Microbial production of bioactive chemicals for human health
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Bioactive compounds are a collection of compounds that have certain effects on humans and animals that consume them. Since many bioactive compounds are beneficial to human health, they are attracting increasing attention in modern life, and their rising consumption stimulates the development of novel production modes that are more efficient than the traditional way, which relies on chemical synthesis or extraction from natural tissues. Among the emerging production techniques, biotechnology-based generation using fermentation of genetically engineered microorganisms shows great potential as an alternative to the current manufacturing systems, and has actually been applied for the industrial supply of some bioactive compounds. In this review, we highlight the microbial production of some typical bioactive compounds, including polyphenols, polysaccharides, amino acids, and vitamins. Also, we discuss the underlying disadvantages of some microbial systems and point out the future directions for system optimization.

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Current Opinion in Food Science 2020, 32:9–16
This review comes from a themed issue on Functional foods and nutrition
Edited by Andreas Schieber

Introduction
Bioactive compounds, such as polyphenols, polysaccharides, amino acids, and vitamins, are a wide range of naturally present chemicals that can modulate metabolic processes of human bodies and result in the promotion of better health [1*]. Bioactive compounds are generally found in fruits, vegetables, and whole grains, as well as in other natural sources, and have shown some beneficial effects, including antiaging, prevention of cardiovascular diseases, and protection against chronic diseases such as diabetes, mellitus, cancer, and neurodegenerative diseases [3,4]. Because of these roles on human health, there is a growing demand on the consumption of bioactive compounds in daily life, which are incorporated in various nutraceuticals, beverages, and foods.

Traditionally, the industrial supply of bioactive compounds relies on extraction from natural tissues such as fruits, vegetables, cereals, and so on [5,6], which has some limitations, including seasonal and regional fluctuations in chemical content in plants, and the difficulty in quality control of agricultural products [7]. Thus, stable and sustainable production using modern biotechnological methods is necessary for the industrial generation of bioactive compounds.

Biotechnological production of bioactive compounds has been extensively studied since the development of cell culture technology, metabolic engineering, systems biology, and synthetic biology [8]. This method generally uses genetically modified organisms or tissues instead of wild-type organisms to produce target compounds in fermenters with tightly controlled process conditions. Among these production hosts, microbes have been attracting increasing attention owing to their distinguished properties compared with other organisms or tissues, such as fast growth, easy cultivation, and facile genetic modifications [1*,9]. For example, in food industry, biotechnological production of monosodium glutamate using engineered Corynebacterium glutamicum has been conducted for several decades and can meet the needs of the world market [10]. In this review, we will highlight the recent progress on microbial generation of four classes of typical bioactive compounds, which include polyphenols, polysaccharides, amino acids, and vitamins, and will point out some critical issues in future studies.

Microbial production of polyphenols
Polyphenols, with flavonoids, stilbenes and curcuminoids being the typical representatives (Figure 1), are a large class of compounds with plant origin containing one or more phenolic hydroxyl groups, which are derived from the aromatic amino acids phenylalanine or tyrosine [11]. They have significant antioxidant and anti-inflammatory activities, and can help to prevent heart disease and cancer. Thus, polyphenols and polyphenol-containing
materials are widely used as nutraceuticals, food additives, and potential drug candidates. The sustainable bioproduction of polyphenols mainly utilizes engineered recombinant microorganisms, among which the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae* are most commonly used because of their fast adaptation to fermentation conditions and the availability of well-defined genetic manipulation techniques [1,11]. In recent years, biogeneration of polyphenols with complicated structures has been made possible in microbes with the discovery and elucidation of various complex biosynthetic pathways as well as the development of novel metabolic engineering tools (Table 1).

To increase the biotransformation efficiency of recombinant microorganisms, many metabolic engineering strategies have been implemented, including selection of suitable gene orthologs, optimization of gene expression and enzyme folding, engineering of promoters and transporters, regulation or redistribution of metabolic flux, removal of regulatory restrictions, enzyme colocalization or compartmentalization, and pathway balancing, and so on. [9,12]. These combinatorial metabolic engineering approaches have greatly increased the production titers of polyphenols in recombinant strains, and in some cases the titers can reach g/L level, moving such processes closer to industrial realization.

Recombinant production of polyphenols generally relies on the feeding of phenylpropanoic acids as substrates [13,14]. To decrease the overall cost of microbial production, attempts to use glucose as a cheaper substrate have been reported. One way to achieve this is to direct the metabolic flux of phenylalanine or tyrosine toward the target polyphenol compounds while enhancing the intracellular phenylalanine/tyrosine pathway [15,16]. Another way is to obtain de novo production via the coculture of multiple strains each one of which expresses one part of the whole pathway, leading to the complete biosynthesis of the target polyphenolic compound [17]. In a coculture containing four *E. coli* strains, the entire anthocyanin pathway from tyrosine was split into

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**Figure 1**

Major groups of polyphenols as bioactive compounds, including flavonoids, stilbenes, and curcuminoïds.

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four modules, employing an efficient tyrosine-producing strain as the first strain, an efficient flavanone producer as the second strain, a strain that produces almost 1 g/L of flavan-3-ols from flavanone precursors as a third strain, and finally an anthocyanin producing strain from flavan-3-ols [17]. This artificial microbial community gave rise to the direct production of ~10 mg/L pelargonidin 3-O-glucoside from glucose, which is impossible by a monoculture. Although the de novo production of some polyphenols has been achieved, the production titers and the productivity are very low.

Fermentative production of polyphenols such as anthocyanins using engineered plant cell lines has been achieved successfully (Figure 1) [18]. Since the whole pathways are present in plant cells, the engineering focuses on enhancing the accumulation of target molecules, reducing the generation of byproducts, and improving the growth of suspension cells. Modern fermentation technologies enable precise and tight control of process conditions, product accumulation, and cell growth, providing an evolved way of acquiring polyphenols for industrial needs and avoiding the concerns on biosafety and social recognition.

**Microbial production of polysaccharides**
Microbial polysaccharides are sugar-based biopolymers naturally produced by microbes for biological functions such as intracellular storage of energy, support of cellular structure, maintenance of cell metabolism within biofilms, intercellular communication, surface adherence, host defense, and so on [19]. Of all these microbial polysaccharides, the most industrially relevant class is the extracellular polysaccharides (EPSs) due to their secretion into the extracellular environment and ease of separation from the microbial cells [20]. The EPSs are mostly synthesized by bacteria and fungi, and have found applications in industry as food additives, medicine supplements, thickeners, and so on. Their industrial production largely relies on fermentation of the engineered strains (selected mutants of the wild-type producing strains) under defined conditions followed by purification of the products.

Among all the industrially used EPSs, xanthan (also known as xanthan gum) is the mostly studied, and is one of the few microbial EPSs that are approved worldwide for use in food products without any quantity limitation [21]. Xanthan is a viscos polysaccharide that is used widely as food additive and stabilizer of toothpastes, drugs, fertilizers, ink, and so on. Xanthan structurally consists of β-1,4-linked glucose with a mannose-glucuronic acid-mannose trimer branching off the C3 hydroxyl group of every other glucose residue, and the mannose residue can be modified with acetate or pyruvate. The biosynthesis of xanthan occurs in *Xanthomonas*

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**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Strain</th>
<th>Substrate</th>
<th>Titer</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curcuminoid</td>
<td><em>E. coli</em> coculture</td>
<td>Glucose</td>
<td>13.8 mg/L</td>
<td>[52]</td>
</tr>
<tr>
<td>Cyanidin 3-O-glucoside</td>
<td><em>E. coli</em></td>
<td>Catechin</td>
<td>439 mg/L</td>
<td>[53]</td>
</tr>
<tr>
<td>Calistephin</td>
<td><em>E. coli</em> polyculture</td>
<td>Glucose</td>
<td>10 mg/L</td>
<td>[17]</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td><em>E. coli</em></td>
<td>Tyrosine</td>
<td>107 mg/L</td>
<td>[54]</td>
</tr>
<tr>
<td>Naringenin</td>
<td><em>S. cerevisiae</em></td>
<td>Glucose</td>
<td>54 mg/L</td>
<td>[55]</td>
</tr>
<tr>
<td>Cyanidin 3-O-glucoside</td>
<td><em>S. cerevisiae</em></td>
<td>Glucose</td>
<td>1.55 mg/L</td>
<td>[51]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td><em>E. coli</em></td>
<td>Coumaric acid</td>
<td>2.3 g/L</td>
<td>[13]</td>
</tr>
<tr>
<td>Pterostilbene</td>
<td><em>C. glutamicum</em></td>
<td>Coumaric acid</td>
<td>42 mg/L</td>
<td>[56]</td>
</tr>
<tr>
<td>Quercetin</td>
<td><em>C. glutamicum</em></td>
<td>Caffeic acid</td>
<td>10 mg/L</td>
<td>[56]</td>
</tr>
<tr>
<td>Amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glutamate</td>
<td><em>C. glutamicum</em></td>
<td>Glucose</td>
<td>37</td>
<td>[57]</td>
</tr>
<tr>
<td>L-Lysine</td>
<td><em>C. glutamicum</em></td>
<td>Glucose</td>
<td>120</td>
<td>[58**]</td>
</tr>
<tr>
<td>L-Threonine</td>
<td><em>E. coli</em></td>
<td>Glucose</td>
<td>82</td>
<td>[59]</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td><em>E. coli</em></td>
<td>Glucose</td>
<td>61.3</td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td><em>Pseudomonas denitrificans</em></td>
<td>Sucrose</td>
<td>214 mg/L</td>
<td>[45]</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td><em>B. subtilis</em></td>
<td>Molasses, starch</td>
<td>&gt; 26 g/L</td>
<td>[60]</td>
</tr>
<tr>
<td>Vitamin C</td>
<td><em>A. gossypii</em></td>
<td>Soy flour</td>
<td>&gt; 20 g/L</td>
<td>[46]</td>
</tr>
<tr>
<td>Gluconobacter oxydans</td>
<td><em>Ketogulonicigenium vulgare</em>, <em>Bacillus endophyticus</em></td>
<td>Glucose</td>
<td>112 g/L</td>
<td>[51]</td>
</tr>
<tr>
<td>Pro-vitamin A</td>
<td><em>Blakeslea trispora</em></td>
<td>Glucose</td>
<td>0.95 mg/L</td>
<td>[47]</td>
</tr>
<tr>
<td>Pro-vitamin A</td>
<td><em>E. coli</em></td>
<td>Glucose</td>
<td>3.2 g/L</td>
<td>[49]</td>
</tr>
<tr>
<td>Pro-vitamin A</td>
<td><em>S. cerevisiae</em></td>
<td>Glucose</td>
<td>5.9 mg/g (dry weight)</td>
<td>[48]</td>
</tr>
</tbody>
</table>
*campestris*, a plant pathogenic bacterium, where the pentasaccharide monomer is synthesized on the cell membrane and transferred to the periplasm for polymerization, followed by secretion into the medium [21,22]. This process is catalyzed and regulated by 12 enzymes, the genes of which are clustered together in the *gum* operon in the bacterial genome; however, the detailed mechanism of the whole process is still under exploration. The industrial production of xanthan relies on batch aerobic fermentation using glucose syrup or starch as the carbon source, and corn steep liquor or ammonia as the nitrogen source [23]. As the final product is highly viscous, the supply and transfer of oxygen is critically important, and a high stirring speed is necessary, which is energetically inefficient. Novel techniques aiming to reduce the viscosity in the fermenter, such as emulsion fermentation, although showing enhanced productivity, cannot reduce energy costs due to difficulties associated with product recovery. Other techniques, such as bubble column reactors, foam reactors, jet reactors, and so on, can only marginally increase the productivity and reduce the overall cost.

Sphingans are a class of structurally similar polysaccharides produced by the *Sphingomonas* species, and four members of this class have industrial applications, that is, gellan, welan, sanxan, and diutan [24]. The repeating unit of these polymers consists of tetrasaccharides of rhamnose, glucose, glucuronic acid and glucose (diutan contains mannose instead of rhamnose) with or without saccharide branches or acetate/glycerol decorations. Their biosynthesis is similar to that of xanthan, with all the relevant genes organized in a sphingan operon in the bacterial genome [24]. The industrial fermentation utilizes glucose syrup or liquefied starch as the carbon source, and organic or inorganic nitrogen as the nitrogen source. After fermentation, the culture is traditionally heated and filtered to remove the host cells, and the produced polysaccharides are precipitated. To simplify this procedure and to improve the yield, the producing hosts are engineered such that they can be lysed by lysozyme, the proteins be degraded by proteases, and the cellular components be removed by isopropanol/water washes, giving rise to purified sphingans, which can be used as gelling agents in the food industry, concrete additives in the construction industry, and so on.

Hyaluronic acid, famous for its water-binding capacity and its applications in cosmetics, skin care products, and eye surgery, is a heteropolysaccharide with an ever-increasing world market [25]. It consists of alternating N-acetylmannosamine and glucuronic acid with β-1,4 linkages. Hyaluronic acid is naturally produced by *Streptococcus* species such as *S. pyogenes* [26], while the industrial production utilizes safe bacterial strains such as *C. glutamicum* that contain recombinantly expressed hyaluronate synthase, which forms a glycosidic bond between UDP-N-acetylglucosamine and UDP-glucuronic acid and releases the UDP moiety [27,28]. Hyaluronic acid is sensitive to both acidic and alkaline pHs, making its purification difficult and expensive. In industry, a combinatorial method of ultrafiltration, diafiltration and microfiltration is mainly adopted for its purification and concentration [28,29].

Pullulan is an α-homopolysaccharide produced by the mold *Aurobasidium pullulans*, which forms a dark pigment and is hard to be separated from pullulan [30]. The industrial fermentation uses an engineered strain of *A. pullulans* without pigment formation, and the purified pullulan is mainly used as an oxygen barrier and a carrier of flavor in food, and as a drug carrier in the pharmaceutical industry [30].

Another important class of polysaccharides is the sulfated glycosaminoglycans that include four major types of polysaccharides: heparin and heparosan, chondroitin sulfates, dermatan sulfate, and keratan sulfate. These sulfated polysaccharides have been synthesized microbially (in engineered *E. coli* or *Bacillus subtilis*), enzymatically, or chemoenzymatically [31–33]. Extensive reviews for biotechnological production of these polysaccharides have recently been published [34,35].

**Microbial production of amino acids**

Amino acids are basic building blocks of proteins and are important for human health. Humans and animals cannot synthesize all the basic amino acids and therefore need to obtain essential amino acids externally. In total, eight amino acids are required for humans, including leucine, isoleucine, valine, threonine, lysine, methionine, phenylalanine, and tryptophan. Although they can be obtained via protein-rich food, sometimes these amino acids have to be supplemented directly for people who are weak or have problems associated with protein digestion. Moreover, amino acids are widely used in food, chemical, and animal feed industries. For example, monosodium glutamate is used as the flavor enhancer umami; valine and glycine are used to synthesize pesticides and the herbicide glyphosate; phenylalanine and aspartate are used to synthesize the sweetener aspartame [36–39]. Owing to the rising consumption of meat, amino acids are required to feed animals for faster and better growth.

Amino acids, except glycline, methionine, and aspartate, are produced microbially in industry, and the most commonly used production strains are *C. glutamicum* and *E. coli* (Table 1). *C. glutamicum* is resistant to phage infections and is a Generally Regarded As Safe (GRAS) microorganism while *E. coli* is not [40]. In contrast, *E. coli* has an advantage of a higher fermentation temperature than *C. glutamicum*, requiring lower costs for environment cooling. To find out the best strains for industrial use, cells are conventionally subjected to random mutagenesis such as UV irradiation, followed by large-scale screening.
which is labor-intensive and has low success rates [41]. With the rapid development of metabolic engineering, systems biology, and synthetic biology, the toolbox in developing amino acid-producing industrial strains is greatly expanded [42**]. By comparing the global information of the wild-type strains and the mutants, including gene expression, enzyme activity, metabolic fluxes, and cellular metabolism, potential targets can be identified rapidly, thus accelerating strain development. In addition, the emerging tools for gene manipulation, such as CRISPR-Cas systems, can rapidly incorporate various modifications into the genome, further facilitating strain improvement [43]. Through these approaches, commercially viable strains have been developed for the production of glutamate, lysine, threonine, phenylalanine, and so on, with glutamate and lysine having the largest annual production by E. coli and C. glutamicum (Table 1).

**Microbial production of vitamins**

Vitamins are organic compounds that perform specific biological roles for the maintenance of normal metabolism in humans. These compounds cannot be synthesized by our bodies and have to be supplied in small amounts in the diet.

In industry, many vitamins are now produced by microorganisms, including cobalamin (vitamin B12), riboflavin (vitamin B2), ascorbic acid (vitamin C), and β-carotene (pro-vitamin A) (Figure 2). Some other vitamins are commercially produced by chemical synthesis since their structures are simple or the overall costs are low via organic synthesis [44**]. The microbial production titers of vitamins vary greatly, depending on the production strains, the biosynthetic pathway capacity, and the fermentation processes (Table 1).

At present, the industrial cobalamin production uses *Pseudomonas denitrificans* with a production titer of ~200 mg/L; in contrast, the chemical synthesis of cobalamin consists of ~70 reactions, making it difficult for industrialization [45]. Riboflavin is commercially produced using *Ashbya gossypii* or *B. subtilis* with production titers of >20 g/L [46]. Most β-carotene is industrially synthesized by *Blakeslea trispora* (a filamentous fungus). Other microbial production hosts include genetically engineered *E. coli* and *S. cerevisiae* with titers reaching >2 g/L [47–49]. Vitamin C fermentation relies on a consortium of *Ketogulonicigenium vulgare* and *Bacillus endophyticus*, in which *B. endophyticus* supports the growth and production efficiency of *K. vulgare*, and *K. vulgare* is responsible for the biosynthesis of 2-keto-L-gulonic acid from sorbose, which as a precursor is produced by *Gluconobacter oxydans* from sorbitol in a single step biotransformation [50]. A titer of 100 g/L can be reached for

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**Figure 2**

Structures of some typical vitamins produced by engineered microbes, including vitamin B12, B2, vitamin C, and pro-vitamin A (β-carotene). R = 5′-deoxyadenosyl, Me, OH, CN.
2-keto-L-gulonic acid, which is then chemically transformed to vitamin C [51].

**Conclusions**

Bioactive compounds are crucial to human health and represent an increasing market. The traditional way of producing these compounds through chemical synthesis or extraction from natural tissues cannot meet the needs sustainably or environmentally friendly in many cases. Microbial production of polyphenols, polysaccharides, amino acids and vitamins has made great progress, demonstrating the feasibility and significance of microbial production methods in producing bioactive compounds. However, the current technology is still confronted with relatively high energy input and is not waste-free. The future study can focus on more efficient downstream processes, such as recycling of the gas, steam, and water wastes for the generation of heat and other types of energy, and concomitant reduction of waste production and the development of green processes. Additionally, recombinant microbial production systems often encounter problems associated with the expression of heterologous genes/enzymes, such as low expression levels, insolubility of the expressed enzymes, enzyme instability, and so on. However, the emerging genetic manipulation tools and metabolic engineering strategies, and the increasing understanding of physiology and metabolism of microbial hosts will one day enable efficient recombinant production of more bioactive compounds.

From the perspectives of novel products, with the increasing concerns associated with pathogen contamination and environmental pollution in animal husbandry, and with the increasing demand of artificial meat, the microbial production technologies can be utilized to make high-value plant proteins or animal proteins as dietary supplements for humans.

**Conflict of interest statement**

Nothing declared.

**References and recommended reading**

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

- of special interest
- of outstanding interest


This review summarizes the recent achievements in microbial production of polyphenol compounds.


4. de Jesus Raposo MF, de Morais RMSC, de Morais AMMB: Health applications of bioactive compounds from marine microalgae. Life Sci 2013, 93:479-486.


This work describes engineering of E. coli to produce the polyphenol compound resveratrol with a very high yield.


This report demonstrates de novo production of anthocyanins in yeast through extensive metabolic engineering of native pathways and introduced anthocyanin biosynthetic pathway.


This study shows overproduction of hyaluronic acids by engineered C. glutamicum.


This review examines progress towards the preparation of glycosaminoglycan through chemical synthesis, chemoenzymatic synthesis and metabolic engineering.


This review covers major engineering strategies and systems biology technologies to improve production ability of E. coli and C. glutamicum. Also, this review shows comprehensive metabolic engineering for overproduction of glutamate, lysine, threonine, and tryptophan.


This review describes the microbial production of major B vitamins, such as metabolic engineering strategies, current challenges, and future efforts.


