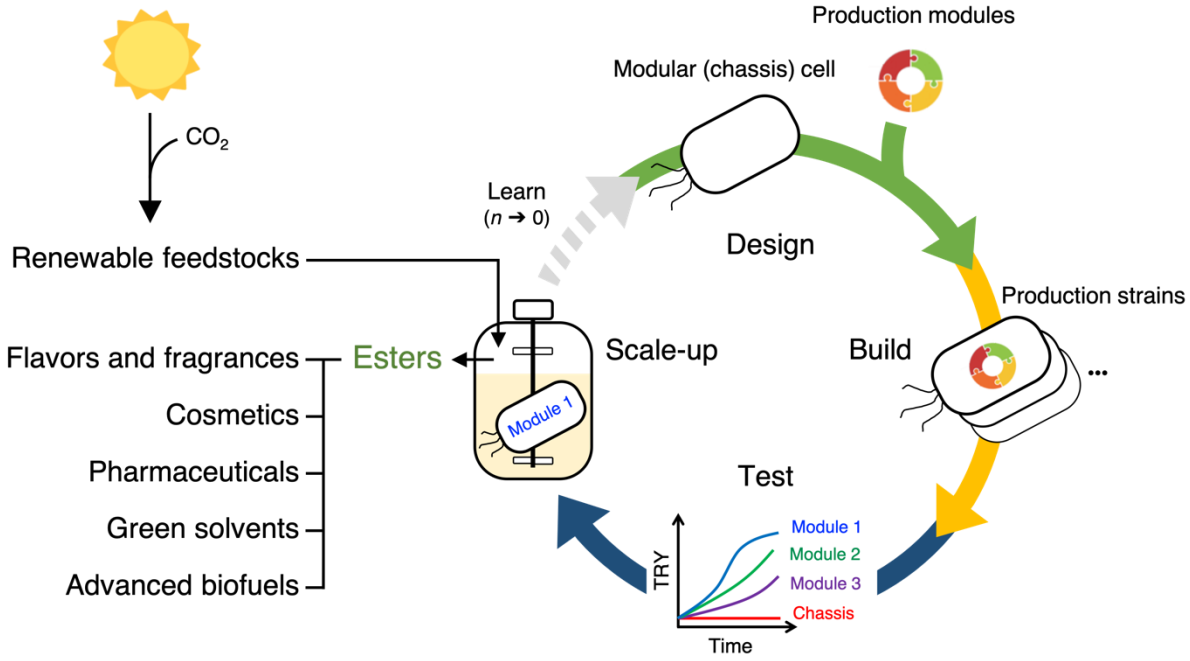

Production modules
Submodule1 (SM1): Acyl-CoA synthesis
Submodule2 (SM2): Alcohol synthesis
Submodule3 (SM3): Ester condensation
Submodule4 (SM4): Plasmid backbone
Modular (chassis) cell
Learn
(n → 0)
Design
Production strains
Build
Scale-up
Module 1
Test
TRY
Time
Module 1
Module 2
Module 3
Chassis

Figure 4.


CBP fermentation:
Mono or Mixed cultures
Pretreatments
Recovery
Esters
Flavors and fragrances
Cosmetics
Pharmaceuticals
Green solvents
Advanced biofuels
CO2
Biomass feedstocks
Substrates
Esters
Tm ≥ 50°C
Enzyme production
Biomass hydrolysis
Precursor production
Ester production

Graphical abstract



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•• of outstanding interest

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