

## Microbial production of value-added nutraceuticals

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Nutraceuticals are important natural bioactive compounds that confer health-promoting and medical benefits to humans. Globally growing demands for value-added nutraceuticals for prevention and treatment of human diseases have rendered nutraceuticals a multi-billion dollar market. However, supply limitations and extraction difficulties from natural sources such as plants, animals or fungi, restrict the large-scale use of nutraceuticals. Metabolic engineering via microbial production platforms has been advanced as an eco-friendly alternative approach for production of value-added nutraceuticals from simple carbon sources. Microbial platforms like the most widely used *Escherichia coli* and *Saccharomyces cerevisiae* have been engineered as versatile cell factories for production of diverse and complex value-added chemicals such as phytochemicals, prebiotics, polysaccharides and poly amino acids. This review highlights the recent progresses in biological production of value-added nutraceuticals via metabolic engineering approaches.

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prevention and treatment of disease' [1]. Later on, the concept of nutraceutical was modified as 'a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food' [2]. Nutraceuticals are now more specific to structurally and functionally diverse bioactive compounds that exert long-term medicinal or physiological benefits other than purely nutritional or direct pharmaceutical effects, which distinguishes them from functional foods and drugs. They can be derived from plants (e.g. phytochemicals, vitamins), from animals (e.g. polysaccharides), from microorganisms (e.g. poly amino acids) and from marine sources (e.g. glucosamine and chitosan). Nutraceuticals are widely used for their health-promoting or disease-preventing properties especially in preventing aging-associated diseases including oxidative stress, depression, inflammation, arthritis, osteoporosis, gastrointestinal diseases, cardiovascular diseases, diabetes and cancer [3]. The rising demands and interests in maintaining human wellness through diet have greatly promoted the growth of the nutraceutical market. According to Global Information Inc.' recent report, the global nutraceutical market was estimated to exceed \$171.8 billion in 2014 and reach \$241.1 billion by 2019, while the US market alone was about \$75.9 billion [3].

However, the expanding nutraceutical market can hardly be fulfilled by the productivity of conventional nutraceutical industries. Direct extraction strategies are limited by the availability and cost of raw materials, the quality control of supplies, and the low content and purity of nutraceuticals. Although chemical synthesis is an alternative approach, it is limited to producing simple biochemicals and is infeasible for complex biochemicals especially those that are chemically unfavorable [4]. To address these issues, microbial-based metabolic engineering is an appealing approach and has achieved great progress on production of value-added nutraceuticals in very recent years. The rapid elucidation of biosynthetic pathways for natural products and the genetic amenability of microorganisms have enabled the development of microbial hosts for production of various nutraceuticals. By further genetic manipulation of host cells and optimization of the culture conditions or fermentation processes, metabolic engineering makes it possible to scale up the production of nutraceuticals from simple carbon sources. As a matter of fact, certain commercial *E. coli* strains and food-grade *S. cerevisiae* have been industrially used as cell factories to produce natural products Generally Recognized As Safe (GRAS). This review covers recent

## Introduction

Nutraceutical, a hybrid term from 'nutrition' and 'pharmaceutical' coined by Stephen L. DeFelice in 1989, was defined as 'any substance that is a food or a part of food and provides medical or health benefits, including the

advances in nutraceutical production via microbial-based metabolic engineering, including the well-studied phytochemicals, as well as prebiotics, polysaccharides and poly amino acids.

## Phytochemicals

Phytochemicals are a broad spectrum of plant-derived bioactive secondary metabolites that are commonly found in fruits, vegetables, beans and grains. They are involved in plant defenses against biotic or abiotic stresses, and also exhibit health-protecting or disease-preventing effects on humans [3]. Phytochemicals is a vast and very important repertoire for nutraceuticals, which include polyphenolic compounds (flavonoids, isoflavonoids, stilbenoids and curcuminoids), alkaloids, terpenoids (monoterpenes, diterpenes, tetraterpenes, polyterpenes, steroid saponins, lycopene and carotenoids) and their derived compounds.

### Polyphenolic compounds

Amongst the phytochemicals, polyphenolic compounds have been used for decades as effective antioxidants and food ingredients in the nutraceutical industry. Polyphenolic scaffolds such as flavonoids and stilbenoids are derived from the phenylpropanoic pathway that extends from aromatic amino acids L-phenylalanine and L-tyrosine (Figure 1) [5]. L-Phenylalanine and L-tyrosine are converted to pivotal intermediates cinnamic acid and *p*-coumaric acid via phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL), which are further converted to cinnamoyl-CoA and *p*-coumaroyl-CoA by 4-coumaroyl-CoA ligase (4CL), respectively. Then, three moles of malonyl-CoA condense with cinnamoyl-CoA or *p*-coumaroyl-CoA to construct the backbone flavanones, stilbenes or curcuminoids by type III polyketide synthases, such as chalcone synthase (CHS), curcuminoid synthase (CUS) or stilbene synthase (STS) [6]. With the action of tailoring enzymes such as hydroxylases, *O*-methyltransferases, glucosyltransferase, lipases and prenyltransferase, diverse polyphenolic compounds are generated [7].

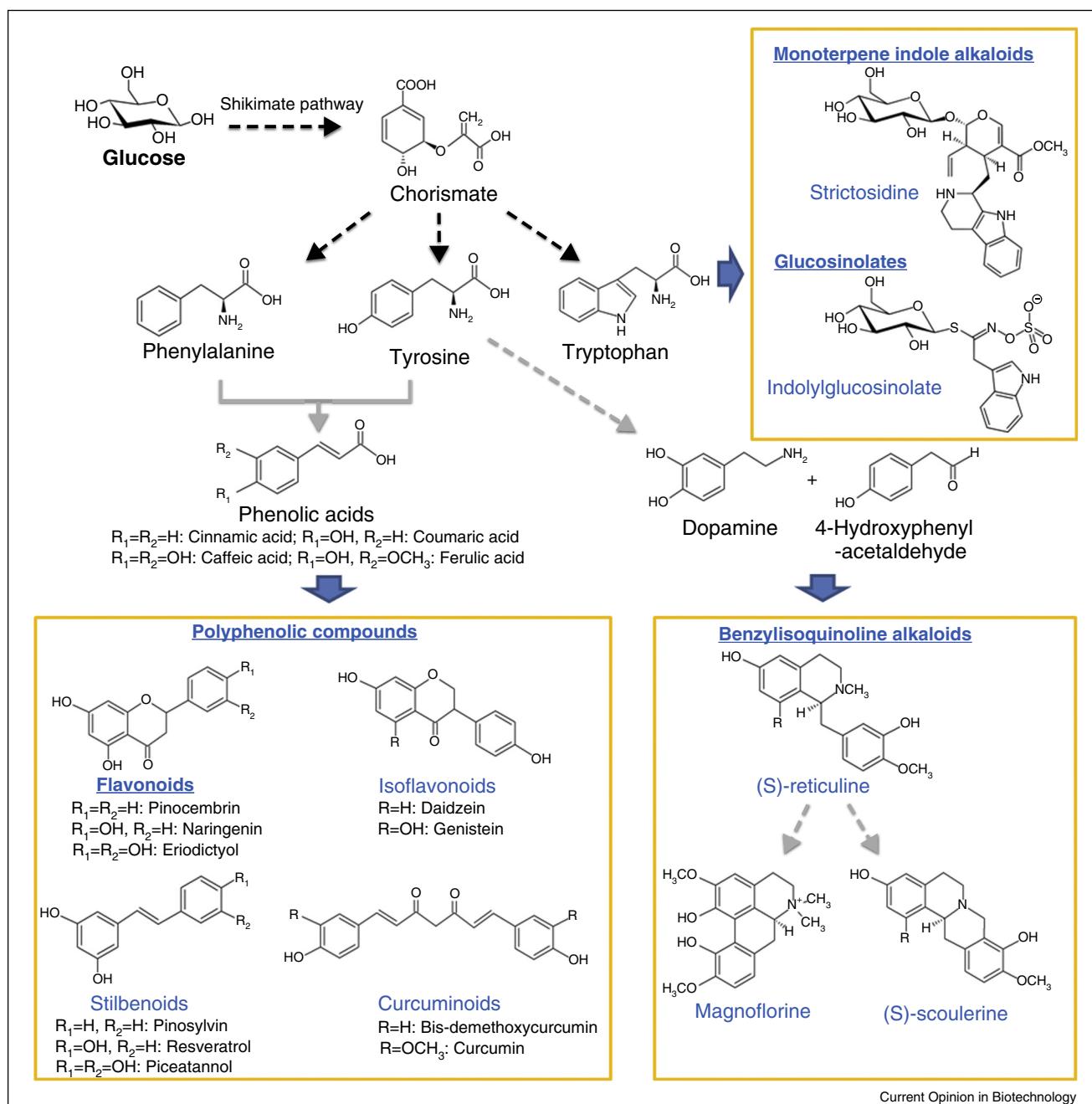
*E. coli* and *S. cerevisiae* have generally been developed as platform organisms for *de novo* or semi-*de novo* production of almost all kinds of polyphenolic compounds (Table 1). Some of the biggest advantages of *E. coli* include its fast growth and ease of genetic manipulation; for *S. cerevisiae* its Generally Regarded As Safe (GRAS) status and its ability to functionally express plant metabolic enzymes. In all cases, in order to achieve polyphenolic compound production in microbes, plant-originated enzymes are firstly overexpressed via either codon optimization or construction of enzyme chimeras that facilitate bacterial expression [8]. The first strategy is feeding precursors to the heterologous hosts to produce corresponding polyphenolic compounds. For example, supplementation with phenylalanine to *E. coli* expressing heterologous pathways produced chrysanthemic acid (9.4 mg/L) or galangin (1.1 mg/L) while supplementation with tyrosine led to

the production of apigenin (13 mg/L) or kaempferol (15.1 mg/L) (Table 1) [9]. This feeding approach is widely used when precursors are low or cannot be biosynthesized in microbes. Flavonoid glucosides like luteolin 4'-*O*- and 7'-*O*-glucosides were produced by feeding luteolin to *E. coli* expressing glycosyltransferases UGT71G1 [10]. By expressing glycosyltransferases with different substrate specificities, quercetin 3-*O*-glucoside, quercetin-3-*O*-glucuronide, quercetin 3-*O*-galactoside and quercetin-3-*O*-rhamnoside were respectively produced (Table 1) [10–12]. The second approach is *de novo* biosynthesis of phenolic compounds from simple carbon sources like glucose and glycerol, which is more appealing for industrial applications. *E. coli* hosts can be easily metabolically engineered to overproduce L-phenylalanine and L-tyrosine from simple carbon sources. The intracellular concentration of malonyl-CoA, the extender unit for polyphenolic backbones, can be greatly enhanced in *E. coli* by overexpressing acetyl-CoA carboxylase (ACC) or genome-scale metabolic optimization or by repressing malonyl-CoA consumption pathways using antisense RNA [13,14••]. *E. coli* has been successfully utilized for *de novo* biosynthesis of certain polyphenolic compounds such as pinocembrin, pinosylvin, sakuranetin and ponciretin [15–17]. Furthermore, substrate promiscuity of authentic or engineered enzymes has provided with new possibilities to produce novel or non-natural polyphenolic compounds [18•,19].

### Alkaloids

Alkaloids are amino acid-derived nitrogenous compounds that have important therapeutic values, including anti-cancer and antimalarial effects [5]. For a long time, production of alkaloids was limited to plants because of their complex structure and long biosynthetic pathways. The three most well-known alkaloids are benzylisoquinoline alkaloids (BIAs) derived from tyrosine, monoterpene indole alkaloids (MIAs) derived from tryptophan and glucosinolates. In recent years, elucidation of many BIA biosynthetic pathways enabled the reconstruction of BIA pathways in both *E. coli* and *S. cerevisiae* [20•,21,22]. (S)-reticuline, a branch-point intermediate for BIA, was biosynthesized from simple carbon sources with titer of 46.0 mg/L [20•]. Bacteria consortia of *E. coli* producing (S)-reticuline and *S. cerevisiae* producing either monooxygenase CYP80G2 or the berberine bridge enzyme (BBE) yielded 7.2 mg/L magnoflorine and 8.3 mg/L scoulerine, respectively (Figure 1) [23]. *S. cerevisiae* was also enabled to produce (R,S)-reticuline and was further engineered to generate scoulerine, tetrahydrocolumbamine and tetrahydroberberine from (S)-reticuline and salutaridine from (R)-reticuline [24]. However, metabolic engineering of MIA alkaloids in microbes is limited. Recently, *de novo* production of the MIA alkaloid strictosidine has been achieved in yeast by introducing 21 new genes and deleting 3 genes in the yeast genome (Table 1) [25••]. Glucosinolates are sulfur-rich, amino acid-derived natural compounds. Tryptophan-derived indolylgluco-

Figure 1



Examples of microbial-based production of phytochemicals from aromatic amino acids. Arrows represent the metabolic flux from primary shikimate pathway to target phytochemicals. Polyphenolic compounds are derived from phenylalanine or tyrosine, benzylisoquinoline alkaloids (BIA) are derived from tyrosine, monoterpene indole alkaloids (MIA) and indolylglucosinolate (IG) are derived from tryptophan.

sinolate (IG) has recently been produced in *S. cerevisiae* by inserting eight plant genes into the genome, which represents a rare example of glucosinolate production in microbes (Figure 1) [26<sup>••</sup>]. Although plant platforms are more suitable for scalable alkaloid production, metabolic engineering in microbes has shown potential capability

for cost-efficient production of these plant-derived compounds [7,25<sup>••</sup>].

#### Terpenoids

Terpenoids are present in green foods, soy plants and cereals, and serve as the largest class of phytonutrients with anti-inflammatory, anti-infectious and anti-cancer

**Table 1****Biological production of nutraceuticals via metabolic engineering in different hosts.**

Nutraceuticals	Heterologous hosts	Fed precursors	Titers (mg/L)	References
<b>Polyphenolic compounds</b>				
Apigenin (Flavone)	<i>E. coli</i>	Tyrosine	13	[9]
Chrysin (Flavone)	<i>E. coli</i>	Phenylalanine	9.4	[9]
Pinocembrin (Flavanone)	<i>E. coli</i>	Glucose	40.2	[15]
Eriodictyol (Flavanone)	<i>E. coli</i>	Tyrosine	107	[51]
Naringenin (Flavanone)	<i>S. cerevisiae</i>	Glucose	108.9	[52*]
Catechin (Flavanol)	<i>E. coli</i>	Eriodictyol	910.9	[53]
Genistein (Isoflavonone)	<i>E. coli-S. cerevisiae</i>	Tyrosine	6	[54]
Resveratrol (Stilbenoid)	<i>E. coli</i>	Tyrosine	35.02	[55]
Pinosylvin (Stilbenoid)	<i>E. coli</i>	Glucose	70	[16]
Curcumin (Curcuminoid)	<i>E. coli</i>	Tyrosine	0.6	[56]
Caffeic acid	<i>E. coli</i>	Glucose and glycerol	766.68	[57]
<b>Flavonoid derivatives</b>				
Sakuranetin	<i>E. coli</i>	Glucose	42.5	[17]
Ponciretin	<i>E. coli</i>	Glucose	40.1	[17]
7-O-Methyl aromadendrin	<i>E. coli</i>	p-coumaric acid	2.7	[58]
Luteolin 4-O-glucosides	<i>E. coli</i>	Luteolin	10.86	[10]
Luteolin 7-O-glucosides	<i>E. coli</i>	Luteolin	6.52	[10]
Luteolin-7-O-glucuronide	<i>E. coli</i>	Luteolin	300	[11]
Kaempferol 3-O-glucoside	<i>E. coli</i>	Naringenin	109.3	[10]
Kaempferol-3-O-rhamnoside	<i>E. coli</i>	Kaempferol	150	[12]
Quercetin 3-O-glucoside	<i>E. coli</i>	Quercetin	11.54	[10]
Quercetin-3-O-glucuronide	<i>E. coli</i>	Quercetin	687	[11]
Quercetin-3-O-rhamnoside	<i>E. coli</i>	Quercetin	200	[12]
Anthocyanin	<i>E. coli</i>	Catechin	110	[59]
Genistein glucosides	<i>E. coli</i>	Genistein	37.29	[60]
Genistin	<i>S. cerevisiae</i>	Genistein	—	[61]
Daidzin	<i>S. cerevisiae</i>	Daidzein	—	[8]
3-Hydroxydaidzein	<i>E. coli</i>	Daidzein	75	[61]
<b>Alkaloids</b>				
(S)-reticuline (Benzylisoquinoline alkaloid)	<i>E. coli</i>	Glycerol	46	[20**]
Strictosidine (Monoterpene indole alkaloid)	<i>S. cerevisiae</i>	Glucose	0.53	[25**]
Indolylglucosinolate (Glucosinolate)	<i>S. cerevisiae</i>	Glucose	1.07	[26**]
<b>Terpenoids</b>				
Lycopene	<i>E. coli</i>	Glucose	18 mg/g	[62]
β-Carotene	<i>E. coli</i>	Glucose	2100	[27]
Zeaxanthin	<i>E. coli</i>	Glucose	43.46	[28]
Astaxanthin	<i>E. coli</i>	Glucose	1.4 mg/g	[29]
<b>Prebiotics</b>				
2-Fucosyllactose	<i>E. coli</i>	Lactose and glycerol	20280	[39*]
Galactooligosaccharides	<i>Lactococcus lactis</i>	Lactose	197000	[37]
<b>Polysaccharides</b>				
Heparosan	<i>E. coli</i>	Glycerol	1880	[46]
Hyaluronan	<i>Streptomyces albulus</i>	Sucrose	6200	[42]
Scleroglucan	<i>Sclerotium rolfsii</i>	Uridine Mono-phosphate	22320	[63]
Chondroitin	<i>E. coli</i>	Glucose	2400	[45**]
<b>Poly amino acids</b>				
Poly-ε-L-lysine	<i>Streptomyces</i> sp. M-Z18	Glucose and glycerol	35140	[64]
Poly-γ-glutamic acid	<i>Bacillus subtilis</i> BL53	L-glutamic acid and glycerol	17000	[65]
Cyanophycin	<i>E. coli</i>	Yeast extract	120 mg/g	[48]

properties [3,7]. Terpenoids are isopentenyl pyrophosphate (IPP) or dimethylallyl pyrophosphate (DMAPP) derived compounds of diverse carbon skeletons, including monoterpenes ( $C_{10}$ , e.g. menthol), sesquiterpenes ( $C_{15}$ , e.g. artemisinin), diterpenes ( $C_{20}$ , e.g. paclitaxel), triterpenes ( $C_{30}$ , e.g. steroids), tetraterpenes ( $C_{40}$ , carotenoids) and polyterpenes [5]. Microbial production of terpenoids

such as taxadiene (intermediate of anticancer drug paclitaxel) and artemisinic acid (precursor of antimalarial drug artemisinin) exemplified the advances and successes of metabolic engineering for production of value-added terpenoid pharmaceuticals. In nutraceutical industries, tetra-terpene carotenoids such as β-carotene, lutein, lycopene, α-carotene and astaxanthin serve as natural food colorants

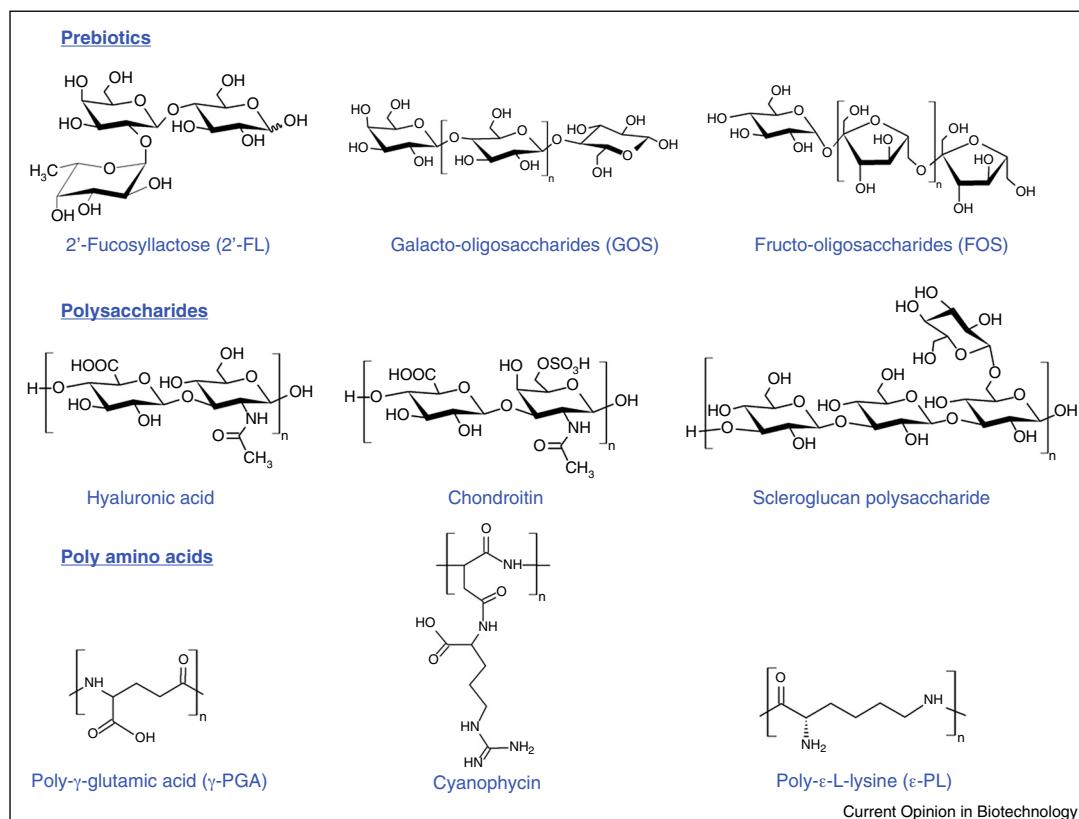
and feed supplements [5]. Combinatorial carotenoid biosynthesis in heterologous non-carotenogenic hosts like *E. coli* and *S. cerevisiae* has been achieved for decades. Since then, metabolic engineering efforts have mainly focused on large-scale production of carotenoids. Strain optimization via systematic and combinatorial gene knock-outs significantly increased lycopene production in *E. coli* [6]. Engineering central metabolic modules of *E. coli* to increase ATP and NADPH supplies improved  $\beta$ -carotene production to 2.1 g/L  $\beta$ -carotene with a yield of 60 mg/g DCW [27]. Lycopene derived zeaxanthin was biosynthesized and its production improved to 43.46 mg/L via protein fusion approaches for coordinating expression of lycopene  $\beta$ -cyclase gene *crtY* and  $\beta$ -carotene 3-hydroxylase gene *crtZ* [28]. A plasmid-free *E. coli* strain with the xanthophyll biosynthetic genes chromosomally integrated produced astaxanthin with a yield of 1.4 mg/g DCW [29]. Besides conventional carotenoids, discovery and production of new carotenoids with health-promoting benefits have also increased the pool of available carotenoids. Novel carotenoids such as 4-ketozeinoxanthin and the rare C<sub>50</sub> carotenoids sarcinaxanthin, decaprenoxanthin and sarprenoxanthin have been produced in lycopene-producing *E. coli* hosts [30,31]. Recently, a non-natural purple carotenoid C<sub>50</sub>-astaxanthin was produced in *E. coli* using

mutants of the first two pathway enzymes, farnesyl di-phosphate synthase (FDS) and carotenoid synthase (CrtM), with shifted size-range substrate selectivities to enhance production of C<sub>50</sub> backbone while suppress non-target compounds [32<sup>\*\*</sup>].

## Prebiotics

A prebiotic is defined as 'a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota' by The Food and Agriculture Organization of the United Nations (FAO) [33]. Prebiotics are non-digestible saccharide polymers that contain 3–10 monomeric sugar units (Figure 2). The beneficial effects of prebiotics on humans lie in their modulation on diversity and metabolic activity of the gut microbiota, which may consequently have significant impact on the host immune system. Thus, prebiotics could be used to treat inflammatory diseases by boosting probiotics like *Bifidobacteria* or *Lactobacillus* species within the gut microbiota [34]. Representative prebiotics include inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS). Inulin and inulin-type fructans, also known as soluble dietary fibers, could be produced by probiotic *Lactobacillus gasseri* strains [35]. GOS are lactose-derived galactose polymers with shorter

Figure 2



Representatives of microbial-produced prebiotics, polysaccharides and poly amino acids.

polymer chains. GOS could be produced by *Kluyveromyces lactis* with a titer of 177 g/L from 400 g/L lactose [36]. Expression of codon-optimized  $\beta$ -galactosidase from the hyperthermophile *Sulfolobus solfataricus* in *Lactococcus lactis* efficiently converted lactose to GOS [37]. 2'-Fucosyllactose (2'-FL) is one of the most abundant oligosaccharides in human milk and has been approved as nutritional additive in term infant and toddler formulas. By increasing the availability GDP-L-fucose and over-expression of fucosyltransferase, 2'-FL could be largely produced from lactose and glycerol in *E. coli* [38,39\*].

### Polysaccharides

Polysaccharides are sugar polymers of versatile structures that can be largely produced by several bacteria, yeast and fungi or extracted from plant and animal tissues (Figure 2). Microbial polysaccharides have been recognized as a source for nutraceuticals owing to their health-beneficial properties. Bacterial polysaccharides such as xanthan, gellan, dextrans and alginate can be commercialized via microbial production and purification [40]. Exopolysaccharides (EPS) are preferentially produced via metabolic engineering of *Lactococcus* and *Streptococcus* for their wide use in dairy products. Especially, bioactive fungal polysaccharides have demonstrated extensive immunostimulating, antitumor, antimicrobial, antioxidant, hypocholesterolaemic and hypoglycaemic benefits, thus indicating great potential in both nutraceutical and pharmaceutical applications [41]. Potential antitumor and anti-viral polysaccharide scleroglucan is an extracellular glucan excreted by mycelia of *Sclerotium rolfsii*, whose yields could be enhanced via addition of L-lysine and uridine monophosphate (UMP) [41]. Instead of extraction from animal tissues, several animal polysaccharides like hyaluronic acid (HA), chondroitin and heparosan have also been produced by microbial hosts. Microbial production of hyaluronic acid has been achieved in various hosts including *E. coli*, *Lactococcus lactis* and *Streptomyces albulus* [42–44]. Medicinally important polysaccharides such as heparosan and chondroitin can be produced by engineered *E. coli* reaching a relatively high titer of 1.88 g/L and 2.4 g/L, respectively (Table 1) [45\*\*,46].

### Poly amino acids

Poly amino acids are synthesized in microorganisms from one or two type of amino acids by ribosome-independent enzymatic processes that distinguish them from protein synthesis. Three kinds of poly amino acids, namely poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA), poly- $\epsilon$ -L-lysine ( $\epsilon$ -PL) and multi-L-arginyl-poly(L-aspartic acid) (cyanophycin), are present in nature (Figure 2).  $\gamma$ -PGA is a water-soluble and biodegradable polymer that can be used as a degradable biomaterial such as drug carriers or hydrogels.  $\gamma$ -PGA can be largely produced (20–50 g/L) by *Bacillus* species when feeding L-glutamic acid. In order to achieve  $\gamma$ -PGA production from glucose, optimization of co-cultivation of *Corynebacterium glutamicum*

and *Bacillus subtilis* could maximally produce 32.8 g/L  $\gamma$ -PGA [47]. Cyanophycin was produced from equimolar amounts of arginine and aspartic acid by cyanobacteria and some other chemotrophic bacteria (e.g. *Acinetobacter calcoaceticus*), which can be used as a precursor of dipeptides for nutritional and therapeutic applications. Over-expression of cyanophycin synthetase *cphA* from *Synechocystis* sp. PCC 6803 in *E. coli* achieved cyanophycin production with productivity of 120 mg/g CDW [48].  $\epsilon$ -PL is a microbial-derived homo-poly amino acid that is synthesized by polymerization of lysine via  $\epsilon$ -PL synthetase (PLS).  $\epsilon$ -PL possesses antibacterial and anticancer activities and has been approved as food preservative or dietary agent in Japan and United States [49].  $\epsilon$ -PL was produced by *Streptomyces* species such as *Streptomyces albulus*. Production of  $\epsilon$ -PL in *Streptomyces* sp. M-Z18 could reach 35.14 g/L when using glucose and glycerol as carbon sources [64].

### Conclusions

The last few decades have witnessed the remarkable progresses of nutraceuticals production via metabolic engineering of microbial-based platforms, which is in response to the great demands for value-added nutraceuticals to fortify humans against diseases. Metabolic engineering in microbes made possible not only the laboratory-scale preparation but also industrial-scale production of complex natural products such as carotenoids from simple carbon sources. Especially, with the elucidation of biosynthetic pathways and development of synthetic biology strategies, novel or more complicated value-added compounds could be produced in microbes in a naturally developed manner. The amenability of genetic manipulation and tolerance of heterologous enzymes enabled microbes to produce diverse nutraceuticals via harnessing their native metabolic networks. One of the major limitations of microbial platform is the efficiency of plant or animal-derived enzymes that limit the productivity of the target compounds. While other non-microbial production platforms such as algae and plant-derived cell cultures have also been developed to produce natural or non-natural value-added nutraceuticals, there is little doubt that continuing advances in metabolic engineering and synthetic biology will allow the production of virtually any nutraceutical through a microbial host.

### Competing interests

The authors declare no competing interests.

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Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

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