Application of Microbial Cell Factories

Subjects: Biotechnology & Applied Microbiology

Contributor: Rida Chaudhary, Ali Nawaz, Mireille Fouillaud, Laurent Dufossé, Ikram ul Haq, Hamid Mukhtar

Microbial cell factories are becoming a fundamental technology for pharmaceutical, food, and chemical industries to satisfy the welfare of an increasing global population and socio-economic development. Microorganisms are used for the production of various products, including carboxylic acids, amino acids, vitamins, enzymes, plant natural products, carotenoids, biogas, and other biofuels. About 52% of FDA-approved chemical entities were naturally derived products during the period of 1981–2006. The production of varied value-added macromolecules and metabolites was witnessed in the last decade by microbial cell factories (MCFs), with titers changing from μg/L to mg/L. Moreover, the introduction of metabolic engineering approaches improved the rate, titer, and yield of industrially vital compounds by manipulating the host metabolism, physiology, stress response, carbon–energy balance, and the annihilation of an undesirable ATP sink. Due to MCFs, the industrial biotechnology sector is increasing expeditiously, and numerous biocommodities are also in production.

Keywords: microbial cell factories ; biodiversity ; designing ; pathway construction ; robustness ; systems metabolic engineering

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1. Carboxylic Acids

Carboxylic acids are considered as appealing bio-renewable chemicals due to their flexibility and utilization as precursors for varied industrial chemicals. Currently, conventional chemical processes are used to achieve carboxylic acid synthesis from petroleum resources. However, an excessive reliance on petrochemical-based raw materials and the requirement of toxic metals, including organic solvents and heavy metal catalysts, have questioned the proficiency of chemical processes in terms of exacerbating the environmental burden. As a result of these concerns, deriving carboxylic acids from renewable microbial sources becomes apparent [1]. Carboxylic acids can be produced by several microbial groups, for instance, acetic acid production from *A. cerevisiae*, *A. aceti*, *Gluconacetobacter liquefaciens*, *G. entanii*, *Komagataeibacter intermedius*, and *K. hansenii* [2], and the synthesis of succinic acid from *Anaerobiospirillum succiniciproducens*, *M. succiniciproducens*, and *Actinobacillus succinogenes* [3]. Acetic acid bacteria (AAB) play a major role in the production of food and beverages, such as kombucha and vinegar [4]. Butyric acid also has vast applications in animal feed, food additives, perfumes, and varnishes. In nature, several *Clostridium* species synthesize butyric acid, particularly, *C. thermobutyricum*, *C. butyricum*, *C. pasteurianum*., and *C. acetobutylicum* [5].

With the advancements in metabolic engineering, the production of carboxylic acids from metabolically engineered microbial systems has gained much attention to achieve the desired yield and titer. Several non-conventionally engineered microbes have been demonstrated as superior hosts for carboxylic acid biosynthesis. For instance, the enhanced production of propionic acid, which is widely used as a food additive, antimicrobial preservative, and an intermediate for producing polymers, pesticides, and flavorings, can be achieved by engineering *P. acidipropionici* sp. CGMCC 1.2232 [6], *P. freudenreichii* subsp*. Shermanii* DSM 4902 [7], and *P. jensenii* sp. ATCC 4868 [8]. The FDA has designated several *Propionibacterium* species (derived from dairy goods) as generally regarded as safe (GRAS), electing them as a suitable approach for producing propionic acid in the food industry [9]. Lactic acid (2-hydroxypropanoic acid) serves as an additive in the food, pharmaceutical, and textile industries. Moreover, it is also used as a synthetic intermediate to manufacture biodegradable polymers [10]. Lactic acid bacteria (LAB), including *Lactobacilli* and *Lactococci* sp., are highly favored microbes for lactic acid biosynthesis and have achieved GRAS status as well [1]. Engineering microbial species of *C. utilis* [11] and *L. lactis* LM0230 [12] also increased lactic acid production. Citric acid is broadly used in the cosmetics, food, and detergent industries owing to its buffering characteristics, water solubility, and reduced toxicity. Presently, microbial fermentation using *Y. lipolytica* and *A. niger* is being carried out for citric acid production on an industrial scale [13]. *A. succinogens* also produce succinic acid naturally [14]; however, several microbial strains can also be metabolically engineered for enhanced production, such as engineering *L. plantarum* sp. NCIMB 8826 [15], *C. glutamicum*, [16], and *C. synechococcus elongates* sp. PCC 7942 [17], which provide higher succinic acid yields. Succinic acid serves as a precursor for preparing polyester, a surfactant, and several other valuable derivatives. Notably, succinic acid is among the few examples of biosynthesizing carboxylic acids by industrial manufacturers, for instance, Reverdia and BioAmber successfully commercialized succinic acid, accounting for half of the global annual production [18].

2. Vitamins

Vitamins are essential nutrients that play a crucial role in maintaining optimal health and metabolic activities. Vitamins show wide-ranging applications in the food, feed, cosmetics, and pharmaceutical industries. The requirement of pressurized reactors and high temperatures, along with the safety concerns regarding increased pollution from hazardous waste, have shifted the focus toward utilizing microbial sources instead of chemical synthesis methods for the production of valuable vitamins [19]. Several microbes serve as cell factories for this purpose, for instance, *E. coli* has emerged as a preferred approach for the production of vitamin B1 (also known as thiamine), which has vast applications in preventing skin inflammation and eczema [20]. Furthermore, the emergence of synthetic biology and metabolic engineering approaches has further strengthened the concept of utilizing microbial cell factories for the production of vitamins, such as the increased yields of vitamin B1 and vitamin A (β-carotene), which can be attained from *A. oryzae* and *Y. lipolytica* by overexpressing genes and heterologous enzymes [21][22], respectively. L-ascorbic acid (LAA), also known as vitamin C, can also be produced from several microbial species. Vitamin C is an antioxidant and also acts as a significant co-factor in multiple reactions that occur in the body. LAA is presently manufactured commercially by utilizing *B. megaterium* and *G. oxydans* [19]. *P. shermanii*, and *P. denitrificans* produce cobalamin (vitamin B12), while vitamin K can be attained from *Sinorhizobium meliloti*, *B. subtilis* [23][24][25][26]. Recently, MK-7, which is an effective subtype of vitamin K produced from *B. subtilis* natto, became an FDA certified-safe food [26]. From the perspectives of the absorption rate, biological activity, and safety concerns, the biological synthesis of vitamins by using microbial strains is a promising approach for achieving a significant yield improvement of vitamins.

3. Amino Acids

Amino acids serve as attractive metabolites in the food industry and pharmaceutical fields. They also play a role in enhancing flavor formation in several fermented foods. At present, *C. glutamicum*, *P. ananatis*, and *E. coli* are extensively utilized host bacterial strains for the industrial production of amino acids. Moreover, a glutamate secreting bacterial isolate, *C. glutamicum*, is a preferred bacterium to be exploited in engineering approaches for the increased production of lysine, glutamate, and flavor-active amino acids at vast scales [27][28]. Lysine and valine are two vital amino acids with varied applications. Lysine is an essential amino acid and is used as a feed additive, with a market size reaching around 1 million tons per annum [28], while valine, a branched amino acid, is an essential nutrient for animals. Valine is also utilized as a raw material for the production of various herbicides and drugs, with an annual fermentative production of 500 tons [29]. *E. coli* and *C. glutamicum* are considered as the most powerful industrial microbes for both lysine and valine production [30][31][32][33]. Considering the fact that industrial processes often operate at high temperatures, thermotolerant microbes have also been developed. For instance, thermotolerant bacteria, *C. efficiens* and *B. methanolicus*, are being developed as lysine producers [34]. Another semi-essential amino acid, L-Arginine (L-Arg), has widespread applications in the pharmaceutical industry as it promotes the secretion of insulin, growth hormones, and prolactin [27][35]. Several engineered microbes, including *B. subtilis*, *E. coli*, *C. glutamicum*, *S. cerevisiae*, and *C. crenatum*, have been used as model organisms for achieving L-Arg overproduction [36]. Moreover, L-ornithine has also been synthesized by a high-glutamate-producing strain, *C. glutamicum* S9114, through pathway engineering [37]. L-Ornithine is a non-protein amino acid and is universally used for the treatment of trauma and liver protection, in addition to strengthening the heart. High-level L-ornithine production is also achieved by carrying out modular pathway rewiring [38]. Constructing microbial workhorses on a large scale aims at satisfying the demand for amino acids as bulk biochemicals. Moreover, amino acid secretion from microbial sources is economically and industrially relevant to biotechnological fermentation processes, as it involves the simplified extraction and purification of metabolites.

4. Plant Natural Products

Plant natural products exhibit significant pharmacological activities and have widespread utilization in healthcare products, food additives, and cosmetics. Most PNPs are extracted from cultivated plants; however, the yield is restricted due to complex processing steps, a long growth cycle, climate change, and seasonal availability, making the process quite unsustainable. Moreover, a complex PNP structure also affects its chemical synthesis efficiency [39]. With the development of modern approaches, the biosynthesis of PNPs from microbial alternatives has gained considerable attention. Several microbial species have been utilized for PNP production, including artemisinin, resveratrol, and many carotenoids as well. Artemisinin is referred to as an effective pharmaceutical compound for treating malaria. It is naturally synthesized by plant *A. annua*; however, the concentration obtained from the plant source is very low, i.e., 0.01–1.1%, and improving the yield through total organic synthesis or plant breeding remains a contest, shifting the focus toward production via microbial sources [40]. Several microbial strains have been genetically engineered for its production. For meeting large-scale demands, the de novo reconstitution of artemisinin biosynthesis in heterologous microbes, including *S. cerevisiae* and *E. coli*, has noteworthy achievements, for instance, engineering the carbon flux and genotype of *S. cerevisiae* yields high quantities of artemisinic acid. Moreover, *S. cerevisiae* has also demonstrated itself as a heterologous host for producing artemisinin precursors, such as artemisinic acid, dihydro-artemisinic acid, and amorphadiene [41].

Resveratrol is a polyphenolic compound and is also found in various plant species, such as peanuts, grapes, cranberries, and bush berries. It also exhibits wide applications in the cosmetic, health, and medicine industries, and is reported to reduce the risks of cancer, heart diseases, and diabetes [42]. It is among the fastest-growing nutritional supplements in the flavonoid market [43]. Like artemisinin, the resveratrol concentration in plants is limited and is commercially unsustainable as well. Engineered strains of *E. coli* and budding yeast *S. cerevisiae* result in increased resveratrol titers by the introduction or modification of STS enzymes (stilbene synthase) [44][45]. Evolva has effectively developed the production of resveratrol on an industrial scale by utilizing a yeast cell factory [40]. The two strains serve as platform organisms and excellent hosts for the production of a wide array of PNPs, such as isoprenoids (l-histidine, l-phenylalanine, and l-tyrosine), phenylpropanoids (flavonoids, coumarins, and lignans), and alkaloids (carotenoids and sterols) [46]. Although the eco-friendly and resource-conserving synthesis of PNPs has gained much attention, several challenges are associated with this approach as well, regarding their large-scale application. Moreover, a low enzyme catalytic activity, poor precursor supply, and unknown PNP biosynthesis pathways have limited the heterologous production of microorganisms due to the high fermentative costs [40].

5. Carotenoids

Carotenoids are lipid-soluble, naturally occurring pigments and are widely used in industries owing to their antioxidant activities and capacity as natural colorants [47]. By the year 2025, the carotenoids global market is anticipated to reach USD 1.68 billion [48]. Photosynthetic cyanobacteria serve as natural microbial factories for the synthesis of carotenoid pigments as cellular antioxidants. Many successful demonstrations have also been reported for genetically engineering cyanobacteria to achieve an improved carotenoid content [49]. At the industrial scale, the filamentous fungi *Phycomyces blakesleeanus* and *Blakeslea trispora* serve as potential candidates for carotenoid production [50]. Moreover, other carotenogenic microbes, including *Haematococcus pluvialis* and *X. dendrorhous*, have also been widely exploited in large-scale processes. Moreover, transforming carotenoid genes in non-carotenogenic microbes, such as *C. utilis*, *E. coli*, *S. cerevisiae*, and *Zymomonas mobilis*, can elevate carotenoid production levels [51]. Several recombinant or engineered microbes also demonstrated the successful production of carotenoids, including recombinant *S. elongatus* sp. PCC 7942 [52], *Planococcus faecalis* [53], *Halobacillus halophilus* [54], *B. indicus*, and *B. firmus* [55].

Cyanobacteria produce a diverse range of metabolites in the carotenoid biosynthetic pathway, and zeaxanthin is among these principal carotenoids. Zeaxanthin is a naturally occurring xanthophyll carotenoid and is widely produced by algae, plants, and microorganisms. It is utilized in the food, nutraceutical, and pharmaceutical industries due to its antioxidant and anti-cancer properties. Moreover, it also plays a critical role in preventing cataracts and macular degeneration. Many bacterial isolates, predominantly belonging to the *Paracoccus* and *Flavobacterium* genera, demonstrate the active accumulation of zeaxanthin. The *Flavobacteriaceae* family primarily comprises zeaxanthin-producing bacteria, including *Kordia aquimaris* sp. CC-AMZ-301T 150 [56], *Aquibacter zeaxanthinifaciens* sp. CC-AMZ-304T 151 [57], *G. oceani* sp. CC-AMSZ-TT 152 [58], and *G. planctonica* sp. CC-AMWZ-3T 153 [59]. Also, non-photosynthetic *Flavobacterium* sp. produces an abundant amount of zeaxanthin, accounting for 95% of total carotenoid production [60].

Another carotenoid, astaxanthin, can also be produced from microbial workhorses. Astaxanthin is an ideal source of pigmentation in the food and aquaculture industries. It also exhibits several health benefits owing to its anti-inflammatory, anti-cancer, antioxidant, and neuroprotective activities [61]. Several microbial strains serve as cell factories for astaxanthin biosynthesis, such as *Brevundimonas* sp. [62], *Paracoccus* sp. [63], and *Sphingomonas* sp. [64]. Among yeast species, *X. dendrorhous* is a major astaxanthin producer [65]. Metabolic engineering has developed potential commercial interests in enhancing carotenoids' yield and titer to attain sustainable production. As an instance, *E. coli* and *S. cerevisiae* have also been exploited as cell factories for astaxanthin production by carrying out modular engineering and the membrane-fused expression of β-carotene hydroxylase [66][67][68]. Another xanthophyll pigment, i.e., lutein, can also be biosynthesized in *S. cerevisiae*. The lutein pigment is widely used in aquaculture, healthcare, food processing, and poultry farming industries [69]. The production of carotenoids by microbes provides an insight into the economically viable, environmentally friendly, and renewable production of natural products, depicting the industrial viability of MCFs for carotenoid biosynthesis.

6. Flavors and Fragrances

In addition to several valuable chemicals, flavor and aroma compounds are also synthesized by MCFs, which include indole, terpenoids, β-Ionone, geraniol, 3-phenylpropanol, vanillin, and patchoulol. The global flavor and fragrance market was valued at USD 28,193.1 million in 2019 and is anticipated to achieve USD 35,914.3 million by 2027 with a CAGR of 4.7% [70][71]. Moreover, the cosmetic industry has presented an even higher market value and is expected to reach USD 363.80 billion by 2030 [72]. Thus, the inclination toward utilizing natural and sustainable products drives the focus of ingredient and fragrance firms toward finding alternative sources to create natural products, i.e., microbial biosynthesis [73].

Indole is a nitrogen-containing aromatic compound and is famed for its jasmine-like floral odor [74]. *Corynebacterium* strains display the sustainable production of indole from tryptophan to achieve industrially pertinent indole titers [75]. Terpenoids are vital aroma components for perfumery and flavor products. β-Ionone, a fragrant terpenoid, possesses a pleasing floral scent. Microbial fermentation using GRAS species, such as *Y. lipolytica*, is an effective approach for β-Ionone production as it is oleaginous, economically viable, and a sustainable natural aroma compound [76]. Several monoterpenoids can be generated by using recombinant microbial strains, for instance, the metabolic engineering of *P. putida* to produce geranic acid. Geraniol is an acyclic monoterpene alcohol and it is a standard constituent of many fragrance and flavor products. *E. coli* engineering for expressing the heterologous mevalonate pathway can result in an increased titer of geraniol [77]. *E. coli* cells have also been engineered to produce 3-Phenylpropanol and vanillin. Both flavor compounds are used in the beverages, food, fragrance, and cosmetic industries [78][79]. Moreover, patchoulol is a naturally occurring sesquiterpene and is found naturally in *Pogostemon cablin*. It has a wide range of applications in cosmetics and daily use products, including shampoo, hair spray, perfumes, and essential oils. Its limited production from natural resources shifted the focus toward engineered microbes, such as mitochondria-engineered yeast cells (*S. cerevisise*), which reported the elevated production of patchoulol [80]. Thus, a consequential upsurge in aroma chemicals requires efficient renewable production strategies. For this purpose, biotechnological production from microbial sources is regarded as the best alternative to generate flavor and fragrant molecules [81].

7. Bioenergy

Increasing energy and food demands, the deterioration of the environment, and climate change are the major challenges of this era. Among several other natural sources, biofuels also have immense potential to harmonize the trilemma of food, energy, and environment. Using MCFs for the production of bioenergy is not a new concept, yet it has received great attention for creating novel bioproducts with better productivity rates [82]. Several microbes serve as bioenergy sources, such as the production of 1-butanol, which is a potential fuel and chemical feedstock, by both native and engineered *Clostridium* species [83], and bioethanol production from *Pichia stipites* sp., *C. albicans*, and *S. cerevisiae* sp. KL17 [84][85][86]. Apart from fuels, microbes also serve as factories for biogas and biohydrogen production. Methanogens are integral to biogas production and high-performance methanogens include *Methanocaldococcoccus jannaschii* and the methanococci *Methanotorris igneus* [87]. Methanogens can also be employed as autobiocatalysts for converting molecular hydrogen (H_2) and carbon dioxide (CO_2) to biological CO_2-based methane, also referred to as the CO_2-BMP process. This process has varied applications, comprising the decentralized production of energy, power-to-gas applications, and biogas upgrading [88]. The most studied microbe for this purpose is *Methanothermobacter marburgensis*. It has several advantages, including flexibility toward substrate gas impurities and elevated CH_4 productivity rates [89]. Biohydrogen is also an environmentally benign fuel, having no CO_2 emissions during combustion. It can be produced by the biological conversion processes of photofermentation and dark fermentation. Several microbes can be utilized for biohydrogen production, including *Caldicellulosiruptor saccharolyticus* [90], *Desulfurococcus amylolyticus* DSM 16532, and *Rhodobacter sphaeroides* [91]. Moreover, biodiesel, a derivative of fatty acids, such as methyl, ethyl, or propyl esters (fatty acid methyl esters (FAMEs), fatty acid ethyl esters (FAEEs), and fatty acid propyl esters (FAPEs)) has an annual consumption rate of 2 billion gallons. Several studies have reported the engineered *E. coli* strain as an effective microbial source for producing structurally tailored fatty esters (i.e., biodiesel) [92][93][94]

The metabolic engineering of microbes has led to the production of next-generation biofuels. The biofuel molecules with petroleum replica structures exhibit greater advantages than others as they possess elevated energy densities and can also be utilized as drop-in fuels because of no modification requirements in the internal combustion engines, destined for petro-diesel and gasoline [92]. These biofuels are referred to as fourth-generation (4G) or advanced biofuels. Few microorganisms naturally produce such biofuels, for example, the production of butanol from *B. subtilis*, *P. putida*, and *Clostridium species*. [93]. However, at times, these native producers face limitations in terms of their slow growth rates, low production titers, and the overall yield of the product. Moreover, it is also a challenge to engineer native microbes because of ineffective genetic elements, the unidentified endogenous regulation of synthetic pathways, redox potential, cellular toxicity, and the presence of inhibitors. Metabolic pathway engineering impels the need to reconstruct biofuel biosynthesis in genetically tractable microbes to carry out the heterologous synthesis of 4G biofuels [94]. The point mutation of the cydC gene in *E. coli* has the capacity to completely restore the production of D-limonene and biogasoline, along with the platform chemical isopentenol [95].

MCFs are attracting considerable attention as next-generation energy sources because of their intended use for recovering energy in the form of electricity. Microbial fuel cells convert solar or chemical energy to electrical energy by utilizing microbial cell factories as catalysts [96]. Several electricigens are used for MFCs' construction as pure cultures, including *Natrialba magadii*, *Haloferax volcanii*, *R. sphaeroides* [97], *Rhodospirillum rubrum* [98], and *Acidiphilium cryptum* [99]. However, pure cultures mandate the requirement of strict operating settings and selective substrates. Therefore, using miscellaneous consortiums as anodic biocatalysts is a preferred approach, as mixed communities are highly suitable for complex substrates as well. Several co-cultured electricigens reported high peak densities, including co-cultures of *Geobacter sulfurreducens* + *C. cellulolyticum* [100], *Klebsiella pneumonia* + *Lipomyces starkeyi* [101], and *P. aeruginosa* + *K. variicola* [102], demonstrating the potential of mixed cultures for effective electricity generation.

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