Recent advances in modular co-culture engineering for synthesis of natural products

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The microbial production of natural products has been traditionally accomplished in a single organism engineered to accommodate target biosynthetic pathways. Often times, such approaches result in large metabolic burdens as key cofactors, precursor metabolites and energy are channeled to pathways of structurally complex chemicals. Recently, modular co-culture engineering has emerged as a new approach to efficiently conduct heterologous biosynthesis and greatly enhance the production of natural products. This review highlights recent advances that leverage Escherichia coli-based modular co-culture engineering for making natural products. Potential future perspectives for studies in this promising field are addressed as well.

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Introduction
Natural products discovered in plants, animals and microbes have been applied in many important aspects of human life since ancient times for different purposes. Because of their enormous molecular diversity and broad bioactivities, their roles include functional foods, fragrances, and pharmaceuticals. Many of these natural products have found applications as drugs such as artemisinin and paclitaxel for effective treatment of diseases [1,2]. However, the supply of natural products has been dramatically limited by the lack of effective methods for their production. Many natural products have complex chemical structures that make the associated chemical syntheses rather inefficient, complicated, and costly [3]. Even though common commercial sources that rely on extraction from native producers exist, such production methods are problematic because of their long-term sustainability and low overall abundance in their hosts [4]. The emerging fields of synthetic biology and metabolic engineering have substantially enhanced our abilities to program microbial organisms to provide alternative means for overproducing bioactive and high-value chemicals to chemical synthesis and traditional extraction [5,6].

Over the past few decades, researchers have produced a variety of natural compounds through constructing, regulating and optimizing the metabolic pathways of model host microorganisms including Escherichia coli and Saccharomyces cerevisiae. The majority of synthetic biology and metabolic engineering efforts on the heterologous production of chemicals was accomplished using a consolidated approach that relied on a single host cell in monoculture fermentation [7]. However, it has been challenging to provide an optimal environment for functionally expressing all enzymes involved in the complete biosynthetic pathways of complex compounds [5]. Recently, modular co-culture engineering approaches have been employed to make up for deficiencies of monoculture fermentation and improve biosynthesis efficiency of natural products [7].

Owing to features such as rapid growth, simple cultured conditions, easy genetic manipulation, and well characterized biochemistry, E. coli has become one of the most preferred production hosts and has been used extensively for the production of natural products both in monocultures and co-cultures [8]. Therefore, in this review, we will focus on current advances in modular co-culture engineering together with accompanying applications involving E. coli-based hosts for production of natural products. Using their biosynthetic origins, natural compounds were mainly grouped in four categories in this review for convenience: polyphenols, alkaloids, terpenoids, and other chemicals. Finally, we also discuss how the design and construction strategies of modular co-culture engineering have played important roles in producing natural products.
Advantages of modular co-culture engineering

Microbial consortia are ubiquitous in nature and are responsible for a variety of complex activities. In industry, they have been utilized in many fields for decades, especially in the food and pharmaceutical industries [9]. Microbial cocultures can also be engineered in order to produce complex chemicals at high yields [10]. Modular co-culture metabolic engineering involves the modularization of a complete biosynthetic pathway, with individual modules accommodated in different hosts for expressing associated genes to achieve desired biosynthesis [11]. Compared with monoculture fermentation, modular co-culture engineering has many advantages, such as: (i) it can overcome metabolic burden by division of labor and allows optimization of pathways in a modular fashion [10], (ii) it allows flexibility in balancing the metabolic fluxes between individual modules by easily controlling the ratio of engineered strains [12], (iii) it can prevent the potential inhibition exercised by metabolic intermediates upon susceptible enzymes [13], (iv) it provides well-suited environments for functional overexpression of all pathway genes, (v) it increases substrate utilization and the yield of target compounds [14], and (vi) due to its modular nature, it allows the facile production of a variety of chemicals by simply mixing and matching different microbial strains [15].

Modular co-culture engineering for synthesis of natural products

Recently, an increasing number of researchers have devoted themselves to the biosynthesis of natural products using modular co-culture engineering because of its benefits. Recent advances involving the synthesis of natural products by co-culture fermentation with E. coli-E. coli and E. coli-other species are summarized in Table 1.

Biosynthesis of polyphenols in co-culture system

As a diverse family of plant secondary metabolites, polyphenols are synthesized from the aromatic amino acids, phenylalanine and tyrosine [34]. They have been commonly used as health-promoting natural products owing to their bioactivities, such as antioxidant, anti-inflammatory, and antiviral effects. [35]. As shown in Table 1, polyphenols are currently the most studied natural products biosynthesized by modular co-culture engineering, including resveratrol, naringenin and afzelechin. For the biosynthetic route of phenylalanine, a cytochrome

Table 1

<table>
<thead>
<tr>
<th>Co-culture system</th>
<th>Natural product</th>
<th>Carbon source/precursors</th>
<th>Fold increase over mono-culture/production</th>
<th>Compound class</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli-E. coli</td>
<td>Resveratrol</td>
<td>Glycerol</td>
<td>22 mg/L</td>
<td>Phenylpropanoids</td>
<td>[16]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Rosmarinic acid</td>
<td>Glucose, Yeast extract</td>
<td>38-fold/172 mg/L</td>
<td>Phenylpropanoids</td>
<td>[17**]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>(-)-Afzelechin</td>
<td>Glucose</td>
<td>26.1 mg/L</td>
<td>Phenylpropanoids</td>
<td>[18]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Callistephen</td>
<td>Glucose</td>
<td>9.5 mg/L</td>
<td>Phenylpropanoids</td>
<td>[18]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>(+)-Afzelechin</td>
<td>Glycerol/p-Coumaric acid</td>
<td>970-fold/40.7 mg/L</td>
<td>Phenylpropanoids</td>
<td>[19]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Resveratrolisole,</td>
<td>glucose/p-Coumaric acid</td>
<td>3.2-fold/92.3 mg/L</td>
<td>Phenylpropanoids</td>
<td>[20]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Apigenin</td>
<td>Glucose/p-Coumaric acid</td>
<td>2.5-fold/16.6 mg/L</td>
<td>Phenylpropanoids</td>
<td>[21]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Bisdemethoxycurcumin</td>
<td>Glucose</td>
<td>6.28 mg/L</td>
<td>Phenylpropanoids</td>
<td>[22]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Naringenin</td>
<td>Glucose</td>
<td>41.5 mg/L</td>
<td>Phenylpropanoids</td>
<td>[23]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Pinene</td>
<td>Sucrose, Yeast extract,</td>
<td>1.9-fold/64.9 mg/L</td>
<td>Terpenoids</td>
<td>[24**]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Salidroside</td>
<td>Glucose, Xylose</td>
<td>6.03 g/L</td>
<td>Phenylethanoid (glycosides)</td>
<td>[25**]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Salicylate 2-O-β-d-glucoside</td>
<td>Glucose, Glycerol</td>
<td>2.5 g/L</td>
<td>Phenols glycosides</td>
<td>[26]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Caffeoylmalic acid</td>
<td>Glucose</td>
<td>3-fold/570.1 mg/L</td>
<td>Phenylpropanoids</td>
<td>[27]</td>
</tr>
<tr>
<td>E. coli-E. coli</td>
<td>Caffeylethanol,</td>
<td>Glucose, Yeast extract,</td>
<td>12-fold/401 mg/L, 124.9 mg/L</td>
<td>Phenylpropanoids</td>
<td>[28]</td>
</tr>
<tr>
<td>E. coli-S. cerevisiae</td>
<td>Oxygenated taxanes</td>
<td>Glucose, Xylose, Yeast extract</td>
<td>33 mg/L</td>
<td>Terpenoids</td>
<td>[29]</td>
</tr>
<tr>
<td>E. coli-S. cerevisiae</td>
<td>Magnoflorine,</td>
<td>Glucose, Yeast extract,</td>
<td>7.2 mg/L, 8.3 mg/L</td>
<td>Alkaloids</td>
<td>[30]</td>
</tr>
<tr>
<td>E. coli-S. cerevisiae</td>
<td>Scoulerine,</td>
<td>Peptone/Dopamine</td>
<td></td>
<td>Phenylpropanoids</td>
<td>[31]</td>
</tr>
<tr>
<td>E. coli-Streptomyces</td>
<td>Naringenin</td>
<td>Xylose, Yeast extract</td>
<td>8-fold/21.1 mg/L</td>
<td>Phenylpropanoids</td>
<td>[32**]</td>
</tr>
<tr>
<td>E. coli-C. glutamicum</td>
<td>Pipeolic acid</td>
<td>Starch</td>
<td>439.1 mg/L</td>
<td>Amino acid</td>
<td>[33**]</td>
</tr>
</tbody>
</table>
P450-dependent cinnamate-4-hydroxylase plays a critical role in the production of p-coumaric acid [36]. It is well established in the literature that the heterologous expression of P450 enzymes in E. coli remains an engineering conundrum [36]. Therefore, the transformation of tyrosine to p-coumaric acid (p-CoA) has been widely used as an alternative approach for the biosynthesis of flavonoids with multiple hydroxylations on the B ring (Figure 1).

One typical example for modular co-culture engineering is the recent achievement of balancing the non-linear rosmarinic acid (RA) biosynthetic pathway [17**]. In this study, a rationally designed microbial co-culture system comprises three metabolically engineered E. coli strain modules (upstream modules: caffeic acid module and salvianic acid A module; downstream module: rosmarinic acid module) was developed for the production of natural compound rosmarinic acid whose biosynthesis involves a complex diverging-converging pathway [17**]. In the caffeic acid module, caffeic acid can be derived from 4-hydroxy phenylpyruvate by using three enzymes: tyrosine aminotransferase (TyrB), tyrosine ammonia lyase (TAL) and 4-hydroxyphenylacetate 3-hydroxylase (HpaBC) (Figure 1). In the salvianic acid A module, salvianic acid A is produced by two-step enzymatic reactions from 4-hydroxy phenylpyruvate: HpaBC and D-lactate dehydrogenase (Figure 1). For the formation of rosmarinic acid, caffeic acid needs to be converted to caffeoyl CoA by 4CL, and caffeoyl CoA is further catalyzed to salvianic acid A by rosmarinic acid synthase (Figure 1). The pathway was modularized in three individual strains allowing for the segregation of the caffeic acid module and salvianic acid A module in order to minimize the competition for the upstream carbon flux [17**]. This strategy also facilitated flexible adjustment of the biosynthetic ability of each independent module by controlling the subpopulation ratio of co-culture strains. Compared with the mono-culture, this engineered modular co-culture resulted in the bioproduction of 172 mg/L rosmarinic acid, a 38-fold increase compared to the mono-culture approach [17**].

As one of the key precursors of most polyphenols, p-coumaroyl-CoA can be condensed with malonyl-CoA by stilbene synthase (STS) and curcumin synthase and converted into resveratrol and bisdemethoxycurcumin in a single step, respectively (Figure 1). To some extent, malonyl-CoA is a key precursor whose supply is essential for the high yield of target product in the aromatic polyketide biosynthetic pathway. For the rapid production of bisdemethoxycurcumin, Fang et al. constructed an E. coli-E. coli co-culture system where an E. coli module was used to produce p-coumaric acid from glucose and the other was responsible for the conversion of p-coumaric acid to a target compound [22*]. In this study, four different strategies were tested to enhance the intracellular malonyl-CoA pool in one engineered E. coli module, and 6.28 mg/L of bisdemethoxycurcumin was produced after introducing malonyl-CoA synthetase and dicarboxylate carrier protein, and optimizing the inoculation ratio and induction point [22*].

In addition, p-coumaric acid can be converted to naringenin which is a very important intermediate of flavonoids in the presence of CHS and CHI. Jones et al. presented an E. coli co-culture platform for the efficient production of afzelechin and (+)-catechin through modularizing associated pathways involving six enzymes into two distinct modules: an upstream malonyl-CoA-dependent module with 4CL, CHS, CHI and a downstream NADPH-dependent module with F3H, DFR, and LAR [19]. In another study, Jones et al. constructed a four recombinant E. coli co-culture system collectively expressing fifteen enzymes for the biosynthesis of pelargonidin-3-O-glucoside callistephenin [18*]. In addition to the malonyl-CoA-dependent module for the biosynthesis of intermediate naringenin and an NADPH-dependent module for the biosynthesis of flavan-3-ol intermediates, the TAL module for the production of phenylpropanoic acids and the anthocyanin module were introduced into this 4-strain polyculture system (Figure 1), resulting in a de novo titer of 9.5 mg/L callistephenin [18*]. This synthetic microbial consortia approach enables the functional expression of enzymes in long length biosynthetic pathways by division of labor.

Many natural products exist in their glycosylated form since glycosylated molecules in plants usually have better stability, solubility, and enhanced bioactivity [37]. Resveratrol glucosides were recently produced through an E. coli co-culture consisting of an upstream module, expressing 4CL and STS for transforming p-coumaric acid to resveratrol and a downstream module expressing the glucosyltransferase PaGT3 to generate glycosylated products resveratrolside and polydatin (Figure 1) [20].

Thuan et al. presented an engineered E. coli co-culture system to synthesize apigenin. Similar to the strategy for obtaining resveratrol glucosides, this system was also compartmentalized into two modules: an apigenin strain containing a biosynthetic pathway (4CL, CHS, CHI and FNSI) for the production of apigenin and a glycosylation strain for heterogeneous expression of PaGT3 [21*]. In all cases, the glycosylation modules were engineered by increasing the flux to UDP-glucose.

In another study, a syntrophic E. coli co-culture was designed and constructed for the de novo biosynthesis of salidroside [25**]. The pathway of salidroside was convergently divided into the aglycone strain for the production of tyrosol and the glycoside strain for biosynthesis of UDP-glucose and salidroside. The phenylalanine-deficient strain was constructed to favor the consumption of xylose over glucose and the tyrosine-deficient strain was engineered to use exclusively glucose
The biosynthetic pathways of natural products produced (a): polyphenols; (b): alkaloids; (c): terpenoids) by modular co-culture-based fermentations.

TyrB: tyrosine aminotransferase, TAL: tyrosine ammonia lyase, ß-LDH: ß-lactate dehydrogenase, HpaBC: 4-hydroxyphenylacetate 3-hydroxylase, D-LDH: D-lactate dehydrogenase, 4CL: 4-coumaroyl-CoA ligase, 40MT: 4-hydroxy-3-methoxystilbene 6-O-methyltransferase, 6OMT: norcoclaurine 6-O-methyltransferase, ANS: anthocyanidin-3-O-glucoside, 3GT: 3-O-glycosyltransferase, STS: stilbene synthase, CUS, curcumin synthase, MAO: monoamine oxidase, NCS: norcoclaurine synthase, 60MT: norcoclaurine 6-O-methyltransferase, CNMT: coacaldehyde-N-methyltransferase, 4'OMT: 3'-hydroxy-N-
as a carbon source [25**]. The two strains in this synthetic consortium were co-cultured by mutually cross-feeding of tyrosine and phenylalanine, resulting in a stable system and efficient production of salidroside.

Moreover, modular co-culture biosynthesis has been extended for bioproduction of O-methylated phenylpropanoids. Most of methylated phenylpropanoids exhibited significantly better physicochemical properties and higher biological activity than their original counterparts [38]. The corresponding system was developed by co-culturing the engineered E. coli producing phenylpropanoids along with Streptomyces venezuelae expressing a methyltransferase SaOMT2 from Streptomyces avermitilis, resulting in high-yield microbial production of di- and trimethylated phenylpropanoids including sakuranetin and genkwanin for the first time [32*].

**Consortia process for bioproduction of alkaloids**

Alkaloids are a class of natural products that contain nitrogen moieties. Most of alkaloids derive from amines that are produced by the decarboxylation of amino acids [39]. Although the production of some alkaloids, like noscapine, stricotsidine and opioids, has been tried in mono-culture, to our knowledge, there has been only one study that reported on the biosynthesis of alkaloids using an E. coli co-culture [30, 40–42]. Considering some plants enzymes are not functionally expressed in bacteria, Minami et al. biosynthesized plant benzylisoquinoline alkaloids by using a co-culture platform composed of an E. coli module for the production of reticuline from dopamine by expressing associated enzymes (MAO, NCS, 6OMT, CNMT and 4’OMT) and an S. cerevisiae module with desired biosynthetic genes of BBE or CYP80G2/CNMT (Figure 1), resulting in 7.2 mg/L of magforline or 8.3 mg/L of scoulerine [30]. This example of the reconstruction of an alkaid pathway in co-culture system may provide some guidance for the production of similar plant-derived alkaloids in the future.

**Co-culture fermentation for synthesis of terpenoids**

Terpenoids represent a numerous group of structurally diverse chemicals that are formed by five-carbon building blocks (isoprene) [4]. The biosynthesis of terpenoids has been explored mostly in a single microbe because of their complex structures [43]. In one study, the production of α-pinene was improved by 1.9-fold (166.5 mg/L) using a combined strategy of tolerance, evolution and E. coli-E. coli modular co-culture engineering [24**]. In this study, pinene tolerance was improved by using adaptive laboratory evolution after atmospheric and room temperature plasma mutagenesis and overexpression of efflux pumps [24**]. Then a geranyl pyrophosphate synthase variant (GPPS1990G1L175P) with better enzymatic properties was obtained by directed evolution that included both error-prone PCR and DNA shuffling. To coordinate the expression of cascade enzymes, the tunable intergenic regions (TIGR) approach for tuning the expression of multiple genes within operons by generating libraries of tunable intergenic regions was further applied in the mevalonate (MEV) pathway, leading to a sevenfold enhanced yield of mevalonate [24**, 44]. To improve overall pinene production, modules composed of the MEV pathway and the PINE pathway were integrated into the chromosome of an optimized pinene tolerance strain and then evolved to a higher copy number using triclason induction, respectively.

In another study, Zhou et al. developed E. coli-S. cerevisiae synthetic consortia to synthesize oxygenated taxanes and other oxygenated isoprenoids by expressing the taxadiene biosynthetic pathway in E. coli and taxadinene-oxygenating cytochrome P450s in S. cerevisiae (Figure 1) [29]. To control population densities of the two strains in the mutualistic consortium, E. coli was engineered to grow on xylose and produce acetate which severs as the sole carbon source for S. cerevisiae. A similar tactic was also applied in the production of naringenin from xylose with co-culture of E. coli and S. cerevisiae [31].

**Production of other chemicals by cocultivation system**

Recently, synthetic E. coli-Corynebacterium glutamicum consortia were constructed to biosynthesize lysine and value-added products derived from L-lysine, such as piperocol acid and cadaverine from starch or sucrose [33**]. The commensalism-based synthetic consortia employed an L-lysine autotrophic, naturally sucrose-negative E. coli and a fructose importer deleted (ptsF) C. glutamicum mutant for the production of lysine from sucrose. The mutualistic synthetic consortia composed of a lysine autotrophic, α-amylase secreting E. coli and naturally amylose-negative C. glutamicum was utilized for biosynthesis of piperocol acid and cadaverine from starch. This study provides some insight into how to design and optimize synthetic microbial consortia consisting of C. glutamicum and E. coli.

In general, modularization and optimization of target biosynthetic pathways to benefit the co-culture fermentation are the strategies most commonly used in the examples summarized in the previous paragraphs. However, based on metabolic fluxes and mutual relations.
among the co-culture species, diverse strategies were further rationally designed in order to further develop desired modular co-culture systems for the efficient production of specific natural compounds.

Conclusions and future perspectives
To date, more and more applications of modularization co-culture strategies to achieve division of labor have been demonstrated, indicating that modular co-culture engineering provides an effective and robust toolkit for biosynthesis of various biochemicals, especially of the more structurally complex natural products with long biosynthetic pathways. This new approach not only significantly expedites the achievement of higher product yields but also facilitates biosynthetic pathway refactoring for obtaining a wide range of natural products in a plug-and-play fashion [45]. Despite the advantages of modular co-culture engineering, several challenges need to be overcome for industrializing such approaches on a large scale. The foremost of issues is to enhance the stability of the synthetic microbial consortia throughout the fermentation process. To that extend, the strategies of commensalism-based synthetic consortia and mutualistic synthetic consortia have been shown to be effective methods to achieve this goal [35]. Another key issue that also needs careful consideration is the media composition, especially when the engineered consortia involve two or multiple different species. In addition, it is important to take advantage of the versatile abilities and advantages that these species provide, particularly involving metabolic flux and metabolites, for reconstruction and design of optimal pathways. Therefore, novel approaches should be implemented within the modular co-culture field, such as directed evolution to enhance enzymatic properties, 13C-metabolic flux analysis to determine metabolic flux distributions [46], and computational biology to rationally engineer robust co-culture systems [47].

Although a few natural products were successfully produced by modular co-culture engineering in recent years, great efforts are necessary to bring such approaches to large-scale industrial applications. It is worth mentioning that nowadays most of these co-culture studies only focused on polyphenols, perhaps because of the ease these molecules enter and exit their cellular producers. One direction for future development is sugar-functionalyzed natural products. Nature glycosides are usually conjugated with various sugar moieties such as glucose, galactose, glucuronic acid, rhamnose, xylose, arabinose, and N-acetyl-glucosamine [48]. In the future, it is possible to establish versatile NDP-sugar modules which can be rationally assembled into co-culture systems. In addition, the biosynthesis of complex structural natural products such as terpenoids and alkaloids will be of particular interest in co-cultures with long and non-linear pathways involving multiple cytochrome P450s. With continued progress in modular co-culture engineering, we expect a large array of valuable and bioactive natural products to be produced in the future.

Conflicts of interest statement
Nothing declared.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

17. Li Z, Wang X, Zhang H: Balancing the non-linear rosmarinic acid biosynthetic pathway by modular co-culture engineering. *Metab Eng* 2019, 54:1-11. The authors developed a three-strain co-culture system for biosynthesis of complex natural product rosmarinic acid by balancing the diverging-converging pathway, resulting in a yield of 172 mg/L RA and 38-fold biosynthesis improvement over the parent strain in mono-culture system.


21. Thuan NH, Chaudhary AK, Van Cuong D, Cuong NX: Engineering co-culture system for production of apigetrin in *Escherichia coli*. *J Ind Microbiol Biotechnol* 2018, 45:175-185. This paper described genetically engineered *E. coli*-based system to the de novo synthesis of apigetrin by optimizing the initial inoculum ratio of stains and co-culture system conditions such as temperature and media component.

22. Fang Z, Jones JA, Zhou J, Koffas MAG: Engineering *Escherichia coli* co-cultures for production of curcuminoids from glucose. *Biotechnol J* 2018, 13:1-8. Bisdemethoxycurcumin was produced through engineered *E. coli*-E. coli co-culture fermentation. In this study, four different strategies related to improving intracellular precursors, malonyl-CoA pool were constructed in *E. coli*.


