Vitamins and Vitamin-like Compounds: Microbial Production

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Defining Statement
Applications and Market
Fat-Soluble Compounds

Glossary

BCC Business Communications Company Inc., a leading US information resource producing detailed market research reports, newsletters, and conferences.
FDA Food and Drug Administration, an agency of the United States Department of Health and Human Services responsible for regulating inter alia food and dietary supplements.
oleoresin A coloring or flavoring plant extract obtained by solvent extraction.
PUFAs Polyunsaturated fatty acids, a chemical group of substances containing some essential nutrients originally termed vitamin F.

Abbreviations

2KGA 2-keto-L-gluconic acid
AMP adenosine monophosphate
BCC Business Communications Company Inc.
DHBP 3,4-dihydroxy-2-butanoic 4-phosphate
DRL 6,7-dimethyl-8-ribityllumazine
FAD flavin adenine dinucleotide
FDA Food and Drug Administration
FMN flavin mononucleotide

Defining Statement

Studies on the worldwide nutritional status repeatedly reveal vitamin deficiencies and a need for their dietary supplementation. Also, with an increasing number of reported beneficial health effects, the growing vitamin market demands cost-efficient production processes, employing genetically engineered microorganisms as alternatives to chemical synthesis.

Applications and Market

Vitamins, a chemically heterogeneous group of organic compounds essential for an organism, are by definition substances that are not or only inadequately provided by the organism’s anabolism. These nutrients fulfill catalytic or hormone-like functions, and thus a daily dietary intake of small amounts satisfies the requirements. Apart from a catalogue of symptoms caused by vitamin deficiency,
nowadays vitamins have gained additional attention due to numerous preventative health benefits, for example, in neoplastic diseases like cancer or degenerative diseases like rheumatism. In developed countries, a more than sufficient and seasonally independent food supply of all vitamins and vitamin-like compounds is available – if only the consumer chooses the right diet. However, studies repeatedly reveal inadequate nutrient intakes, even if situations creating increased requirements like pregnancy, certain pre-existing medical conditions, and an unhealthy lifestyle are made allowances for. A balanced and varied diet should contain nutrient-rich fruit and vegetables, milk and grain products, as well as sea fish and low-fat meat. According to the large-scale eVe Study, nearly 50% of the Spanish population need to adjust their usual food pattern toward a more nutrient-dense, healthier diet. Shortages in single vitamins or combinations of riboflavin, folate, vitamin A, vitamin D, vitamin E, and vitamin C were found in a high proportion of the 10 208 participants. Although the pattern of inadequate vitamin intake varies between populations, it is a worldwide problem causing a demand for additional, industrially produced vitamins.

After the isolation of all 13 vitamin groups by extraction techniques in the 1930s and 1940s, structure determinations of the vitamins led to the development of chemical synthesis procedures, culminating in several Nobel prizes. However, today vitamins are produced by chemical synthesis, extraction chemistry, biotechnical or combined methods, in which biotechnical procedures comprise fermentation or bioconversion processes. Production scales and methods of syntheses of these compounds within the scope of this article are compared in Table 1. Although only the polyunsaturated fatty acid (PUFA) arachidonic acid, vitamin B2, and vitamin B12 are produced exclusively by biotechnical processes, the development of further microbial techniques to replace chemical synthesis is also pursued. Several factors like increasing ecological awareness and environmental constraints, sustainability of renewable resources, and consumer demand for naturally produced products have an impact. However, regarding the latter, no scientific data support a significant difference between chemically and natural derived vitamins, as stated by the Health and Consumer Protection Directorate-General of the European Commission in 2003 for the pigmentation of fish by astaxanthin synthesized chemically or originating from the natural source Xanthophyllomyces dendrorhous. But, increasing costs of fossil resources and disposal of chemical waste count in favor of biotechnical processes, since not only is there a need for an efficient process of manufacture but also one that makes a handsome profit for a company in the international market.

This article focuses on vitamins and vitamin-like compounds, whose microbial production already is commercially relevant, with emphasis on the production of vitamin B2. Subsequently, the current state of industrial development of other vitamins is discussed and the impact of the so-called white biotechnology on our daily life and on industry is considered in the section titled ‘Conclusions and outlook’.

The world market value of vitamins and vitamin-like compounds is estimated at nearly US$2.3 billion by 2007. As a result of the entry of Chinese competitors in the market sales fell from US$2.7 billion in 1999 even though the market was still growing by approximately 4% annually. Interestingly, however, the market volume of vitamins is still a multiple of that of antibiotics. The leading vitamins are E, C, and A, summing up to more than 65% of total sales in 2002. In general, the majority of industrially produced vitamins are used in animal feed, with a share of almost 50%. The supplement and food industries follow with 22 and 17%, respectively, and 11% is used in cosmetic products. The percentage in pharmaceutical and technical applications is marginal.

An increasing demand for feed vitamins is caused by the growing world population going along with factory farming ashore and at sea, in which the fodder, especially conserved feed, needs vitamin supplementation to enable a healthy and competitive livestock. Moreover, people directly consume almost 40% of all vitamins produced either as dietary supplements or as fortified foods. Indeed

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical synthesis</th>
<th>Extraction (chemical)</th>
<th>Microbial</th>
<th>Combined</th>
<th>World production (tons year⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Fat soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>PUFAs</td>
<td>+</td>
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<tr>
<td>Water soluble</td>
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<tr>
<td>Vitamin B2</td>
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<td>Vitamin B12</td>
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<td>Vitamin C</td>
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eating habits, lifestyle, some medical conditions, and food processing or preservation methods render additional vitamin intake useful. Additionally, the positive appeal of vitamin enriched products is boosted by scientific and public reports about their beneficial health effects. Thus nowadays vitamin fortification of industrially produced food to increase nutritional value is very common, with breakfast cereals and fruit juices being typical examples. In parallel, the market for dietary supplements in the form of tablets, powder, or liquids is rapidly growing: according to a Forsa survey in 2000 every third German regularly consumed dietary vitamins with annual costs of €300 on average. The consideration of vitamins as a panacea creates a never-ending debate about the pros and cons of dietary supplementation of vitamins. Pharmaceutical applications like drip-feeding and prescription of vitamins account for less than 1% of the vitamin market.

In contrast to the pharmaceutical industry where highly purified vitamins are applied the feed industry, being the principal client, takes an additional advantage of microbial production: biomass residua rich in lipids and proteins represent extra nutrients and thus less purified vitamins have a positive feeding effect. In case of vitamin B<sub>2</sub> and the carotenoid astaxanthin, even poor qualities of such vitamins that have residual biomass are being sold, and for which the extraction procedure is simplified and the chemical load is reduced. Nevertheless, although all vitamin productions in organisms must be licensed they must also have GRAS (generally regarded as safe) status.

Microorganisms used in industrial scale production of vitamins belong to bacteria, microalgae, or fungi. In classical approaches microbial vitamin production starts with a natural overproducer, such as the fungus Ashbya gossypii, rich in vitamin B<sub>2</sub>. To improve the yield successive rounds of mutagenesis and selection are carried out along with optimization of process parameters. Apart from classical strain improvement, modern biotechnology allows genetic engineering of many microorganisms that are already approved for use in other industrial processes. Their metabolic engineering for application in a vitamin manufacturing process is facilitated by increased knowledge of the biochemical and regulatory pathways in these microorganisms. In-depth analyses of enzymes and their substrates with regard to flux control and genome-wide transcriptional analysis allow further optimization if genetic engineering is feasible and the genomic DNA sequence is known. Nevertheless, most industrial strains still are based on improvement of natural overproducers. Only a few, most impressively Bacillus subtilis in the vitamin B<sub>2</sub> production process, are a result of sophisticated genetic engineering (Table 2).

The efficiency of microbial vitamin production is very diverse. Final product titers range from about 100 mg l<sup>-1</sup> to 100 G l<sup>-1</sup>. The yield, that is, conversion of the carbon source into the final product, ranges from inefficient to highly efficient. Especially when phototrophic microorganisms are used, turnover time and concentrations are low, a large fermentation area is needed, and downstream processing is cost-intensive. Although strains and production processes are continuously optimized, some biotechnical procedures are used only for production of niche products in comparison to chemical methods. Examples include high-quality stereo-specific carotenoids and PUFA’s as dietary supplements in infant formulas.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Microorganisms applied for large-scale production of vitamins and vitamin-like compounds</th>
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<tr>
<td>Compound</td>
<td>Production organism</td>
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<tr>
<td>Fat soluble</td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td></td>
</tr>
<tr>
<td>/β-Carotene</td>
<td>Blakeslea trispora&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Astaxanthin</td>
<td>Xanthophyllomyces dendrorhous&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dunaliella salina&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUFAs</td>
<td></td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>Mortierella alpina</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>Thraustochytrium</td>
</tr>
<tr>
<td>Water soluble</td>
<td></td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Pseudomonas denitrificans&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Gluconobacter suboxydans&lt;sup&gt;a&lt;/sup&gt; and Ketogulonicigenium vulgare&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Natural overproducer modified by strain improvement.
<sup>b</sup>Original name: Phaffia rhodozyma.
<sup>c</sup>The bioprocess yields 2-keto-L-glucarate from which L-ascorbic acid, the γ-lactone, is derived by a chemical downstream step.
<sup>d</sup>Original name: Gluconobacter oxidans.
Fat-Soluble Compounds

Carotenoids

Carotenoids are organic pigments naturally produced by microorganisms and plants. They fulfill diverse biological functions like energy transfer in photosynthesis, protection, species-specific coloration, and supply of precursors, for example, in the synthesis of vitamin A. They consist of a long carbon backbone derived from isoprenoid moieties. In many cases, both termini form ring systems. The extended system of conjugated carbon–carbon double bonds is responsible for the light absorption and the bright color of the carotenoids. The over 600 different carotenoids described so far fall into two main groups: the carotenes, nonoxidized hydrocarbon carotenoids, and the xanthophylls, oxygenated carotene derivatives. Following the isoprenoid pathway, natural carotenoid synthesis either starts from acetyl-CoA via mevalonate or from deoxyxylulose-5-phosphate, a condensation product of pyruvate and glyceraldehyde-3-phosphate.

Less than a dozen carotenoids are produced industrially from vegetable sources or petals, by fermentation, or by chemical synthesis, the latter providing for the bulk amount of carotenoid demand worldwide. Lutein-containing oleoresins from Tagetes grown in large scale mainly in India and China are extensively used for egg yolk and broiler pigmentation. Despite numerous intensive efforts to develop classically improved or genetically engineered carotenoid production strains, microbial carotenoid production is very limited, although the construction of the first recombinant carotenoid-producing Escherichia coli strain dates back almost 20 years ago. By contrast a sheer unlimited source for commercial β-carotene production could be crude palm oil, if cheap extraction methods to recover the highly diluted product from the oil could be developed.

Apart from being used as food colorants and animal feed supplements for poultry and aquaculture, carotenoids play an increasing role in cosmetic and pharmaceutical applications due to their antioxidant properties. The pigments are often regarded as the driving force of the nutraceutical boom, since they not only exhibit significant anticarcinogenic activities but also promote ocular health, can improve immune response, and prevent chronic degenerative diseases.

β-Carotene

The nutritional role of carotenoids in human diet is best understood in the context of their provitamin A activity. About 50 carotenoids are known that can function as provitamin A, of which β-carotene is the most efficient one. There are several colorful fruits and vegetables rich in the red-colored β-carotene, examples of vegetables include carrots and sweet potatoes, but the best source is food derived from animals, especially liver and whole milk. A daily intake of 3–6 mg of β-carotene is estimated to ensure a beneficial blood concentration of retinol equivalents, the animal form of vitamin A.

β-Carotene is an important industrially produced carotenoid. As estimated in a BCC (Business Communications Company Inc.) report of 2005, β-carotene accounted for roughly a quarter of the total carotenoid sales in 2004, totaling about US$242 million. DSM Nutritional Products (Switzerland) and BASF (Germany) dominate the market with their chemical synthesis processes, but Chinese competitors are catching up.

Microorganisms account for about 15% of industrial β-carotene production. Vitan Ltd. (Ukraine) and Vitatene (Spain) use the heterothallic zygomycete Blakeslea trispora, producing, after intensive classical strain improvement by random mutagenesis and selection, according to patent literature, up to 7 G L⁻¹ β-carotene in large-scale fermentations. Pigment production and accumulation is stimulated by several sex-specific pheromones, for example, trisporic acids, the synthesis of which commence after inoculation of the production fermenters with both B. trispora mating partners. The high β-carotene accumulation of 4% or more within the B. trispora biomass might be facilitated by the cell’s high triglyceride content. Filtration after the fermentation process, solvent extraction of the dried biomass, and crystallization result in a pure product.

An alternative natural β-carotene producer of commercial interest is the halophilic green microalga Dunaliella salina. It accumulates the pigments in oil globules in the chloroplast interthylakoid spaces, protecting them against photoinhibition and photodestruction. AquaCarotene Ltd. and Cognis take advantage of large, shallow ponds with high salinity in Western Australia that are exposed to intense solar radiation, in which the microalgae are cultivated. Other companies like Nature Beta Technologies with a production site in Israel employ open channels in the form of oblong raceways agitated by paddle wheels. Closed tubular systems are in an experimental stage. Excessive pigment formation in D. salina is achieved by numerous stress factors like high temperature, lack of nitrogen and phosphate but excess of carbon, high light intensity, and high salt concentration, the latter two having the highest impact. Whereas in open ponds β-carotene accumulates in the 100 mg m⁻² range, the obviously more capital-intensive raceway ponds can deliver up to 15 G m⁻². An average yearly productivity of around 200 mg m⁻² day⁻¹ has been reported. Harvesting the biomass present at low concentration in the corrosive cultivation brine, for example, by filtration after flocculation, requires special attention. Dried D. salina biomass for sale contains 10–16% carotenoids, mainly β-carotene. In addition crystalline material obtained after extraction with edible oil is also sold.
**Astaxanthin**

Astaxanthin is biosynthesized by providing both $\beta$-ionone rings of $\beta$-carotene with hydroxy- and o xo-moieties at C3 and C4, respectively. The intense dark red xanthophyll, which has no provitamin A activity, plays a role in pigmentation of various animals, conspicuous examples include wild and pen-reared salmon and red crabs, which compulsorily require astaxanthin in their diet. Although recent studies point to superior antioxidant properties compared with $\beta$-carotene, astaxanthin applications in nutraceuticals, cosmetics, and food industry are still scarce. The predominant use of the xanthophyll is as a pigment source, most notably in aquaculture of salmon and trout. Improved fertility and enhanced immune response are also ascribed to astaxanthin supplementation of the fish diet.

A BCC report released in 2005 estimated the astaxanthin market to be US$234 million in 2004. Astaxanthin sales account for 25% of the total carotenoid sales, similar to $\beta$-carotene. Chemical synthesis supplies the major part of commercial astaxanthin. A minor amount of astaxanthin originates from solvent extraction of crustacean waste or krill.

Microbial astaxanthin production, occupying a small proportion of the market, is based on the chromophyte microalga *Haematococcus pluvialis* and the basidiomycete yeast *X. dendrorhous*, originally named *Phaffia rhodozyma*.

*H. pluvialis* is grown heterotrophically under low irradiation in closed systems with an excess of organic carbon source such as acetate to achieve a high yield of green biomass, which is thereafter subjected to environmental and nutrient stress to induce aplanospore formation and astaxanthin accumulation. *H. pluvialis* cultures easily fall prey to contaminating microorganisms and are sensitive to extreme environmental conditions. A selective cultivation environment is not available. Therefore, the second, reddening cultivation stage can only be operated for periods not exceeding 5–6 days if open systems are used. Closed photobioreactors in the form of transparent tubes or illuminated stirred tanks might be more efficient systems, but the increased investment and operation costs have to be taken into consideration. *H. pluvialis* biomass is harvested by settling and subsequent centrifugation. After drying and fracturing of the thick cell wall to ensure bioavailability of the enclosed pigment, the biomass contains 1.5–3% of astaxanthin as the $3\delta, 3'\delta$ optical isomer. Several companies with production sites in Hawaii (Fuji, Mera, Cyanotech), Israel (Algatech), and Sweden (BioReal AB) offer *H. pluvialis* astaxanthin in the form of a dried algal powder or an oleoresin extract.

*X. dendrorhous* provides the $3\delta, 3'\delta$ optical isomer of astaxanthin. Mutants selected for increased astaxanthin productivity are cultivated in conventional stirred tank reactors. After harvesting, disintegration, and drying, biomass containing 0.5–1% pigment is marketed by companies like ADM (Ecotone) or Igene (Aquasta) (both in the United States). Although the *X. dendrorhous* genes encoding the enzymes of the biosynthetic pathway have been cloned and successfully overexpressed in *Saccharomyces cerevisiae* production of astaxanthin using metabolically engineered strains is difficult because unwished side products appear and the metabolic flux is too low.

**Polyunsaturated Fatty Acids**

PUFAs are vitamin-like compounds having important structural functions in cell membranes and lipid tissue, and serve as precursors for the biosynthesis of several hormones like leukotrienes, prostaglandins, and thromboxanes. PUFAs are alphatic monocarboxylic acids with two to six carbon–carbon double bonds in biologically active cis configuration within their C18–C24 carbon backbone. The double bonds are separated by two single carbon–carbon bonds resulting in a nonconjugated polyene system. PUFAs are categorized as $\omega$-3 or $\omega$-6, depending on the position of the first double bond on C3 or C6, respectively, when counted from the terminal carbon atom of the fatty acid. They are biosynthesized starting from monounsaturated C18 oleic acid by a series of desaturase and elongase reactions. The genes of most of the PUFA biosynthetic enzymes have been cloned from various organisms. Since mammals lack the ability to introduce double bonds at the $\omega$-3 or $\omega$-6 position of the fatty acid carbon backbone, linoleic acid ($18:2$, $\omega$-6) and $\alpha$-linolenic acid ($18:3$, $\omega$-3) are essential for them. Since the long chain PUFAs arachidonic acid ($20:4$, $\omega$-6; ARA) and docosahexaenoic acid ($22:6$, $\omega$-3; DHA) are incorporated in the developing brain, the neuronal tissue and retina of the human fetus and human breast milk contains these PUFAs studies with supplemented infant formula were performed. The correlation between long chain PUFAs content in the cerebral cortex of breastfed infants and better cognitive and visual function of infants fed with infant formula supplemented with long chain PUFAs suggested that the activity of the enzyme system of elongases and desaturases is suboptimal. Unfortunately, assays for both types of enzymes are not developed yet.

Commercial scale microbial processes for the production of DHA and ARA based on natural isolates or classically derived strains are available, which are discussed in some detail here. Attempts to employ molecular engineering techniques in PUFA production have also been published. One example is *S. cerevisiae*, not a natural PUFA producer, that when provided with the appropriate desaturase and elongase genes could perform one or several biosynthetic steps toward eicosapentaenoic acid ($20:5$, $\omega$-3; EPA) or DHA synthesis starting from the corresponding precursor fatty acids. A series of patent
applications from DuPont published in 2004 and 2005 disclosed EPA production in a genetically modified oleogenic yeast, *Yarrowia lipolytica*. Progress has been made to demonstrate that PUFAs, particularly ARA and EPA, can be obtained from transgenic plants, though significant technical obstacles in the development of PUFA-producing oil crops have also been encountered.

**Docosahexaenoic acid**

DHA, a 22-carbon ω-3 fatty acid with six double bonds, is found in membrane phospholipids of different cell types. For example, in the photosensitive part of the retina DHA accounts for more than 60% of the total fatty acid content. It functions as a signal molecule precursor, and has beneficial effects in prevention of cardiovascular diseases, probably by downregulating intracellular mechanisms leading to expression of proatherogenic genes. Fish, wild catch or from aquaculture, are the most important source of DHA in human diet. Fish cannot synthesize DHA by themselves but marine microorganisms at the lower end of the marine food chain are genuine DHA producers. Aquaculture is often fed with fish oil or fish meal, of which more than 1 million tons and 6 million tons, respectively, were produced in 2005. A negligible proportion of the oil is used for products for the human dietary supplement market. The fish meal industry maintains that fish meal and oil is produced from sustainably managed fish stocks. However, in the general perception, in view of the decade-long over-exploitation of common marine fish resulting in ever-reducing catch size, fish oil is not considered a sustainable source of DHA (and other PUFAs) and microbial alternatives have been developed.

The biomass of the marine microalga *Cryptothecodinium cohnii* consists of triglycerides with a high percentage of DHA, which is almost free of other PUFAs. In protracted (200–400 h) lab scale fed-batch fermentations with acetic acid or ethanol as the carbon source, about 100 G l⁻¹ biomass accumulates with a triglyceride content between 30 and 50%. DHA represents about a third of the fatty acids of the triglyceride phase obtained by solvent extraction.

The genera *Schizochytrium*, *Tbrasostechytrium*, and *Ulkenia*, marine heterotrophic protists classified as labyrinthulomycota, are also preferred sources of microbial DHA. In lab scale fermentations with a *Schizochytrium* sp. strain 60 G l⁻¹ dry cell mass was obtained with a fatty acid content of 70%, of which 37% was DHA. DHA-containing products obtained from protist fermentations are offered by Martek Biosciences and Advanced BioNutrition (both in the United States), focusing on the human nutrition and aquaculture market, respectively.

**Arachidonic acid**

ARA, a 20-carbon ω-6 fatty acid with four double bonds, is a natural component of breast milk, supporting neonatal eye and brain development. Breastfed infants have higher ARA blood levels than formula-fed infants. Infant formulas fortified with ARA and DHA, sold in Europe for more than 10 years, after permission by the Food and Drug Administration (FDA) were first introduced in the United States in 2002 and since then have become increasingly popular.

In contrast to DHA, which can be obtained from fish oil or microbial processes, industrial ARA production solely relies on fermentation processes. First publications on the zygomycete *Mortierella alpina* as a suitable ARA producer appeared in the late 1980s. With the *M. alpina* strains IS-4 or ATCC 32222 lab scale processes were developed affording about 10 G l⁻¹ ARA as triglyceride after 8–10 days fermentation runs in conventional stirred tank reactors. The morphology of the fungal biomass in the reactor is of specific concern. It should appear as small pellets with a diameter of 1–2 mm to allow for sufficient mass transfer and to keep the broth viscosity at an acceptable level. After fermentation, the *M. alpina* mycelium is harvested by centrifugation or filtration, dried, and solvent-extracted. Alternatively, the mycelium is homogenized as an aqueous suspension and extracted with a water-immiscible organic solvent.

Fungal ARA is produced and marketed as Arasby by Martek Biosciences (United States). In 2004, Cargill (United States), a leading supplier of agriculture products and food ingredients, entered into a joint venture with Wuhan Allking Bioengineering Co. Ltd. (Hubei Province, China), an ARA producer serving the Chinese infant formula business. Suntory, a leading Japanese beverage manufacturer and a pioneer in microbial PUFA production, provides a fungal ARA oil under the trade name Suntga.

**Water-Soluble Vitamins**

**Riboflavin**

Riboflavin is the common name of 7,8-dimethyl-10-(1’-ribityl)isoalloxazine, also known as vitamin B₂, colorant E101, lactoflavin, lactochrome, or ovoflavin. The latter names referring to the source the vitamin was derived from. The compound is naturally synthesized by plants and most microorganisms, but not by higher eukaryotes. Starting from GTP and ribulose 5-phosphate the riboflavin biosynthesis pathways of fungi and bacteria are similar, albeit the order of two consecutive biosynthetic steps, the reductase and deaminase reactions, is inversed. The genes encoding the riboflavin biosynthetic enzymes are well conserved among bacteria and fungi. Vitamin B₂ has key functions in energy metabolism, maintenance of healthy skin and muscles, support of immune and nervous system, and promotion of cell growth and division. Riboflavin is the precursor for the coenzymes FMN (flavin mononucleotide) and FAD.
(flavin adenine dinucleotide), which are both important electron carriers in biological redox reactions. Furthermore, the two flavo-coenzymes participate in nonredox phenomena like bioluminescence, light sensing, phototropism, DNA protection against UV, and in resetting of the circadian clock. Light sensitivity and poor resorption makes riboflavin deficiency recurrent, as suggested by worldwide surveys on nutritional status, and supplementation is often recommended. Overdosing due to dietary supplementation does not occur owing to the direct excretion of riboflavin in the urine. In industrialized countries processed food is often fortified by the use of riboflavin as a colorant or vitamin supplement. The main application (70%) of commercial riboflavin is in animal feed, since productive livestock, especially poultry and pigs, show growth retardation and diarrhea in case of riboflavin deficiency. According to a report by SRIC, a consulting company in Menlo Park (California), in 2005 the need for industrially produced riboflavin was estimated at 6500–7000 tons per year.

Industrial riboflavin production, a domain of the chemical industry for a long time, followed a six- to eight-step route starting from 3,4-xylidine, barbituric acid, and D-ribose. The latter is provided by the chemical conversion of glucose or more recently by fermentation from glucose using transketolase-deficient Bacillus sp. production strains. The first commercial single-stage riboflavin fermentations came up in the 1940s employing Clostridium acetobutylicum and, more efficient, the fungi Eremothecium ashbyi and A. gossypii. However, all three companies manufacturing riboflavin by fermentation in 1965 shut down their production plants in 1968 as their production processes were competitively disadvantaged to chemical processes. Nevertheless, riboflavin biosynthesis meanwhile has become the paradigm for environmentally superior white biotechnology replacing chemical production processes. Although Merck (Germany) resumed fermentation using A. gossypii in 1974, the success story started with an A. gossypii production plant by BASF (Germany) in 1990. The market share of riboflavin bioprocesses thereafter increased from 5% in 1990 to 75% in 2002, of which initially, the two naturally vitamin B2-overproducing fungi A. gossypii and Candida famata, and later, genetically modified strains of the bacterium B. subtilis were used. However, C. famata is not employed any more and chemical riboflavin production came to an end, leaving only A. gossypii- and B. subtilis-based production methods. Both processes are the outcome of classical strain improvement, genetic engineering, and process optimization.

The wild-type strain of the filamentous fungus A. gossypii, originally isolated as a severe but today negligible cotton pathogen, produces 2 mg riboflavin per gram of biomass, possibly for light-protection of its spores. Adjustment of process parameters allows a production above 100 mg G\(^{-1}\) biomass even by wild-type strains. However, at the industrial scale the performance of A. gossypii production strains was increased by analysis of the pathway from the very first enzyme, an extracellular lipase hydrolyzing plant triglycerides serving as the carbon source, to the carrier systems transporting the vitamin into the vacuole or extracellular space. Within a decade the production process was improved by (1) optimization of culture conditions, that is, different medium compositions for growth and production phases, (2) selection of antimetabolite-resistant mutants, that is, using inhibitors against key enzymes limiting reaction velocity like isocitrate lyase, (3) overexpression of RIB genes, that is, integration of additional copies of RIB encoding the enzyme converting ribulose-5-phosphate into 3,4-dihydroxy-2-butanone-4-phosphate (DHBP), and (4) decreasing the flux to unwanted side products by disruption of genes, for example SHM2 encoding cytosolic serine hydroxymethyl transferase that converts glycine, a precursor of riboflavin, into l-serine. Metabolic design of A. gossypii is possible by self-cloning, gaining stable genomic integrations targetable by homologous recombination. Large-scale fermentation of A. gossypii is advantageous because extracellular hydrolases allow high loads of osmotically inactive carbon sources like triglycerides removing the need for controlled carbon feeding and autolysis of the vitamin-accumulating cells can be controlled by a temperature shift.

B. subtilis, a soil bacterium, strains of which are traditionally used in food preparation in Asia, for example, for the soy bean fermentation product natto, is capable of synthesizing industrially relevant amounts of vitamin B\(_2\) only after genetic engineering. In wild-type strains, riboflavin biosynthesis is minimized to the physiological needs of the bacterium by a tight riboswitch transcriptional attenuation mechanism of the rib genes. A suitable B. subtilis host strain was obtained by resistance screening against three purine analogues (8-azaguanine (S1), decoyinine (S2), and methionine sulfoxide (S3)) (Figure 1), yielding mutants with a deregulated purine synthesis pathway that provided an enhanced availability of the riboflavin precursor GTP. Starting from a purine-analogue resistant strain, a mutant resistant against the riboflavin analogue roseoflavin was selected (S4). Later it turned out that flavo-coenzyme-mediated attenuation of rib gene expression was abolished in this mutant, which expressed a flavokinase with drastically reduced activity causing reduced intracellular FMN and FAD levels. A defect in adenylsuccinate synthetase causing a block in the flux from IMP (inosine monophosphate) to AMP (adenosine monophosphate) was advantageous for purin precursor production, but the resulting adenine auxotrophy was detrimental to riboflavin overproducing strains. Overexpression of the B. subtilis rib operon comprising all relevant rib genes (ribA, ribB, ribH, ribB) by use of a phage promoter, multicopy genomic insertion of the engineered operon, and by providing an additional expression cassette for ribA encoding a bifunctional enzyme catalyzing
two entrance reactions of the converging riboflavin pathway led to a production strain fit for industrial application (GE1 and GE2). Meanwhile, a new generation of marker-free, self-cloned *B. subtilis* production strains have been constructed, achieving high production performance with a single copy of a precisely engineered rib operon. A mutation induced in the *tkt* gene reduces but not completely abolishes the enzymatic activity of the encoded transketolase enzyme, thus allowing higher intracellular concentrations of the intermediate ribulose-5-phosphate.

Rational design of high performance *A. gossypii* or *B. subtilis* production strains to channel a major metabolic flux toward the target compound and to prevent drainage of resources into side or waste products became possible only after a detailed understanding of riboflavin biosynthesis was available. Construction of the strains depended on a filled tool box for mutant screening, DNA synthesis, and recombination. PCR technologies and synthetic genes including their regulatory DNA components facilitated precision engineering to avoid surplus DNA and antibiotic markers in the final production strains.

During the fermentation process, riboflavin is exported from the cells into the medium and crystallizes in the fermentation broth due to its low solubility in neutral aqueous media. In *A. gossypii* additionally a transport into the vacuoles occurs. The long, needle-shaped crystals can easily be recovered from the fermentation broth and separated from the biomass by several rounds of centrifugation and resuspension in water. Microbial riboflavin production at an industrial scale turned out to be both cost-effective and environmentally friendly compared with conventional chemical synthesis: carbon dioxide emissions and use of nonrenewable resources were reduced by 80% each and water emissions by 66%. Fermentation products for food and pharmaceutical applications offer a higher purity as aniline and other impurities found with chemical synthesis are absent. For feed applications, products with reduced purity are accepted since the impurities consist of residual biomass with additional nutritional value.

Major riboflavin producers are DSM Nutritional Products (Switzerland) and Hubei Guangji (Hubei Province, China), both using genetically engineered *B. subtilis* production strains, and BASF (first in Germany but now in South Korea), employing genetically engineered *A. gossypii*.

![Figure 1](image-url) Important steps in manipulation of biochemical and regulatory pathways to obtain riboflavin overproduction by *Bacillus subtilis*. Simplified scheme of metabolic steps (arrows) and relevant regulations (broken lines) from the main carbon source glucose to the targeted product riboflavin. $\uparrow$ and $\downarrow$ mark increased or decreased fluxes or activities, as appropriate, all with a positive effect on riboflavin production. Labels depict metabolic steps or regulations affected in antimetabolite selected mutants (S) or genetically engineered strains (GE). See text for details. gluc-6p, glucose-6-phosphate; ribu-5p, ribulose-5-phosphate; IMP, inosine monophosphate; AMP, adenosine monophosphate; XMP, xanthosine monophosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; DHBP, 3,4-dihydroxy-2-butanone 4-phosphate; DRL, 6,7-dimethyl-8-ribityllumazine; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide.
Industrially produced vitamin B₁₂, used as a nutritional supplement, by definition is an artificial cobalamin compound with a cyano ligand derived from natural cobalamins like adenosyl-, methyl-, or hydroxy cobalamins. However, the term vitamin B₁₂ is often also used for the natural cobalamins. Cobalamins are the chemically most complex of all vitamins, consisting of a 15-membered, planar corrin ring with a central cobalt ion (hence the name), a dimethylbenzimidazole group as the lower ligand, and an adenosyl-, methyl-, hydroxy-, or cyano group as the upper ligand, giving rise to the aforementioned four cobalamin compounds. Vitamin B₁₂ is an essential nutrient for plants and animals, since only some bacteria and archaea can synthesize it de novo. Among enteric bacteria two pathways, an aerobic pathway as in *Pseudomonas denitrificans* and an anaerobic pathway as in *Propionibacterium* or *Salmonella typhimurium*, have been identified for the initial corrin ring formation and the insertion of cobalt. In the prehistoric environment where the atmosphere lacked oxygen today’s corrinoid enzyme originated enabling anaerobic metabolism in which carbon dioxide is reduced to methane. With increasing oxygen in the atmosphere and the evolution of porphyrin compounds like heme or chlorophyll, cobalamin-dependent enzymes gained function in other methyl-dependent reactions. In human physiology, adenosylcobalamin and methylcobalamin are important coenzymes linked to the formation of blood, fat metabolism, and the normal functioning of the nervous system and the brain. Vitamin B₁₂ deficiency causes, among others, several forms of anemia, and has been long used as a diagnostic tool. Recent surveys point to a high proportion of inadequate intake, even in industrial countries, and the need of nutritional supplementation, especially for vegans and elderly people.

Industrial vitamin B₁₂ production has never been carried out by the complex chemical synthesis route, comprising over 70 steps. Instead, cobalamins initially extracted from feces, sapropel, or *Streptomyces* sp. cultures used for antibiotic production were exclusively manufactured by fermentation, employing species of *Bacillus*, *Methanobacterium*, *Propionibacterium*, or *Pseudomonas*. The global production in the range of 3 tons year⁻¹ is low, but a selling price of several thousand euros kg⁻¹ drew a number of Chinese producers into the market that was dominated by the French Sanofi-Aventis. As a result, the market is currently characterized by severe production overcapacities and concomitant price pressure.

Nowadays, the bacterium *P. denitrificans* is almost exclusively used as the production organism. Investigations of the vitamin B₁₂ biosynthesis pathway in *P. denitrificans* and cloning of the 22 *cob* genes of the organism allowed researchers at Rhone Poulenc to construct genetically engineered production strains presumably providing a technological advantage in terms of productivity and yield on raw materials over strains solely obtained by classical strain improvement. Glycine betaine present in significant amounts in sugar beet molasses or the biosynthetic precursor choline chloride are indispensable for high productivity. The fermentation medium also provides a cobalt salt and dimethylbenzimidazole as components of the cobalamin molecule. During the 7-day fermentation run, adenosylcobalamin is predominantly secreted from the biomass and accumulates in the fermentation broth in milligram amounts. The downstream steps comprise filtration, cyanide treatment, chromatography, extraction, and crystallization yielding vitamin B₁₂ in high purity. However, optimization of recombinant B₁₂ synthesis is still under investigation, wherein the most likely host is *P. denitrificans*.

**L-Ascorbic Acid**

Vitamin C probably has the most interesting history of all vitamins. The famous Ebers papyrus from 1550 BC already describes the avitaminose symptoms of scurvy, a formerly common disease among mariners and explorers. Scurvy resulting from a lack of vitamin C in the human diet leads to collagen instability causing *inter alia* loss of teeth and bleeding of all mucous membranes. Vitamin C is chemically defined as L-ascorbic acid, a weak six-carbon sugar acid with a pentagonal ring structure. The exocyclic C₃ has L-configuration, and in higher organisms only the L-enantiomer of ascorbic acid is biologically active. The pivotal functions making vitamin C essential are both the high antioxidant capacity and cofactor effects in the synthesis of collagen, carnitine, and hormones like adrenaline. For use as a food additive, several E numbers represent different chemical forms of vitamin C, that is, E300 (free ascorbic acid), E301–303 (ascorbate salts of sodium, calcium or potassium, respectively), E304 (fatty esters), and E315 (erythorbic acid, a stereoisomer). Starting from D-glucose, L-ascorbic acid biosynthesis follows different routes in plants and animals. L-Galactono-γ-lactone, the ultimate precursor of the plant pathway, is reached via an inversion of the D-glucose carbon skeleton. In animals, L-gulono-γ-lactone is obtained by three epimerization reactions. All plants and most animal species are prototrophic for vitamin C. Nevertheless, humans, higher primates, guinea pigs, and a small number of other animals require vitamin C supplementation of their diet due to a defective GULO gene encoding L-gulono-1,4-lactone oxidase, the last enzyme in the
synthesis pathway in animals. Fungi of the genera Zygomycetes, Ascomycetes, or Basidiomycetes synthesize d-erythrosaccarate, a C5 analogue of ascorbate, from d-arabinose via an inversion pathway.

L-Ascorbic acid and its chemical derivatives are by far the most bulk produced vitamins with about 110,000 tons year⁻¹. Chinese manufacturers entering the vitamin C market in the early 1990s caused a ruinous competition. DSM Nutritional Products Ltd. from Switzerland (formerly Roche Vitamins), the sole Western producer, positioned itself in the premium segment of the market. Chinese companies like Weisheng Pharma, North China Pharmaceutical Company (both in Hebei Province), and Jiangshan Pharmaceutical Co. (Jiangsu Province) became the leading producers for the bulk market. In contrast to other vitamins, feed applications of L-ascorbic acid account for only 10%, whereas the main uses are in the pharmaceutical industry (50%), food (25%), and beverages (15%). Pharmaceutical applications include stimulation of collagen synthesis (especially cosmetic products) and high antioxidant capacity, used for the reported health benefits in the prevention of flu, heart diseases, and cancer, as well as an antidote for poisoning. The food and beverage industry predominantly exploits the antioxidant capacity of L-ascorbic acid to extend durability, prevent discoloration, and to protect flavor and nutrient contents of their products.

Industrial production of vitamin C initially started by extraction from fruit. The first chemical process based on L-xyllosone was carried out in 1933. In 1934, the famous Reichstein–Grüssner process was published. The process starts with a chemical hydrogenation of d-glucose to d-sorbitol, followed by the oxidation of the sugar alcohol to l-sorbose using Gluconobacter sp. strains as biocatalysts. The subsequent chemical steps including acetonization, oxidation, deacetonization, and rearrangement of the oxidation product 2-keto-l-gluconic acid (2KGA) deliver L-ascorbic acid. Although more than 70 years old, the technique with a typical yield of above 50% on starting glucose is still in use, a testimony to the fact that the advantages of long-term process optimization still allows classical methods to compete with more modern microbial-based production methods.

Chinese vitamin C manufacturers produce 2KGA by a two-stage microbial process developed in China in the late 1960s and 1970s. As in the Reichstein process, l-sorbose is provided by Gluconobacter sp. oxidation of d-sorbitol (first stage). In a second fermentation step, replacing the chemical oxidation steps, 2KGA is obtained via l-sorbose employing Ketogulonicigenium sp. strains, in Chinese literature frequently referred to as Gluconobacter oxidans. For efficient oxidation co-cultivation with a helper strain, for example, Bacillus megaterium, is mandatory but the underlying molecular mechanism is still unclear. The execution of the process in two steps is necessary to prevent Ketogulonicigenium sp. from metabolizing d-sorbitol, which would lead to the production of unwanted d-glucose and its oxidation products. Finally, as in the Reichstein process, 2KGA is chemically converted to l-ascorbic acid. The two-stage process can achieve up to 130 G l⁻¹ 2KGA with a yield of above 80% on d-sorbitol. The reduced number of process steps compared to the Reichstein process, less process complexity, lower investment costs, less energy demand, and lower chemical consumption make the two-stage process advantageous.

G. oxidans alone is able to oxidize d-sorbitol to 2KGA, however, even processes based on genetically engineered G. oxidans strains cannot match the space–time yield of mixed culture processes.

Alternative microbial routes to 2KGA synthesis starting from d-glucose via 2,5-diketo-D-gluconate have been developed employing Erwinia sp. and Corynebacterium sp. in a two-step tandem process or by using genetically engineered Erwinia sp. expressing a gene for a Corynebacterium 2,5-diketo-reductase. These processes have yet to make it to industrial realization.

In summary, all vitamin C bioprocesses developed to an industrial stage so far result in the formation of the precursor compound 2KGA, requiring a cost-driving chemical rearrangement step to achieve the final product. Obviously, numerous attempts have been made to design microbial synthesis routes that result in direct synthesis of vitamin C. One approach tried to utilize the biosynthetic capacity of baker’s yeast to synthesize the five-carbon vitamin C analogue d-erythrosaccaric acid. Indeed, genetically engineered S. cerevisiae strains produced minor amounts of L-ascorbic acid starting from L-galactose, which should be supplied from a combination of epimerase and isomerase reactions starting from the Reichstein intermediate l-sorbose. In a second approach, microalgae synthesizing vitamin C according to the plant biosynthetic pathway via L-galactono-γ-lactone yielded up to 2 G l⁻¹, the synthesized vitamin mainly associated with the biomass. Although cheap d-glucose was the fermentation substrate, the reported low productivity renders a commercial application of the microalgae system rather unlikely. However, a promising direct route to vitamin C might has been opened by the discovery of dehydrogenases present in Ketogulonicigenium sp. and Gluconobacter sp. that convert l-sorbose, the partially oxidized biosynthetic intermediate of microbial 2KGA processes, into vitamin C.

Production Efforts for Other Vitamins

R-Pantothenic acid

Efforts on developing microbial production processes for water-soluble vitamins are not limited to the vitamins B₂, B₁₂, and C, as discussed in detail in the previous sections.
The next vitamin to be industrially produced by a fermentation process might be \( R \)-pantothenic acid, also known as vitamin B\(_5\). \( R \)-Pantothenic acid plays a vital role for a multitude of metabolic processes in all living cells, serving among others as a building block for the biosynthesis of coenzyme A. Pantothenate is synthesized by bacteria, fungi, and plants, but not by mammals, including livestock and humans. The current worldwide demand of more than 5000 tons is produced by chemical synthesis involving a racemic resolution step that can be carried out biocatalytically at the industrial stage.

Biosynthesis of pantothenic acid starting from the common metabolic intermediates \( \alpha \)-ketoisovalerate and aspartate is well understood. The products of the \( panB \) and \( panE \) genes are involved in the conversion of \( \alpha \)-ketoisovalerate to \( R \)-pantothenate. The \( panD \) gene product is a pyruvyl-dependent decarboxylase converting aspartate to \( \beta \)-alanine. The ATP-dependent \( panC \) gene product combines the two pantothenic acid precursors into the final product. Biotechnical approaches based on genetically modified \( E. coli \), Corynebacterium glutamicum, and \( B. subtilis \) have been reported. The \( E. coli \) process developed by Takeda Chemical Industries Ltd. (Japan) delivers a respectable \( 66 \text{ G l}^{-1} \) of \( R \)-pantothenic acid in a 72 h fermentation run, but requires \( \beta \)-alanine as a co-substrate. The fact that in the \( E. coli \) process 40% of the molecular mass of pantothenic acid is not provided by the basic carbohydrate fermentation substrate, but originates from the processed chemical \( \beta \)-alanine should not be without disadvantageous economic consequences. The \( B. subtilis \) procedure developed by Omnigene Inc. (United States) shows an even higher productivity with \( 86 \text{ G l}^{-1} \) \( R \)-pantothenic acid accumulating within 48 h of fermentation. Interestingly, sufficient \( \beta \)-alanine is provided endogenously in this process superseding exogenous \( \beta \)-alanine supply.

**\( \delta \)-Biotin**

Biotin, also known as vitamin H or \( B_7 \), is an important catalyst in essential metabolic conversions like gluconeogenesis and fatty acid synthesis. Usually produced in excess by intestinal bacteria, biotin is mainly of cosmetical interest as it strengthens hair and nails. Significant efforts have been made to design a microbial process for \( \delta \)-biotin, which is currently produced in a complex multistep chemical process. Tanabe Seiyaku Ltd. (Japan) in the early 1990s developed a process based on genetically modified bacterium *Serratia marcescens* affording \( 540 \text{ mg l}^{-1} \) of \( \delta \)-biotin during 144 h of fermentation. Biotin-overproducing recombinant *Kurthia* sp. and *B. subtilis* were developed at Nippon Roche (Japan) and Omnigene Inc. (United States), respectively.

Biotin biosynthesis involves pimeloyl-CoA, a derivative of the seven-carbon dicarboxylic pimelic acid. In *B. subtilis* pimelic acid and alanine are converted to dethiobiotin via four biosynthetic steps catalyzed by the gene products of *bioW*, *bioF*, *bioA*, and *bioD*. The subsequent BioB-catalyzed conversion of dethiobiotin to biotin involves the formation of an adenosyl radical. Interestingly, one of the two iron–sulfur clusters of BioB is probably the immediate sulfur source of biotin. This remarkable BioB reaction attracted much scientific attention, but is still incompletely understood. Solving the BioB enigma is regarded as a key milestone toward developing a competitive microbial biotin production process.

**Vitamins \( B_1, B_6 \) and folic acid**

Serious efforts have been devoted to the development of microbial production processes for vitamins \( B_1 \) (thiamine), \( B_6 \) (pyridoxol), and folic acid based on various bacterial host strains including *E. coli*, *B. subtilis*, and *Rhizobium mellioti*. In all cases, genetic engineering approaches were applied to deregulate and overexpress the genes known to be involved in the respective metabolic pathways. Although the engineered strains produced significantly higher levels of the vitamins compared with their wild-type ancestors, their productivities were far too low for commercial application. As in the case of biotin, it seems that an incomplete understanding of the biochemical fundamentals is a serious impediment for the development of competitive fermentation processes for these vitamins.

**Conclusions and Outlook**

All microbial processes discussed in this article are part of white biotechnology, which in contrast to red biotechnology (medical sector) and green biotechnology (genetically engineered crops) deals with industrial application of biological systems to produce chemicals, materials, and energy. Several recent studies estimate the current share of biotechnical processes in various chemical productions to increase from about 5 to 20% by 2010. Fine chemicals with high functionality and a typical annual worldwide demand below 10,000 tons year\(^{-1}\), for example, vitamins, are expected to be a future market for bioprocesses, with an estimated fivefold increase in their world market value to around US$250 billion within the next 10–20 years. By 2010, 30–60% of all fine chemical production is assumed to involve a biocatalytic step, whereas, in the same timeframe, 6–12% of today’s chemically manufactured bulk products and polymers (production greater than 10,000 tons year\(^{-1}\)) is anticipated to be biotechnically produced.

Important products of white biotechnical are food ingredients, vitamins, biocolorants, solvents, degradable bioplastics, and biofuels, in which typically microorganisms or isolated enzymes quickly catalyze even crucial chiral products with a remarkable specificity. This goes along with a reduction in emissions, water usage, energy
demand, and waste. White biotechnical applications for animal or plant cells are also conceivable, but microorganisms are usually preferred due to their faster growth, easier handling, and often relatively easily modifiable genetic material. As in vitamin B<sub>2</sub> production, coexistence of an obviously faster cultivation process with a bacterium and a process applying a more slowly growing filamentous fungus is possible. Hence other advantages like substrate quality or final product concentration have a significant impact. To develop a new bioprocess for vitamin synthesis, in principle two general strategies are feasible, albeit a successful production strain is often the result of both approaches. The first strategy starts with screening for natural overproducers, that is, species already producing the compound of interest in considerable amounts. The yield is then consecutively improved by several rounds of mutagenesis (by chemicals or UV radiation) and selection, for which minimal knowledge about the metabolism and genetic organization of the microorganism employed is sufficient. On the contrary, the second tactic, namely targeted deregulation of metabolism by application of recombinant DNA techniques, is only appropriate for thoroughly studied organisms. Apart from the need for sufficiently effective means of genetic manipulation, pathway elucidation is an important prerequisite for this approach. The economic success story of commercial riboflavin production by <i>B. subtilis</i> is a proof of concept of how satisfactorily the second strategy can work. Moreover, innovative methods like altering metabolic flux and metabolic control studies, as well as transcriptome analyses, advanced screening techniques (such as using novel antimetabolites), and computer simulations further support the re-education of long-time microbial workhorses into production strains of new compounds. However, even for genetically well-known organisms, still some fundamental dilemmas exist. First, elucidation of both the synthesis pathway and the transport processes of product, intermediates, and co-metabolites as yet needs time-consuming and costly basic or pure research. Publication of genomes also is only the beginning of protracted discovery of the function of numerous unknown open reading frames. For instance the genomes of <i>B. subtilis</i> and <i>A. gossypii</i> have been publicly available for years but only riboflavin importer genes of <i>B. subtilis</i> and <i>S. cerevisiae</i> were identified recently. Kinetic studies have shown that in <i>A. gossypii</i> at least an uptake and a separate export system plus a proton gradient-driven system in the vacuolar membrane must exist. Moreover, metabolic enhancement of several key enzymes in compound synthesis and transport is significantly impeded due to the lack of direct enzyme activity assays, either because the enzyme of interest is membrane-bound or owing to a shortage of sufficient test-substrate availability. As described above for vitamins B<sub>12</sub> and B<sub>2</sub> production, actual availability for the microbial cell is the crux of highly efficient fermentation, wherein choice of feedstock as well as supplementation with additional precursors and regulative compounds fundamentally affect the financial survival of the microbial fabrication technique versus chemistry. Likewise, as for vitamin C production with yeasts and microbial vitamin B<sub>5</sub> synthesis, supplementation costs often render a bioprocess uneconomical until genetic engineering overcomes its limitations or changes in the general framework, such as decreasing prices for the supplements needed or increasing production costs of competing chemical synthesis, become effective. However, some microbial production concepts have no chance of success because of the intrinsic characteristics of the available or preferred microorganisms. An example is the production of fat-soluble vitamins, which not only needs precursors but also a storage depot within the cell, hence excluding bacteria in the production process. Nevertheless, microorganisms have already proven to exhibit an outstanding versatility in metabolism, although as yet only a small fraction of existing species has been discovered and classified. Screening for new isolated strains from seawater, soil, or extreme habitats is thus still a common method in the development of novel bioprocesses, in particular for delicate challenges like synthesizing PUFAs. Moreover, unveiling of vitamin-like functions of novel compounds is expected since, additional to the almost six thousand bioactive metabolites described so far in the literature, the potential industrial capacity of marine microorganisms, to name only one group, is estimated by hundreds of compounds having industrially interesting novel structures and properties.

Given the undisputed beneficial effects of an adequate vitamin supply in food and feed, the question raised now is how this demand can be satisfied. Since fossil and natural resources (e.g., fish oil) are depleting, in the long term a transition from a fossil-based to a bio-based economy and society is needed. Apart from microbial production processes, green chemistry offers a short-term cost-efficient perspective, in which plant products, such as oils or sugars, are microbially converted into vitamins. A more direct and long-term approach is the metabolic engineering of the plants themselves to increase their nutritional value. A prime example of this strategy is ‘golden rice’ enriched in provitamin A, manufactured since the late 1990s.

Despite all advantages, there are still many factors that impair the transformation from conventional to biotechnical industry. Many reports conclude that the main barrier in the industry is because of conservative thinking, summoning up the lack of knowledge both on bioprocess alternatives and on environmentally crucial steps of the current processes in plant management, as well as a lack of suppliers and advertising of biotechnical intermediate or end products. Moreover, international competition often
allows little room and little demand for investments in new technology. However, since fossil resources are diminishing, pollution control becoming increasingly expensive, and customers asking for environmentally friendly products, white biotechnical is boosted. Thus Europe and the United States, the current market leaders, are anticipated to increase state-of-the-art research and industrial implementation of new bioprocesses. Although fermentation processes are already being applied in the synthesis of several vitamins and vitamin-like compounds, often the final aim is the total replacement of chemical synthesis by feasible and economic biocatalytic systems using cost-efficient and sustainable raw materials. It remains to be seen in the following years, whether the above described biotechniques are sufficiently efficient to compete with chemical synthesis, imminent or existing combined methods, or upcoming genetically engineered vitamin-enriched crops.

See also: Industrial Biotechnology, (overview); Industrial Fermentation Processes; Metabolic Reconstruction; Pigments, Microbial; Strain Improvement

Further Reading