Microbial bioprocessing of health promoting food supplements

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5.1 Introduction

For centuries, human beings have utilized microorganism for their abilities to produce various bioactive compounds/metabolites of interest, such as food supplements or food ingredients. The food supplements include a vast array of products, i.e., vitamins, minerals, amino acids, polyunsaturated fatty acids (PUFAs), antioxidants (polyphenols, carotenoids), amino acids/peptides and their derivatives, etc., that are synthesized from microorganisms for providing health and nutrition to consumers. The data available from different sources like genomic, transcriptomic, proteomic, and metabolomic studies have provided ways to engineer microorganisms for development of food supplements of commercial importance. Synthetic biology/metabolic engineering envisages the use of genetic parts/tools [promoter, transcription factors, ribosomal binding sites (RBS), degradation tags, and transcriptional terminators] in microbial system for synthesis of biocommodities including food supplements. Microbial synthesis is an alternative to chemical methods for production of food supplements and uses organic feedstocks.

This chapter focuses on the classification and types of food supplements, various industrially important organisms involved, bioprocessing route, market trend, and regulatory issues.

5.2 Classification of food supplements

5.2.1 Food supplements, nutraceuticals, and food additives

A **food supplement** is a preparation that is intended to supply a nutrient that is missing from a diet. It can be vitamins, minerals, amino acids, fatty acids, and other substances. **Nutraceuticals** are supplements that contain a concentrated form of a substance that is

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not in food form but is derived from foods. For example, soy protein is a **food supplement**. However, ipriflavone is the synthetic derivative of isoflavone daidzein found in soy protein and is sold as a nutraceutical. On the other hand, **food additives** are substances that are added to food to maintain or improve the safety, freshness, taste, texture, or appearance of food. Some food additives have been in use for centuries for preservation—such as salt (in meats such as bacon or dried fish), sugar (in marmalade), or sulfur dioxide (in wine).

Food supplements can be categorized into two groups depending on their intended use as per the National Agency of Medicines: (a) Supplements as foodstuff; (b) Food for meticulous uses as a beverage, for different age group of people. Also, the supplements can be categorized as per the origin: supplements of natural or synthetic origin. Table 5.1 summarizes the classification of food supplements of microbial origin, and some examples of food supplements are described below.

Table 5.1 Classification and some examples of food supplements of microbial origin.

Food supplements	Microbial origin	References
Essential Fatty Acids		
[a]Omega-3 LC-PUFAs	*Aurantiochytrium* sp. strain TC 20	Chang et al. (2013)
Microbial lipids	*Rhodosporidium toruloides* DSM 4444	Tsakona et al. (2019)
Gamma Linolenic Acid (GLA)	*Cunninghamella echinulata* and *Mortierella isabellina*	Chatzifragkou et al. (2010)
Microbial lipids	*Mortierella alpina*	Diwan et al. (2018)
Vitamins		
Vitamin B1	*Bacillus subtilis*	Schyns et al. (2005)
Vitamin B1	*Aspergillus oryzae*	Tokui et al. (2011)
Vitamin B2	*Aphis gossypii*	Revuelta et al. (2018)
Vitamin B5	*Corynebacterium glutamicum*	Hüser et al. (2005)
Vitamin B6	*Escherichia coli*	Rosenberg et al. (2017)
Vitamin B12	*Pseudomonas denitrificans*	Li et al. (2008)
Amino acids		
Lysine	*Corynebacterium glutamicum*	Mitsuhashi (2014)
Glutamic acid	*Corynebacterium glutamicum*	Hirasawa and Shimizu (2016)
Essential minerals		
Zn, Ca, and P	*Microbial phytase*	Walk et al. (2013)
Probiotics		
Microflora	*Lactobacillus* genus	Argyri et al. (2013)

[a]Omega-3 LC-PUFAs: Omega-3-long chain polyunsaturated fatty acids.

5.2.1.1 Essential fatty acids

The essential fatty acids such as omega-3 fatty acid, alpha-linolenic acid (ALA), omega-6 fatty acid, and linoleic acid (LA) (Fig. 5.1.) are considered as nutritional supplements and are important for maitaining good health (Ji et al., 2015). The human body cannot synthesize these fatty acids on its own. Therefore, the essential fatty acids must be obtained from diet. For instance, fish oils are rich sources of fatty acids, e.g., docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which are widely used as nutritional supplements to the consumers (Ward and Singh, 2005; Finco et al., 2017). However, a limited number of plant oilseeds are good sources of other essential fatty acids (PUFAs). A promising alternative system for the production of omega-3 lipids is from microbial metabolism of yeast, fungi, or microalgae (protists and dinoflagellates). Marine protists and dinoflagellates such as species of *Crypthecodinium, Thraustochytrium,* and *Schizochytrium* are the rich sources of fatty acids (DHA), whereas microalgae like *Phaeodactylum* and *Monodus* are good sources of EPA (Ward and Singh, 2005). An isolated protists *Aurantiochytrium* sp. strain TC 20 was investigated using small-scale (2 L) bioreactors and found potential for the production of omega-3 long chain PUFAs (Chang et al., 2013). *Yarrowia lipolytica* is a model oleaginous yeast for the production of lipids-derived biofuels, biosynthesis of industrially important metabolites, and the essential fatty acids (Lazar et al., 2018). Chatzifragkou et al. (2010) reported the fungi *Cunninghamella echinulata* and *Mortierella isabellina*, capable of accumulating single cell oil containing γ-linolenic acid, and were cultivated on sugar-based media, at initial substrate concentration 60 g/L. Diwan et al. (2018) studied a nondetoxified rice straw hydrolysate to its application in lipid production from mold *Mortierella alpina*.

5.2.1.2 Vitamins

Vitamins are nutritional compound required in small quantities by the living organisms for growth, metabolism, and development (Ledesma-Amaro et al., 2013). Vitamins if

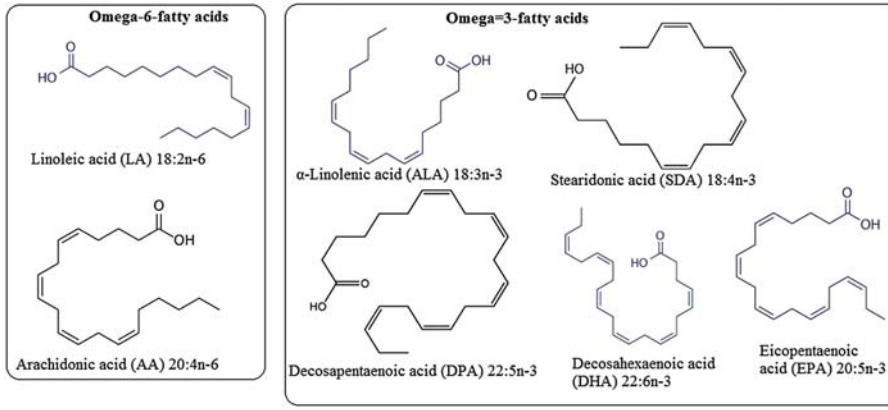

Figure 5.1 Chemical structures of some essential fatty acids.

not being synthesized in adequate amounts by the body should be acquired from food or food supplements. Human beings need 13 types of vitamins (classified as fat soluble (A, D, E, and K) and water soluble (C, B group) in the diet that are essential organic compounds (Acevedo-Rocha et al., 2019). The list of vitamins is as follows: K, C, E, D, A, Vitamin B12, Thiamine (B1), Niacin (B3), Pantothenic acid (B5), Riboflavin (B2), Biotin (B7), Vitamin B6, and Folate (B9). Vitamins such as vitamin E contain tocotrienols and tocopherols, and vitamin K includes both K1 and K2. Vitamin K1 is primarily found in leafy green vegetables, while K2 is most abundant in fermented foods and some animal products. Vitamin K2 may be absorbed better by the body, and some forms may stay in the blood longer than vitamin K1. These two things may cause K1 and K2 to have different effects on human health (Lyzak et al., 2017).

5.2.1.3 Amino acids

Proteins are chain of amino acids. Our body needs 20 different amino acids to grow and function properly. Though all 20 are important, only nine amino acids are classified as essentials. These are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valanine. Unlike nonessential amino acids, essential amino acids cannot be synthesized in our body and must be supplemented through protein-based diet. Certain nonessential amino acids are some time considered as conditionally essential such as arginine (when body is fighting certain disease like cancer). Protein-containing nutrients, either prepared-to-drink or as powder forms are sold as supports to patients suffering from sickness or trauma, and seeking to overcome the old-age sarcopenia (Colonetti et al., 2017), or for those who claim that heavy physical exercise raises amino acids requirements in the body (Stonehouse et al., 2016). Whey protein is a popular food supplement (Naclerio and Larumbe-Zabala, 2016; Colonetti et al., 2017).

5.2.1.4 Minerals

The body needs many minerals; these are called essential minerals. Just like vitamins, essential minerals help your body grow, develop, and stay healthy. The body uses minerals to perform many different functions—from building strong bones to transmitting nerve impulses. Some minerals are even used to make hormones or maintain a normal heartbeat. Essential minerals are sometimes divided up into major minerals (macrominerals) and trace minerals (microminerals). These two groups of minerals are equally important, but trace minerals are needed in smaller amounts than major minerals. A balanced diet usually provides all of the essential minerals. Potassium, sodium, chlorine, phosphorus, calcium, iron, magnesium, and sulfur (all macrominerals) and manganese, zinc, iodine, copper, molybdenum, chromium, cobalt, selenium, fluoride (all microminerals) are the essential minerals for humans. Potentially essential minerals are sold separately and in conjunction with vitamins and some other minerals, as food supplements.

5.2.1.5 Natural products

Nutritional supplements can be developed utilizing either complete materials or extracts from animals, vegetables, fungi, lichens, or algae among other examples as curcumin, *Ginkgo biloba*, cranberry, resveratrol, ginseng, collagen, and glucosamine (Prince, 2017).

5.2.1.6 Probiotics

Food and Agriculture Organization/World Health Organization defined probiotic as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Florou-Paneri et al., 2013). The dominated microflora considered as commercial probiotics are mainly of *Lactobacillus* genus with over 100 species recognized, including *Lactobacillus plantarum, Lb. acidophilus, Lb. rhamnosus, Lb. casei, Lb. bulgaricus, Lb. reuteri, Lb. helveticus,* and *Lb. delbrueckii*. However, *Lactobacillus* is generally recognized as safe (GRAS) organisms (Argyri et al., 2013). In the scientific literature, probiotic population of 10^6–10^7 CFU is established as therapeutic quantities in fermented foods (da Cruz et al., 2009; Behera et al., 2018a, b). While there are various alleged benefits to use probiotics, like preserving gastrointestinal wellbeing, reducing the risk and severity of constipation or diarrhea, and enhancing immune wellness, along with reduced risk and magnitude of severe respiratory tract diseases, not all of these claims are endorsed by adequate therapeutic evidence (Rijkers et al., 2011; Behera et al., 2018a).

5.3 System biology and industrial food microorganisms

Industrial food microbiology is the sciences to manufacture or produce food products in mass quantities. There are multiple ways to manipulate a microorganism (mutation, gene amplification using plasmids and vectors, etc) in order to increase maximum product yields. The plasmids and/or vectors are used to incorporate multiple copies of a specific gene that would allow more enzymes to be produced that eventually cause more product yield. Microorganisms play a big role in the food industry, with multiple ways to be used.

5.3.1 Filamentous fungi

Since primitive period, filamentous fungi and yeasts are utilized to produce beer, wine, bread, and cheese. The 20th century was a golden era for industrial food microbiology that exploited fermentation for production of number of enzymes, vitamins, amino acids, polymers, and many other useful compounds (Fig. 5.2). Filamentous fungi are used in industrial scale for manufacture of a huge range of beneficial products such as recombinant proteins and metabolites, all for the gain of human kind. These metabolic products consist of enzymes, amino acids, organic acids, exopolysaccharides (i.e., pullulan, xanthan), pigments, fatty acids, and food (mushrooms). The improvement of molecular biology strategies gives new methods to use yeasts and molds as microbial factories for manufacturing these high-value products. The choice is basically dependent on production yields and regulatory issues, importantly for fungi used in the food industry. Host lines are commonly selected from among these which have attained the so-called GRAS fame via the U.S. Food and Drug Administration (FDA).

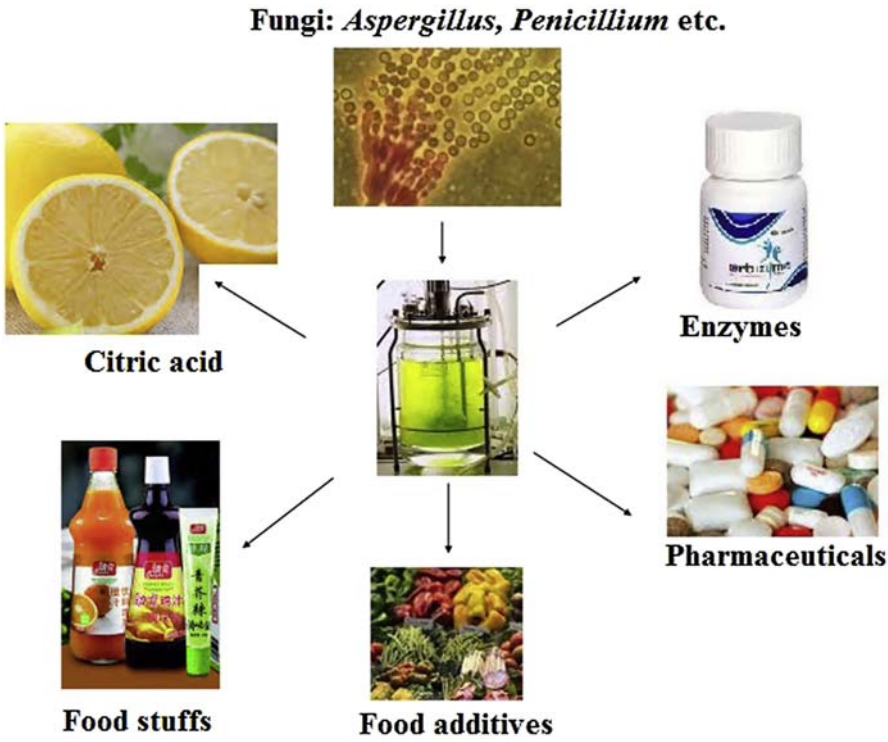

Figure 5.2 Industrial use filamentous fungi batch fermentation.

Filamentous fungi (i.e., *Aspergillus niger, A.oryzae,* and *Rhizopus oryzae*) provide a great opportunity for industrial fermentation. Two particularly important aspects are the high-yield coefficients and the ability to secrete products. The fermentation of soybeans into soy sauce by *A. oryzae* and *Aspergillus sojae* (Luh, 1995) and the layer fermentation of cheese and sausages (Trigueros et al., 1995) are excellent examples.

5.3.2 Industrial yeast

For millennia, yeasts were used to produce fermented foods (breads) and beverages (wine, beer, and sake). Nonetheless, selecting a particular strain of yeast for a particular industrial use is mostly based on historical criteria, instead of science (Steensels et al., 2014).

The development of superior industrial yeasts has resulted in the manipulation of established natural diversity through the use of technologies such as mutagenesis, protoplast fusion, cloning, genome shuffling, and guided evolution to produce artificial variety. In addition, recent technical advancements have allowed the production of high-throughput technologies, known as 'global transcription machinery engineering' (gTME), to stimulate genetic variability, generating a fresh source of genetic diversity for the yeast (Steensels et al., 2014).

Both conventional methods and modern gene manipulation approaches are implemented to produce yeast strains appropriate for work under particular commercial

conditions (Lane and Morrissey, 2010). The age-old yeast *Saccharomyces cerevisiae* is complemented in many applications with the use of less known non-*Saccharomyces* yeasts that are now used widely in food industry. While yeast is synonymous with *S. cerevisiae*, other biotechnologically important yeast strains were implemented to produce manufactured goods beyond conventional foods.

More recently, as industrial species for the heterologous development of enzymes and proteins, *Ogataea (Hansenula) polymorpha, S. cerevisiae, Komagataella (Pichia) pastoris,* and certain other yeast species were created (Branduardi et al., 2008; Johnson, 2013a, 2013b).

5.3.3 Industrial bacteria

Lactic acid bacteria (LAB) are used all over the world for industrial fermentations of milk, meat, fish, and vegetables. Their contribution in these fermentation methods consists of the formation of lactic acid from the available carbon source (matrices) ensuring acidification of the food. However, other than lactic acid forming capacity, LAB have the capability to make a contribution to product characteristics such as flavor, texture, and nutrition. LAB are additionally applied at an industrial scale in the fermentation of food supplements, i.e., mannitol, xylitol, sorbitol, and tagatose, as an end result of metabolic engineering (Monedero et al., 2010; Rice et al., 2019).

Anaerobic bacteria have played a significant role in the advancement of industrial biotechnology.The first mass fermentation was for the manufacturing of Acetone–Butanol–Ethanol (ABE) system by *Clostridium acetobutylicum* in the 1920s (Wolfe, 1999). Anaerobic microorganisms were also used for centuries in food fermentation (Goldstein, 1995). Food products (vinegar, cheese, and beer) created by yeast or bacteria through anaerobic fermentation acquire their properties through the production of substances like carbon dioxide, lactic acid, ethanol, propionic acid, and acetic acid. An approach to the study of chemical compounds and fuels derived from renewable sources is to use conventional food-fermenting bacteria for the chemical industry (Dishisha et al., 2012). One such example is the use of LAB (*Lb. bulgaricus*) in cosmetic, medicinal, and polymer formulations (Datta et al., 1995).

5.4 Bioprocessing (fermentation) technology in food supplement industry

The fermentation technologies are considered as cost competitive compared to chemical synthesis/methods to carry out microbial production of food supplements at an industrial scale (Lv et al., 2019). The various fermentation methods are described below.

5.4.1 Solid-state fermentation

Solid-state fermentation (SsF) is a fermentation process in which microorganism grow on solid materials without the presence of free liquid (Desobgo et al., 2017; Ray and

Behera, 2017). In SsF, the moisture necessary for microbial growth exists in an absorbed state or complexes within the solid matrix. At present, SsF techniques involve in the production of several groups of microbial products such as enzymes (amylase, glucosidase, cellulase, and pectinase), organic acids (citric acids, and lactic acids), microbial secondary metabolites (gibberellic acid, ergot alkaloids, penicillin, and cyclosporin), and other microbial metabolites, such as nucleotides, lipids, vitamins, and amino acids (Zhao et al., 2015; Panda et al., 2016). Some examples of food supplement production in SsF are described below.

A high production of β-glucosidase (27.4 U/mL) was obtained by cultivating the fungus *Lichtheimia ramose* by SsF using wheat bran with 65% of initial substrate moisture (incubated for 96 h at 35°C) (Garcia et al., 2015). SsF has potential to produce lignocellulolytic enzymes (cellulase and xylanase) (Behera and Ray, 2016).

Dhillon et al. (2013) evaluated the potential of different agroindustrial wastes (apple pomace, brewer's spent grain, citrus waste, and sphagnum peat moss) as substrate for solid state citric acid production using *Aspergillus niger* NRRL 2001. Among the four substrates, apple pomace resulted highest citric acid production (61.06 ± 1.9 g/kg dry substrate). Bartkiene et al. (2015) investigated the protein digestibility and formation of lactic acids (LA) during SsF of legumes (lupin and soya bean) using LAB. Protein digestibility of fermented lupin and soya bean was found higher on average by 18.3% and 15.9%, respectively, compared to untreated samples. The LAB produced mainly L-LA (D/L ratio 0.35−0.54), while spontaneous formation gave almost equal amounts of both LA isomers (D/L ratio 0.92−0.98). de Olmos et al. (2015) studied the ability of selected LAB strains (*Lb. paracasei* and *Bifidobacterium longum*) to grow on soy flour substrate with strain-dependent behavior on the SsF system. β-glucosidase activity was evident in both strains, and *Lb. paracasei* was able to increase the free amino acids at the end of fermentation under assayed conditions (50%−80% moisture, temperature incubation 31−43°C).

The production of single cell oils enriched with PUFAs, such as γ-linolenic acid (C18:3 n-6: GLA), is one of the main tasks for biotechnological production of nutritionally important lipids (Čertík et al. 2013). Significant activities in the biosynthesis of Gamma Linolenic Acid (GLA) have been described for species of the lower oleaginous fungi belonging to the genus *Cunninghamella, Mortierella, Mucor, Rhizopus*, and *Thamnidium* (Čertík et al. 2013). Jangbua et al. (2009) studied to maximize the yield of γ-linolenic acid grown on different substrates (polished rice, broken and spent malt grain) by a filamentous fungus, *Mucor rouxii*, using low cost production by SsF. The GLA content was highly accumulated in rice bran (6 g/kg fermented mass) in the fifth day culture grown at 30°C.

5.4.2 Submerged fermentation

In submerged fermentation (SmF), microorganism and substrate are homogeneously distributed in a liquid medium (Naz et al., 2017). For the microbial synthesis of food supplements, the SmF is favored mostly, due to more accessibility to nutrients, sufficient supply of oxygen, and demand of small time duration for the fermentation (Naz et al., 2017; Sharma et al., 2018). Hermansyah et al. (2018) reported the

production lipase by the cultures of *Pseudomonas aeruginosa* on agroindustrial waste product (palm oil mill effluent (POME)) using the SmF. The optimum value of the lipase activity unit (1.327 U/mL) was gained when 3% (v/v) of inoculum, 4 mM of Ca^{2+} ion, 0.4% (v/v) of olive oil, 0.9% (m/v) of peptone, and 0.9% of Tween 80 were added into the medium. Sharma et al. (2018) studied the isolation, purification, and characterization of potential lipase producing bacteria using SmF. The lipase producing bacteria were isolated and identified as *Bacillus methylotrophicus* PS3. The purification procedure (Sephadex G-100 gel column chromatography) resulted in 2.90 fold purification of lipase with 24.10% final yield. Oumer and Abate (2018) compared the production of pectinase by *Bacillus subtilis* in SSF and SmF using agricultural residues as substrate. The production of pectinase was enhanced more than a 6-fold in SmF and a fold in SSF. The highest productivity of pectinase using SmF from *Bacillus subtilis* was 10−66 U/mL whereas in SSF it improved from 800 U/g to 1272 U/g.

5.4.2.1 Batch and fed-batch fermentation

Batch fermentation, a cost-effective process, has been expensively used for the commercial production of various value-added products; during this process, nutrients were provided to the reactor while cells and products remained in the reactor until the end of fermentation (Laopaiboon et al., 2007). Fed-batch process, during which the medium was periodically withdrawn and substituted with fresh medium, was known to enhance the productivity of microbial fermentation as it saved the time for cleaning, sterilization, seed culture, and inoculation processes between batches (Qu et al., 2013). Abdel-Rahman et al. (2015) studied the fed-batch fermentation for the increased production of L-LA from glucose/xylose mixture using *Enterococcus mundtii* QU 25. A high L-LA concentration (129 g/L) with 99.5% optically pure was found in the fed-batch fermentation. A different fermentation processes, including batch, fed-batch, and repeated fed-batch processes by *Schizochytrium* sp., were studied (Qu et al., 2013) and compared for the effective DHA-rich microbial lipids production. The comparison between different processes showed that fed-batch process was a more efficient fermentation strategy for microbial lipids production (18.88 g/L) than the batch process (8.98 g/L).

Batch fermentation has several advantages over continuous fermentation, such as easy control of microbial contaminants and increased product quality per batch (Klasson et al., 1989). However, for large scale production, batch reactors require high capital investments and also require extensive labor (Wee and Ryu, 2009).

5.4.2.2 Continuous fermentation

A Continuous Stirred Tank Reactor (CSTR) can give a more consistent product and provides a steady rate of crude product to be processed in the recovery system. In employing a continuous fermentation process, the microorganism used must be rigid enough to withstand shear while still being easily pumped (Klasson et al., 1989). A continuous fermentation of lignocellulosic hydrolyzates yielded an LA productivity of 6.7 g/L/h using 30 g/L of corn steep liquor and 1.5 g/L of yeast extract as nutrients

(Wee and Ryu, 2009). Silbir et al. (2014) reported the levan (naturally occurring fructan) in continuous fermentation by *Zymomonas mobilis* B-14023. Continuous fermentation processes were performed in packed bed bioreactor using Ca-alginate immobilized *Z. mobilis* cells. The highest levan concentration (31.8 ± 0.21 g/L) was obtained at a dilution rate of 0.14/h while maximum volumetric productivity (6.556 g/L/h) was obtained at a dilution rate of 0.22/h.

5.4.3 Simultaneous saccharification and fermentation

The enzymatic hydrolysis and fermentation steps for microbial food supplements production can be performed as separate hydrolysis and fermentation (SHF) or as simultaneous saccharification and fermentation (SSF). The SSF process offers various advantages over SHF such as the use of a single-reaction vessel for both steps (allowing process integration with reduction of capital cost), rapid processing time, reduced end-product inhibition of hydrolysis, and increased productivity, which is obtained (Marques et al., 2008; Pleissner et al., 2017). Marques et al. (2008) reported the use of recycled paper sludge as an alternative substrate for LA production using *Lb. rhamnosus* ATCC 7469. The maximum production of LA was reported to 73 g/L, corresponding to a maximum productivity of 2.9 g/L/h. Pleissner et al. (2017) reported the food waste as carbon and nitrogen source in SSF using *Lactobacillus* sp. or *Streptococcus* sp. strains for L-LA production. The outcomes revealed a linear relationship between LA concentration and strain used in SSF. *Lactobacillus* sp., strains showed a productivity of 0.27−0.53 g/L/h and yield of 0.07−0.14 g/g, while *Streptococcus* sp., strains more efficiently degraded the food waste material and produced LA at maximum rate of 2.16 g/L/h and a yield of 0.81 g/g dry substrate.

5.5 Metabolic engineering of industrial organisms

Metabolic engineering is one of the most promising and emerging methods for the production of value-added bioproducts (Fig. 5.3). The metabolic pathway of several microorganisms has been successfully engineered for higher production of amino acids, vitamins, colorants, and organic acids compared to conventional methods. Table 5.2 summarizes the metabolic engineering of microorganisms for the production of food additives. Some of the relevant studies have been depicted below.

5.5.1 Production of polyunsaturated fatty acids

Polyunsaturated fatty acids are essential fatty acids required for human development and health and are typically categorized into two major classes: omega-3 (n-3) and omega-6 (n-6) fatty acids with the ω-3 fatty acids being the major focus of most industrial microbial engineering. They have many positive effects on human beings, such as antiinflammatory and antiblood clotting actions, lowering triglyceride level, reducing blood pressure, and reducing the risks of diabetes, some cancers, etc (Ren et al., 2010;

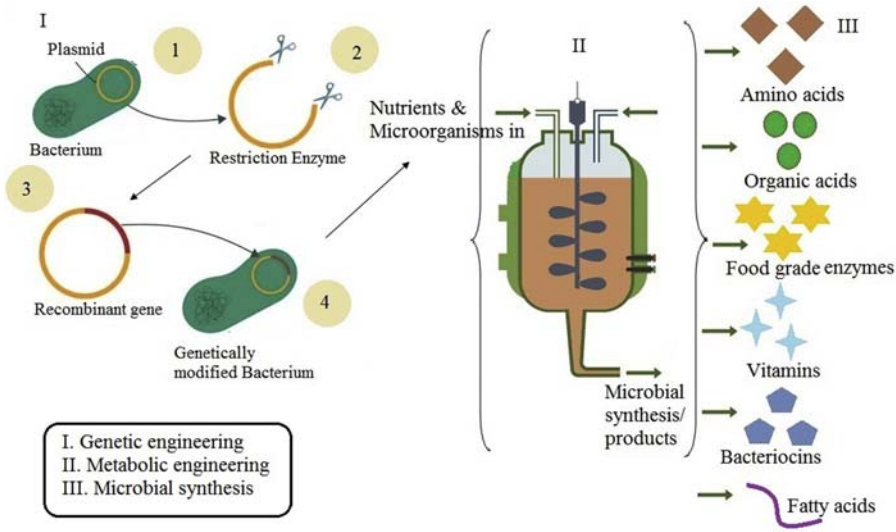

Figure 5.3 Microbial synthesis of Food supplements.

Xie et al., 2015). The human body cannot synthesize these fatty acids on its own. Therefore, the omega-3 fatty acids must be obtained from the diet (Ji et al., 2015). Commercially important ω-3 fatty acids include α-linolenic acid (ALA; C18: 3n − 3), eicosapentaenoic acid (EPA; C20:5n − 3) and docosahexaenoic acid (DHA; C22:6n − 3). Given the increased recognition of health benefits from these molecules, demand for ω-3 PUFAs is growing and expected to reach a global demand of 241 thousand metric tons with a value of $4.96 billion by the year 2020 (Yuan and Alper, 2019).

5.5.1.1 Eicosapentaenoic acid and docosahexaenoic acid

Eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) are two typical omega-3 fatty acids. Their traditional source is derived from cold-water fish oils. Alternatively, novel sources of omega-3 fatty acids can be green manufactured from marine algal or algae-like microbial oils, which could eliminate many of the taste and odor problems associated with fish and discard the shortcomings of fish oil—based process (Gupta et al., 2012). Currently, the most common algae or algae-like microorganism used for the production of DHA belong to the marine members of the families *Thraustochytriaceae* and *Crypthecodiniaceae* (discussed elaborately in Section 7.0).

EPA and DHA biosynthesis is typically pursued through the aerobic desaturase/elongase pathway although production is feasible through an anaerobic polyketide synthase (PKS) pathway (Xue et al., 2013). DuPont researchers used this aerobic pathway in *Yarrowia lipolytica* to generate a strain capable of producing EPA at 56.6% of the total fatty acids and about 15% of the dry cell weight, a value that is

Table 5.2 Metabolic engineering of microorganisms for the production of food additives.

Food additives/ supplements[a]	Engineering organism	Gene/factor involved	References
Essential Fatty Acids			
TGA	*Rhodococcus opacus* PD630	β-glucosidase	Hetzler and Steinbüchel (2013)
ALA	*Yarrowia lipolytica*	Δ12/Δ15-desaturase	Cordova and Alper (2018)
n-3 LC-PUFA	*Methanococcus* sp.	-	Sprague et al. (2017)
Vitamins			
Vitamin C	*Kluyveromyces lactis*	*GME*, *VTC 2,* and *VTC 4*	Rosa et al. (2013)
Vitamin B2	*Eremothecium gossypii*	*RIB*	Revuelta et al. (2016)
Amino acids			
L-arginine	*Corynebacterium glutamicum*	*pgi*/Increasing the NADPH level	Park et al. (2014)
Organic acids			
Citric acid	*Aspergillus niger*	SSADH	Yin et al. (2017)
L-Lactic acid	*Lactobacillus plantarum*	*ldhD*/D-LDH	Okano et al. (2018)
Succinic acid	*Actinobacillus succinogenes*	PCK, MDH, FUM	Guarnieri et al. (2017)
Acetic acid	*Acetobacter pasteurianus*	ADH	Wu et al. (2017)
Enzymes			
Amylase	*Saccharomyces cerevisiae*	Point mutation of *VTA1* gene	Liu et al. (2014)
Lipase	*Lactococcus lactis*	DNA insert	Raftari et al. (2013)
L-asparaginase	*Escherichia coli*	pET vectors, histidine tag	Einsfeldt et al. (2016)

Table 5.2 Metabolic engineering of microorganisms for the production of food additives.—cont'd

Food additives/ supplements[a]	Engineering organism	Gene/factor involved	References
Vitamins			
L-ascorbic acid	*Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*	ALO, LGDH	Sauer et al. (2004)
L-ascorbic acid	*Kluyveromyces lactis*	GME, VTC_2, VTC_4	Rosa et al. (2013)
Thiamine (vitamin B1)	*Bacillus subtilis*	*thiN*[b], *thiT*[c], and *thiW*[d]	Schyns et al. (2005)
Adenosylcobalamin (vitamin B_{12})	*E. coli*	AdoCbi-P	Fang et al. (2018)

[a]TGA: Triacylglycerols; ALA: α-Linolenic acid; Omega-3 LC-PUFAs: Omega-3-long chain polyunsaturated fatty acids; NADPH: Nicotinamide Adenine Dinucleotide Phosphate Hydrogen; SSADH: Succinate-semialdehyde dehydrogenase; D-LDH: D-lactate dehydrogenase; PCK: Phosphoenolpyruvate carboxykinase; MDH: Malate dehydrogenase; FUM: Fumarase; ADH: Alcohol dehydrogenase; ALO: D-arabinono-1,4-lactone oxidase; LGDH: L-galactose dehydrogenase; GME: DP-mannose 3,5-epimerase; VTC_2: GDP-L-galactose phosphorylase; VTC_4: L-galactose-1-phosphate phosphatase; AdoCbi-P: Adenosylcobinamide phosphate.
[b]thiamine pyrophosphokinase.
[c]thiamine permease.
[d]thiamine ABC transporter component.

the highest percentage among known EPA sources. The same group later developed a new commercial strain (*Y. lipolytica* Z5567) that optimized carbon flux toward EPA biosynthesis pathway, eliminated β-oxidation, and fine-tune regulated EPA transportation (Zhu and Jackson, 2015). When cultivated using a two-stage fed-batch fermentation process (using nitrogen-rich medium for growth phase and nitrogen-limiting conditions for oil production), this strain was capable of producing an oil comprising EPA at 50% and 25% dry cell weight (Xie et al., 2015).

In a recent study, the red yeast *Rhodosporidium toruloides* DSM 4444 was used in both in batch and fed-batch mode for production of microbial lipids rich in PUFAs, with oleic acid being the major fatty acid (61.7%, w/w) (Tsakona et al., 2019). Diversified mixed confectionery waste streams were used as the substrate for production of microbial lipids.

Consolidated bioprocessing (CBP) technology was used for coproduction of DHAs and bioethanol from rice straw biomass as substrate. Key points of the process were: (a) CBP of pretreated rice straw biomass to bioethanol (anaerobic fermentation) resulting into 1.8 g/L bioethanol and 29.40% solubilization of rice straw biomass; (b) utilization of spent lignocellulose derived sugars in microalgal fermentation (aerobic fermentation) with subsequent promising cell growth (2.77 g/L), substantial lipids (17.05%), and DHAs production (44.0% of total fatty acids). Other major fatty acids

(as total fatty acid %) were palmitic acid (13.95%), stearic acid (5.07%), EPA (7.24%), and docosapentaenoic acid (16.12%) (Singh et al., 2020).

5.5.1.2 α-Linolenic acid

An additional ω-3 fatty acid, α-linolenic acid (ALA), has been explored also in the oleaginous yeast *Y. lipolytica*. Biosynthesis of ALA requires a Δ15-desaturase to convert native unsaturated fatty acids of oleic acid (C18:1n − 9) and LA (C18: 2n − 6) into the ALA (Cui et al., 2016). Using a previously engineered strain of *Y. lipolytica* that can produce nearly 80% of lipids as an unsaturated C18 s, it was possible to create a platform for ALA biosynthesis. Specifically, heterologous expression of a codon-optimized, bifunctional Δ12/Δ15-desaturase from *Rhodosporidium kratochvilovae* coupled with a low-temperature fermentation (20°C) produced significantly increased ALA content. The resulting strain was capable of producing ALA to upwards of 30% of total fatty and achieving titers of 1.4 g/L ALA in fed-batch fermentation, the highest reported titer in a yeast host (Cordova and Alper, 2018). Collectively, these results highlight the use of microorganisms (especially oleaginous yeasts) for the production of nutritional fatty acids.

5.5.2 Production of amino acids

L-arginine, one of the valuable amino acids with wide range of applications as supplement, is produced through microbial synthesis. An interesting study was carried out for higher yield of L-arginine by engineering the metabolic pathway of *Corynebacterium glutamicum* (Park et al., 2014). The strain improvement of *C. glutamicum* ATCC 21831 was carried out through random mutagenesis. The mutagenic strain improved L-arginine production by increasing the NADPH level since biosynthesis of 1 mole of arginine requires 3 moles of NADPH. In order to generate higher concentration of NADPH, the *pgi* gene responsible for the production of glucose-6-phosphate isomerase was downregulated; as a result, the pentose phosphate pathway was activated in comparatively higher rate for improved production of NADPH. Additionally, it was observed that deletion of *Ncgl 1221 gene* enhanced the conversion of L-glutamate to L-arginine rather than releasing to the medium. Similarly, ornithine is one of the derivatives of arginine and has been reported for many health applications. The production can be improved by overexpression of *argCJBD* gene through plasmids (Shin and Lee, 2014). Lee et al. (2007) have demonstrated the production of higher rate of L-threonine through manipulating the metabolic pathway. Higher yield of L-threonine was observed (0.393/g glucose and 82.4 g/L in fed batch culture) by removal of the genes *thrA*, *lysC*, *thrL,* and *tdh* and mutation of genes *ilv A*, *thrA,* and *lysC* that are responsible for the inhibition of aspartokinase I and III; *thrL* represents transcriptional attenuation regulation, and *tdh* is known for degradation of threonine. Similarly, the gene of acetohydroxy acid synthase isoenzyme III, inhibited by valine, was removed. Also, the *ilvA, leuA,* and *panB* genes were deleted to make more precursors available for the overproduction of valine. Valine generation of 1.3 g/L was observed by overexpression of *ilvBN* genes (Park et al., 2011).

5.5.3 Production of organic acids

Organic acids are mostly used as food additives and preservatives. Among the weak acid groups, citric acid, lactic acid, acetic acid, and succinic acid are very prominent (Panda et al., 2016).

Yin et al. (2017) have reported the production of citric acid by different microorganisms (157 g/L by *Aspergillus niger* H915-1, 117 g/L by *A. niger*A1, and 76 g/L by *A. niger* L2). The higher rate of production of lactic acid by *A. niger* H915-1 was attributed to the mutation of 92 genes which included a succinate-semialdehyde dehydrogenase in the γ-aminobutyric acid shunt pathway. Also, the ATP-citrate lyases, which have important role in citrate synthesis, were upregulated.

A strain of *Lb. plantarum* was improved by deleting certain genes to obtain optically pure lactic acid, i.e., (L)-lactic acid, instead of the racemic mixture (l- and D-), produced by the wild strain. The gene encoding D-LDH (lactate dehydrogenase), *ldhD* was first deleted, and it was found to have no impact on inhibition of D-lactic acid production. However, the conversion of D-lactic acid to L-lactic acid was completely possible when the operon representing lactate racemase (*larA-E*) was disrupted. It may also be noted that the aforesaid mutant produced pure L-lactic acid (99.4% purity) of 87 g/L from 100 g/L glucose (Okano et al., 2018).

Succinic acid is often preferred as a flavoring agent and an acidity regulator in food and beverage industry. A study was conducted to improve the production of succinic acid in *Actinobacillus succinogenes*; the genes *pflB* (pyruvate formate lyase) and *ackA* (acetate kinase) were knocked out which blocks the competitive pathways for the production of formic acid and acetic acid, respectively. However, the mutated strains showed growth defects. Further, three different genes of three different strains were overexpressed phosphoenolpyruvate carboxykinase (PCK), malate dehydrogenase (MDH), fumarase (FUM), and the yield obtained were 31.3, 34.2, and 32.6 g/L, respectively, whereas the wildtype showed a production of 30.6 g/L (Guarnieri et al., 2017). In another study, enzyme subunits I (*adhA*) and II (*adhB*) of pyrroquinoline quinone-dependent alcohol dehydrogenase in *Acetobacter pasteurianus* were overexpressed resulting in higher production yield of acetic acid as compared to the wild strain (Wu et al., 2017).

5.5.4 Production of enzymes

Amylase is one of the important enzymes frequently used in food and beverage industries. Inverse metabolic engineering was applied to *S. cerevisiae* for the higher yield of amylase. Firstly, screening was conducted based on UV-random mutagenesis as well as selection for growth on starch. Later, the genetic mutations linked with overproduction of amylase were detected. S196I point mutation of *VTA1* gene encoding a protein engaged in vacuolar sorting resulted in higher amylase secretion by 35% (Liu et al., 2014). Similarly, genes linked with lipase transport in *Burkholderia cepacia* were amplified and subcloned in pNZ8148 vector; further transformation was done in *Lactococcus lactis* (Raftari et al., 2013). Recombinant lipase expression was observed to be higher ($\sim 152 \cdot 2$ μg/ml/h) than the wild strain. Asparaginase is an enzyme used in reduction of acrylamide during baking and also used as chemotherapeutic agent in

treatment of lymphoblastic leukemia. Einsfeldt et al. (2016) cloned the gene encoding L-asparaginase of *Zymomonas mobilis* in pET vectors along with a histadine tag. The enzyme was found to be expressed in *E. coli*. The recombinant protein was produced (0.13 IU/mL extracellular L-asparaginase and 3.6 IU/mL intracellular L-asparaginase) in a bioreactor after 4h when induced with induction of Isopropyl β-D-1-thiogalactopyranoside.

5.5.5 Production of vitamins

The demands of vitamins are increasing day by day. Hence, research is being conducted globally to enhance the production of microbe-derived vitamins through metabolic engineering. Successful results have been obtained for the production of provitamin A, vitamin C, and B vitamins. A study by Sauer et al. (2004) revealed the production of vitamin C by *S. cerevisiae* and *Zygosaccharomyces bailii* when incubated along with L-galactose, L-galactono-1,4-lactone, or L-gulono-1,4-lactone intermediates (of pathway for vitamin C production); however, in ordinary conditions, yeast do not produce vitamin C (Sauer et al., 2004). Yeast cells overexpressed with D-arabinono-1,4-lactone oxidase and L-galactose dehydrogenase were observed to convert 40% (w/v) of the raw material L-galactose. Vitamin C synthesis was carried out by engineering *Kluyveromyces lactis* with L-galactose biosynthesis pathway genes (L-galactose is the prime intermediate for the synthesis of L-ascorbic acid) of *Arabidopsis thaliana*. The genes isolated were *GME*, encoding GDP-mannose 3, 5 epimerase, *VTC 2* encoding GDP-L-galactose phosphorylase, and *VTC 4* representing L-galactose-1-phosphate phosphatase. The metabolically modified *K. lactis* strains could convert lactose and D-galactose to L-galactose for further synthesis of L-ascorbic acid (Rosa et al., 2013).

Synthesis of vitamin B1 was improved (titer 1.3 mg/L) in *Bacillus subtilis* mutation of salvage thiamine pyrophosphokinase (*thiN*), thiamine permease (*thiT*), and thiamine ABC transporter component (*thiW*) (Schyns et al., 2005). Similarly, threonine aldolase was overexpressed (*GLY1*), serine hydroxymethyltransferase encoding gene (*SHM2*) was disrupted in *Ashbya gossypii* for an overproduction of vitamin B2, i.e., more than 20 g/L (Abbas and Sibirny, 2011). It was also observed that *C. glutamicum* could produce 1000 mg/L of vitamin B5 with β-alanine as fed precursor when *ilvA* gene was inactivated and native ILvBNCD and PanBC enzymes were overexpressed (Hüser et al., 2005). Fang et al. (2018) have engineered an *E. coli* strain metabolically and application of genetic engineering. Genes encoding adenosylcobinamide phosphate (intermediate of vitamin B12 synthesis) were incorporated in *E. coli* for enhanced vitamin B12 yield (more than 250 fold).

Microbial vitamin K production was documented in *Bacillus subtilis*, *Propionibacterium freudenreichii*, and *Flavobacterium* sp., but the vitamin K biosynthesis model microorganism was not developed for mass production. Several researchers have thoroughly studied the process of synthesis of vitamin K_2 (menaquinone, MK) in *B. subtilis* (Mahdinia et al., 2018).

Microorganisms contribute to approximately 15% of the total industrial production of biocommodities. In past few years, many strategic approaches to metabolic engineering were established to generate carotenoids in various species (Bhatia and Victor,

2012) *Saccharomyces cerevisiae* and *Candida subtilis* were engineered to express genes of bacterial carotenoid secretion, supplying between 0.1 and 0.4 mg g/L dry cells with beta-carotene titers. *Escherichia coli* was also biologically engineered to generate the carotenoid using various techniques where gene expression from *Streptococcus pneumoniae* and *Enterococcus faecalis* were most active, reaching 460 mg/L titers (Ajikumar et al., 2010; Zhao et al., 2013). *S. cerevisiae* was updated to express carotenogenic genes through *Xanthophyllomyces dendrorhous*, resulting in levels of beta-carotene exceeding 6.3 mg/g dry cells (Verwaal et al., 2010; Yan et al., 2012). Other hosts that were currently being developed to develop carotenoids such as *Pichia pastoris* (out of which small amounts of β-carotene were developed to date: 0.34 mg/g dry cells) (Araya-Garay et al., 2012) and *Yarrowia lipolytica* (Sabirova et al., 2011).

Vitamin B2 is identified as riboflavin. At the moment, *B. subtilis* and *Eremothecium gossypii* genetically engineered expressing *RIB* genes are industrially used in manufacturing of riboflavin. Perkins et al. (1999) have produced a riboflavin producing strain comprising several versions of transformed *B. subtilis* riboflavin biosynthetic operons (rib operon) incorporated at two various sites in *B. subtilis* chromosome. Vitamin B2 is extracted from the fermented mixture via centrifugation through heat inactivation of the microorganisms.

5.5.6 Release of biominerals

Iron and zinc deficiencies are the major health problems worldwide. Phytic acid is the major storage form of phosphorous in cereals, legumes, oilseeds, and nuts. It is known as a food inhibitor which chelates micronutrients (i.e., iron, zinc, calcium, manganese, etc.) preventing them to be bioavailabe for monogastric animals, including humans because they lack enzyme phytase in their digestive tract. Some filamentous fungi (i.e., *Aspergillus ficuum* NRRL 3135, *Aspergillus oryzae*), yeast (*S. cerevisiae*), and bacteria (*B. subtilis* Natto and LAB) facilitate release of essential minerals from food matrices by virtue of possessing phytase (Singh et al., 2017). The phytase active LAB isolates belong to the species *Lactobacillus panis, Lactobacillus reuteri, Lactobacillus fermentum*, *Lactobacillus plantarum,* and *Pediococcus pentosaceus*. In a recent study, the highest extracellular phytase production was found in *Lb. panis* with a volumetric phytase activity of 140 U/mL. Phytate degradation in whole-wheat dough fermented with *Lb. panis* or *Lb. fermentum* was 90% and 70%, respectively (Nuobariene et al., 2015).

5.5.7 Production of bacteriocins

Bacteriocins are ribosomally blended antimicrobial peptides created by microscopic organisms. Numerous bacteriocins delivered by food grade lactic acids microscopic organisms display the possibility to control deterioration and pathogenic microbes in nourishment. Uncommonly few bacteriocins alongside their local antibacterial property likewise display extra enemy of viral and hostile to parasitic properties. Bacteriocins are by and large created by Gram + ve, Gram - ve and archaea microorganisms. Bacteriocins from Gram + ve bacteria particularly from lactic acid microbes (LAB)

have been completely explored considering about their incredible biosafety and expansive mechanical applications. LAB communicating bacteriocins were segregated from matured milk and milk items, rumen of creatures, and soil utilizing conceded opposition examine. Nisin is the main bacteriocin that has got FDA endorsement for application as a sustenance additive, which is created by *Lactococcus lactis* subsp. *Lactis*. At present, bacteriocins are exclusively connected in food ventures; however, they have an extraordinary potential to be utilized in different fields, for example, nourishes natural manures, ecological insurance, and individual consideration items. The eventual fate of bacteriocins is to a great extent reliant on getting FDA endorsement for utilization of different bacteriocins notwithstanding nisin to advance the examination and applications (Silva et al., 2018).

5.6 Other bioactive compounds used as food supplements

5.6.1 Food flavors

Flavor is typically the results of the presence of many volatile and nonvolatile elements possessing numerous chemical properties. The nonvolatile compounds contribute principally to the taste, while volatile ones influence aroma and flavor. These volatile compounds include alcohols, aldehydes, esters, dicarbonyls, short- to medium-chain free fatty acids, methyl group ketones, lactones, phenoplast compounds, and sulfur compounds (Longo and Sanromán, 2006).

5.6.1.1 Lactones

Lactone flavors with fruity, milky, coconut, and other aromas are widely used in the food and fragrance industries. *Trichoderma viridae* creates a trademark coconut flavor because of the generation of 6-pentyl-2-pyrone. The primary part of peach flavor is 4-decalactone which can be contributed by *Sporobolomyces odorus*. Both δ- and γ-lactones are utilized generally as flavor and scent compounds. A manufactured case of lactone creation is the microbial change of ricinoleic acid through incomplete β-oxidation toward γ-decalactone, which include a peach-like smell, by yeasts, such as *Yarrowia lipolytica* and *Sporidiobolus salmonicolor* (Vandamme and Soetaert, 2002; Lee et al., 2018).

The fatty acid biosynthetic (FAS-B) pathway of *Brevibacterium ammoniagenes* was used to produce triacetic acid lactone (TAL) from glucose rather than a petroleum-based raw material. The ketoreductase (KR) domain of the FAS-B was inactivated by mutating its key catalytic residue to enable it to produce TAL. It was assumed that the KR domain would include the sequence from amino acid residue 2051 to 2319. This sequence turned out to be a member of the short-chain dehydrogenase/reductase (SDR) super family. A replacement in the sequence was made to inactivate it. *Saccharomyces cerevisiae* yeast was transformed to express the modified

FAS-B and also phosphopantethiene tranferase (PPT1) from *B. ammoniagenes*. The yeast was able to produce TAL in vivo (Zhao, 2004).

A novel method to produce flavor lactones from abundant nonhydroxylated fatty acids using yeast cell factories was described. Oleaginous yeast *Yarrowia lipolytica* was engineered to perform hydroxylation of fatty acids and chain-shortening via β-oxidation to preferentially 12- or 10- carbons. The strains could produce γ-dodecalactone from oleic acid and δ-decalactone from LA. Through metabolic engineering, the titer was improved 4-fold, and the final strain produced 282 mg/L γ-dodecalactone in a fed-batch bioreactor (Braga and Belo, 2016).

5.6.1.2 Aromatic esters

Many sectors of industry, mainly food, cosmetics, and pharmaceutics, have increased their interest in esters due to their flavor property. Flavor esters that possess an aromatic ring in their molecular structure are also known as aromatic esters. These esters are widely found in nature (fruits and plants), and the synthetic (i.e., via chemical) and microbial routes (i.e., via fermentation/bioprocessing) are suitable for their biocatalysis. Almeida et al. recorded 94 distinct esters as being distinguished in brew. The majority of the esters found in brew are shaped through essential fermentation (Almeida et al., 2017). Several yeasts such as *S. cerevisiae, Pichia anomala*, *Candida utilis*, *Kluyveromyces marxianus,* and *Candida utilis* were found to age glucose to ethyl acetic acid derivation when developed on a medium constrained in iron. Ethanol could be changed over into either ethyl acetic acid derivation or acetaldehyde. Two pathways might be utilized to develop esters: (1) the alcoholysis of acyl-CoA components and (2) the immediate esterification of an organic acid with an alcohol (Loser et al., 2014).

5.6.1.3 Carbonyls

Diacetyl is one of the most significant carbonyls that imparts a nut-like flavor and can be extensively used as a food ingredient. The most significant diacetyl producing organisms are *Leuconostoc citrovorum*, *Leu. creamoris*, *Leu. dextranicum*, *Streptococcus lactis* subspecies *diacetylactis*, *S. thermophilus,* and certain strains of *Proprionibacterium shermani* (Chen et al., 2017).

In a recent study, diacetyl could be produced from the nonenzymatic oxidative decarboxylation of α-acetolactate during 2, 3-butanediol fermentation. In this study, the 2, 3-butanediol biosynthetic pathway in *Enterobacter cloacae* subsp. *dissolvens* strain SDM, a good candidate for microbial 2, 3-butanediol production, was reconstructed for diacetyl production. To enhance the accumulation of the precursor of diacetyl, the α-acetolactate decarboxylase encoding gene (*budA*) was knocked out in strain SDM. Subsequently, the two diacetyl reductases DR-I (*gdh*) and DR-II (*budC*) encoding genes were inactivated in strain SDM individually or in combination to decrease the reduction of diacetyl. Although the engineered strain *E. cloacae* SDM (Δ*budA*Δ*budC*) was found to have a good ability for diacetyl production, more α-acetolactate than diacetyl was produced simultaneously. In the end, by using the

metabolically engineered strain *E. cloacae* SDM (Δ*budA*Δ*budC*), diacetyl at a concentration of 1.45 g/L was obtained with a high productivity (0.13 g/L/h) (Zhang et al., 2015).

5.7 Microalgae in food supplements

Among the new passages in the food supplements segment, bioproducts containing microalgae as is representing a quickly growing business sector. The showcased items are essentially founded on three creation strains, i.e., *Spirulina* and *Chlorella*, followed by *Klamath*. It is a composite circumstance, since two of them are cyanobacteria and the subsequent one is eukaryotic (Klein-Marcuschamer et al., 2013; Koyande et al., 2019). Algal oil is still significantly more expensive than fish oil applications, although many groups are improving both the cost and quality of omega-3 oil from algal sources (Koyande et al., 2019).

Production of DHA mostly belong to the marine members of the families *Thraustochytriaceae* and *Crypthecodiniaceae*. The *Thraustochytrids* include the genera, *Schizochytrium* and *Ulkenia*, whereas dinoflagellate *Crypthecodinium* is a genus of the family Crypthecodiniaceae (Klok et al., 2014). Members of these genera are widely dispersed in the oceans of the world. By heterotrophically culturing these microorganisms, the omega-3 biotechnological processes for DHA production have gone into industrial scale (Ren et al., 2010). However, the production of EPA is still being restricted to laboratory scale. The traditionally used EPA producers are the algae *Phaeodactylum tricornutum*, *Nannochloropsis*, and *Nitzchia* (Wen and Chen, 2003). The relatively low accumulated biomass and slow growth rate of these algae hindered the industrial EPA production.

5.8 Food supplements global trend, environmental and regulatory issues

The estimated global food supplements market size is between 74.14 and 123.28 billion USD in FY 2019−20 and is projected to expand at a CAGR of 6.34%−8.2% during the forecasted period. The market is backed by rising health awareness globally among consumers of all age groups and growing awareness toward calorie reduction and weight loss in major markets including the US, China, and Italy is expected to promote food supplement, in addition, changing lifestyles and food habits are driving the product demand. Positive outlook toward sports nutrition market is also among major driving factors. Individual food supplements market analysis showed that global essential fatty acids market is expected to reach around USD 9.15 billion by 2026, growing at a CAGR of 9.8% between 2019 and 2026 (https://www.zionmarketresearch.com/report/essential-fatty-acids-market).Essential amino acids are generally produced through processes such as fermentation, which is the most widely used process and accounts for

major share in global production (9.3 Million Tons in 2019) of amino acids(https://www.grandviewresearch.com/industry-analysis/amino-acids-market).

Many scientific and regulatory challenges exist in research on the safety, quality, and efficacy of food supplements are common to all countries as the marketplace for them becomes increasingly global. Some of the key issues that commonly arise are (1) evaluating evidence for product claims as the market for food supplements has increased, (2) distinguishing between a food supplement and other categories such as conventional foods and biologics. To address these issues, US Congress passed the Dietary Supplements Health and Education Act (DSHEA) 1994, which defined dietary supplement as a product taken by mouth containing a dietary ingredient intended to supplement the diet. DSHEA then granted the U.S. FDA authority to establish regulations regarding dietary supplement manufacturing, regulating health claims, and labeling of dietary supplements, and creating governmental bodies to encourage research on supplements. On the other hand, the European Union (EU) in 2002 has created a legal and regulatory framework named as the "Food Supplements Directive 2002/46/EC" containing a list of nutrients and their chemical forms able to be used in food supplements. However, the maximum levels and conditions of use for other substances, such as botanicals, botanical preparations, and bioactive substances, such as lutein and glucosamine, are not harmonized and, therefore, fall under national legislation. While these products must comply with a series of European laws, the compositions of these products are still largely subject to national legislation, resulting in numerous trade barriers even between EU member states.

5.9 Challenges, future prospectives and concluding remarks

Challenges are basically the acceptance of these products worldwide with various diverse ethics, beliefs, habits, diet, and cultures. In agreement with the legislation, the labeling of these health supplements should embrace. Affirmation that it is a health/food supplement after the name of the product, the recommended dose of the product for daily consumption, the statement that the supplements do not substitute a balanced diet, statement about the storage of the product away from children are some of the critical issues. Until now, the spotlight has been on maximal biomass production while the dose effects of probiotics have not been extensively studied. The physiological state of the cells has also to be well thought-out to take advantage of health benefits. In this regard, technologies and operating conditions may be of significant importance and should be assessed.The exploitation of these microorganisms at a massive scale should be conducted to accomplish a better yield of food supplements for easy and cheap availability. Furthermore, screening of proficient isolates and subsequent efforts regarding strain improvement should be facilitated. Nutrition and health will continue to be the driving forces for exploiting the potential of microorganisms, to arrive at even more efficient processes for health supplements production.

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