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review

Microbial Production of Food Grade Pigments

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Summary

The controversial topic of synthetic dyes in food has been discussed for many years. The scrutiny and negative assessment of synthetic food dyes by the modern consumer have raised a strong interest in natural colouring alternatives. Nature is rich in colours (minerals, plants, microalgae, *etc.*), and pigment-producing microorganisms (fungi, yeasts, bacteria) are quite common. Among the molecules produced by microorganisms are carotenoids, melanins, flavins, quinones, and more specifically monascins, violacein or indigo. The success of any pigment produced by fermentation depends upon its acceptability on the market, regulatory approval, and the size of the capital investment required to bring the product to market. A few years ago, some expressed doubts about the successful commercialization of fermentation-derived food grade pigments because of the high capital investment requirements for fermentation facilities and the extensive and lengthy toxicity studies required by regulatory agencies. Public perception of biotechnology-derived products also had to be taken into account. Nowadays some fermentative food grade pigments are on the market: *Monascus* pigments, astaxanthin from *Xanthophyllomyces dendrorhous*, Arpink Red from *Penicillium oxalicum*, riboflavin from *Ashbya gossypii*, β-carotene from *Blakeslea trispora*. The successful marketing of pigments derived from algae or extracted from plants, both as a food colour and a nutritional supplement, reflects the presence and importance of niche markets in which consumers are willing to pay a premium for »all natural ingredients«.

Key words: microbial pigment, food ingredient, carotenoid, *Monascus*, *Brevibacterium*

Introduction

Colour plays a special role with the food we eat. For example, when confronted with an unattractive colour, the consumer assumes that the food is poor or spoiled. On the other hand, products with atypical colour – for example, green cheese or blue drink – in most cases are rejected by the consumers (*1*). Typically, one associates colours with food items such as cherry with red, lemon with yellow, or orange with carrot. Therefore, colours can serve as the primary identification of food and are also a protective measure against the consumption of spoiled food. Colours of foods create physiological and psychological expectations and attitudes that are developed by experience, tradition, education and environment: »We inevitably eat with our eyes« (*2*).

The controversial topic of synthetic dyes in food has been discussed for many years. The scrutiny and negative assessment of synthetic food dyes by the modern consumer have given rise to a strong interest in natural colouring alternatives. Some companies decided to col-

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our food with food, using mainly plant extracts or pigments from plants, *e.g.* red from paprika, beetroots, berries or tomato; yellow from saffron or marigold; orange from annatto; green from leafy vegetables, *etc.* (*3*).

Penetration of the fermentation-derived ingredients into the food industry is increasing year after year. Examples could be taken from the following fields: thickening or gelling agents (xanthan, curdlan, gellan), flavour enhancers (yeast hydrolysate, monosodium glutamate), flavour compounds (gamma-decalactone, diacetyl, methyl ketones), acidulants (lactic acid, citric acid), *etc*. Some fermentation-derived pigments, such as β-carotene from the fungus *Blakeslea trispora* in Europe or pigments from *Monascus* in Asia, were developed later on and are now in use in the food industry (Fig. 1) (*4–6*). Efforts have been made in order to reduce the production costs of fermentation pigments compared to those of synthetic pigments or pigments extracted from natural sources. Innovations will improve the economy of pigment production by isolating new or creating better microorgan-

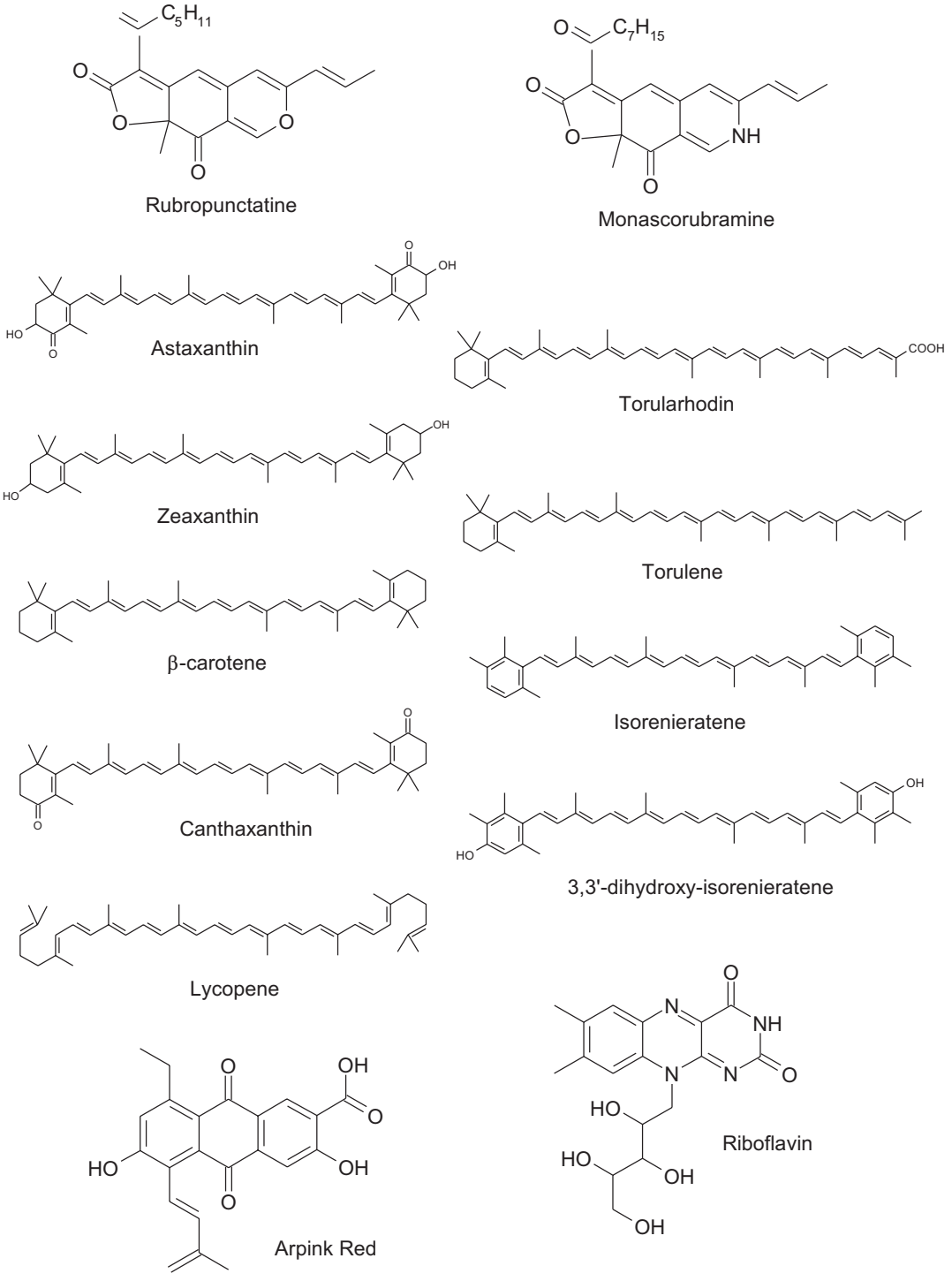

Fig. 1. Chemical formulae of some microbial food grade pigments

isms, by improving the processes. This review focuses on research works related to this field published over the past ten years by private companies or academic laboratories. As recently described by our group (*7*), there is a long way from the Petri dish to the market place, and thus to the food product on store shelves.

Food Grade Pigments from Fungi

Blakeslea trispora: when sexual mating is highly effective for β-carotene production

The source organism, the mould *Blakeslea trispora*, is a plant commensal of tropical plants, some strains of which produce high levels of β-carotene. The fungus exists in (+) and (–) mating type, of which the (+) type synthesises trisporic acid, a precursor of β-carotene. Mating the two types in a specific ratio, the (–) type then produces large amounts of β-carotene. The mould has been shown to be nonpathogenic and nontoxigenic. Standard pathogenicity experiment in mice and the analyses of extracts of several fermentation mashes and of the final product, the β-carotene crystals, were done according to literature, while enzyme immunoassays were performed for 4 mycotoxins.

The production process proceeds essentially in two stages. Glucose and corn steep liquor could be used as carbon and nitrogen sources. By-product of cheese manufacture, *i.e.* whey, has also received consideration (*8*), with strains acclimatized to lactose. For fermentation, seed cultures are produced from the original strain cultures and subsequently used in an aerobic submerged batch fermentation to produce a biomass rich in β-carotene. In the second stage, the recovery process, the biomass is isolated and transformed into a form suitable for isolating β-carotene, which is extracted from the biomass with ethyl acetate, suitably purified and concentrated, and the β-carotene crystallised from the mother liquor. The final product is either crystalline β-carotene (purity>96.0 %) or it is formulated as a 30 % micronised suspension in vegetable oil. The production process is controlled by GMP procedures, adequate hygiene control, and adequate control of the raw materials. The biomass and the final crystalline product comply with an adequate chemical and microbiological specification and the final crystalline product also complies with the JECFA (Joint FAO/WHO Expert Committee on Food Additives) and EU specifications as set out in Directive 95/45/EC for colouring matters in food.

Formerly Gist-Brocades, now DSM was the first company to produce β-carotene from this fermentative source. The product was launched in 1995 at the Food Ingredients Europe business meeting in London. As the first comer on the European market, its experience could be useful for other companies intending to sell fermentative food grade pigments. Following the optimization of the fermentation process, the company addressed many aspects before selling the product:

- **presentation of the microorganism:** fungus isolated from nature, not genetically modified, yield improvement using classical genetics,
- **guidelines for labelling:** natural β-carotene, natural β-carotene from *Blakeslea trispora*, fermentative natural β-carotene, natural β-carotene from a fermentative source,
- **lobbying by other β-carotene producers (nature-identical, mixed carotenes from palm oil, β-carotene from the microalgae *Dunaliella, etc.*):** the EU Health and Consumer Protection Directorate General was asked to give an opinion on the safety of β-carotene from a dried biomass source, obtained from a fermentation process with *Blakeslea trispora* for use as a colouring matter for foodstuffs,
- **safety of the fermentation-produced β-carotene:** HPLC analysis, stability tests and microbiological tests have shown that the β-carotene obtained by fermentation of *Blakeslea trispora* complies with the EC specification for E 160 *aii*, also including the proportions of *cis* and *trans* isomers, and is free of mycotoxins or other toxic metabolites. *In vitro* tests for gene mutations and chromosomal aberrations with the β-carotene produced by the manufacturer in the EU showed it to be free of genotoxic activity. In a 28-day feeding study in rats with the β-carotene manufactured in the EU no adverse findings were noted at a dose of 5 % in the diet, the highest level used. In conclusion, evaluation of the source organism and the production process yielded no grounds to suppose that the final crystalline product, β-carotene, differs from the chemically synthesised β-carotene used as a food colorant. The final crystalline fermentation product has been shown to comply with the specification for β-carotene E 160 *aii* listed in Directive 95/45/EC. The committee considers that β-carotene produced by fermentation of *Blakeslea trispora* is equivalent to the chemically synthesised material used as food colorant and is therefore acceptable for use as a colouring agent for foodstuffs (*9*).

Today there are two other industrial productions of *B. trispora* fungal β-carotene, the first in Russia and Ukraine, the second in León (Spain), where the Vitatene began its activities in 2004 (*10*). The process was developed to get up to 30 mg of β-carotene/g dry mass or about 3 g/L.

Pigments from Monascus, on sale for centuries in Asia

Cultivation of *Monascus* on solid medium has been practiced in Asian countries to produce a red colorant named »Anka«, used as a food ingredient. In a Chinese medical book on herbs published in the first century, this term »ang-kak« or »red mould rice« was first mentioned. Red mould rice has been used as food colorant or spice in cooking. A purple mould was first isolated on potato and linseed cakes and named *Monascus ruber*. This ascomycete was so named as it has only one polyspored ascus. A second strain was isolated from the red mould rice obtained from the market in Java, Indonesia. This fungus was named *Monascus purpureus*. Then several other species were isolated around the world. *Monascus* is often encountered in oriental foods, especially in Southern China, Japan and Southeastern Asia. Currently, more than 50 patents are beeing issued in Ja-

pan, the United States, France and Germany, concerning the use of *Monascus* pigments for food (*11*). Annual consumption of *Monascus* pigments in Japan moved from 100 tonnes in 1981 to 600 tonnes at the end of the nineties and was valued at $1.5 million. New food applications, like the coloration of processed meats (sausage, ham), marine products like fish paste, surimi and tomato ketchup were described.

In the fungus *Monascus*, ankaflavine and monascine are yellow pigments, rubropunctatine and monascorubrine are orange and rubropunctamine and monascorubramine are purple (*12*). The same colour exists in two molecular structures differing in the length of the aliphatic chain. These pigments are produced mainly in the cell-bound state. They have low water solubility, are sensitive to heat, unstable in the pH range from 2 to 10 and fade with light. A number of methods have been patented in order to make water-soluble pigments. The principle is the substitution of the replaceable oxygen in monascorubrine or rubropunctatine by nitrogen of the amino group of various compounds such as aminoacids, peptides and proteins, changing the colour from orange to purple. Stability of the pigments is affected by acidity, temperature, light, oxygen, water activity and time. It was shown that these pigments added to sausages or canned pâté remained stable for 3 months' storage at 4 °C, while their stability ranged from 92 to 98 % (*13*). Thus, the main patents have focused on the solubilization, the stability and the extraction of pigments in solution. The pigments can easily react with amino group-containing compounds in the medium such as proteins, amino acids, or nucleic acids to form water-soluble pigments.

Despite the enormous economic potential of *Monascus* pigment, it has not led to a commercial exploitation in the Western world, mainly because of ignorance and also reluctance to change the opinion of food public agencies. These agencies do not approve *Monascus* pigments for use in the food industry, although they do appear to be nontoxic if correctly used. Thus, even though species of *Monascus* have been consumed in the Far East for many years, this does not help the pigment to gain approval in the EU or the USA.

Arpink Red ™ from Penicillium oxalicum

Many patents from Ascolor Biotech s.r.o. (Czech Republic) relate to a new fungus strain with the properties to produce a red colorant which can be applied in the food and cosmetic industries (*14*). The strain *Penicillium oxalicum* var. Armeniaca CCM 8242, obtained from soil, produces a chromophore of the anthraquinone type. Some strains of the same species are effective as biological control agents, *e.g.* reduction of the incidence of *Fusarium* wilt of tomato under glasshouse and field conditions. Others have been described for production of milk-clotting enzyme.

The cultivation of the fungus in liquid broth requires carbohydrates (such as sucrose, molasses), nitrogen (corn extract, yeast autolysate or extract), zinc sulfate and magnesium sulfate. The optimum conditions for performing the microbiological synthesis are: pH value 5.6 to 6.2, and temperature 27 to 29 °C. On the second day of incubation a red colorant is released into the broth, increasing up to 1.5–2.0 g/L of broth after 3–4 days.

When the biosynthesis of the red colorant is completed, the liquid from the broth is filtered or centrifuged for being separated from the biomass. The liquid is then acidified to pH=3.0–2.5 to precipitate the colorant. The precipitate is dissolved in ethyl alcohol and filtered. After the removal of alcohol, the colorant in the crystalline form is obtained, *i.e.* dark red powder. The colorant gives a raspberry-red colour in aqueous solution, stable at pH over 3.5. Neutral solutions are stable even after 30 min of boiling and colour shade does not change in relation to pH (*14*).

Many toxicological data are available on this red pigment: acute oral toxicity in mice (90-day subchronical toxicological study), acute dermal irritation/corrosion, acute eye irritation/corrosion, anti-tumour effectiveness, micronucleus test in mice, AMES test (*Salmonella typhimurium* reverse mutation assay), estimation of antibiotic activity, results of estimation of 5 mycotoxins. A new patent on Arpink Red was filed in 2001 (*15*) with claims of anti-cancer effects of the anthraquinone derivatives and applications within the food and pharmaceutical fields.

After evaluating all the materials provided by the company Ascolor Biotech s.r.o., the Codex Alimentarius Commission (Rotterdam meeting, March 11–15, 2002) made the following statement: »there will not be any objections to use the red colouring matter Arpink Red« in:

- meat products in the amount up to 100 mg/kg,
- meat and meat product analogues in the amount up to 100 mg/kg,
- nonalcoholic drinks in the amount up to 100 mg/kg,
- alcoholic drinks in the amount up to 200 mg/kg,
- milk products in the amount up to 150 mg/kg,
- ice creams in the amount up to 150 mg/kg,
- confectionery in the amount up to 300 mg/kg.

JECFA evaluation process is in progress and Arpink Red situation was discussed during the 63rd meeting of Joint FAO/WHO Expert Committee on Food Additives in Geneva, June 8–17, 2004.

Riboflavin, the vitamin B_2 but also a yellow food colorant

Riboflavin (vitamin B_2) has a variety of applications as a yellow food colorant. Its use is permitted in most countries. Applications include dressings, sherbet, beverages, instant desserts, ice creams, tablets and other products. Riboflavin has a special affinity for cereal-based products, but its use in these applications is somewhat limited due to its slight odour and naturally bitter taste. There are numerous microorganisms that produce riboflavin fermentatively. Riboflavin fermentation could be classified into three categories: weak overproducers (100 mg/L or less, *e.g. Clostridium acetobutylicum*), moderate overproducers (up to 600 mg/L, *e.g.* yeasts such as *Candida guilliermundii* or *Debaryomyces subglobosus*), and strong overproducers (over 1 g/L, *e.g.* the fungi *Eremothecium ashbyii* and *Ashbya gossypi*) (*16–18*).

β-carotene from Mucor circinelloides

Mucor circinelloides wild type is yellow because it accumulates β-carotene as the main carotenoid. The basic features of the carotenoid pathway, including photocarotenogenesis, are similar in *Phycomyces* and *Mucor* (*19*). *Mucor circinelloides* responds to blue light by activating biosynthesis. Wild type strains grown in darkness contain minimal amounts of β-carotene because of the low levels of transcription of structural genes for carotenogenesis. When exposed to a light pulse, the level of transcription of these genes increases strongly, leading to the formation of high pigment concentrations (*20*). New researches now focus on yeast-like mutants (*M. circinelloides* is a dimorphic fungus that grows either as yeast cells or in a mycelium form) which could be useful in a biotechnological production (*10*).

β-carotene from Phycomyces blakesleeanus

Phycomyces is a potential source of various chemicals including β-carotene. The carotene content of the wild type grown under standard conditions is modest, about 0.05 mg/g dry mass, however, certain mutants accumulate up to 10 mg (*21*). As for *Blakeslea trispora*, sexual stimulation of carotene biosynthesis remains essential to increase yields up to 35 mg/g (*22*). The best strains of *Phycomyces* bear their full carotenogenic potential on solid substrates or in liquid media. Cerdá-Olmedo (*23*) emphasizes that its relative, *Blakeslea trispora*, is more appropriate for production in usual fermentors.

Lycopene factory from corn leftovers

Jones *et al.* (*24*) genetically modified the fungus *Fusarium sporotrichioides* to manufacture the colorant and antioxidant lycopene from the cheap corn fibre material, the leftovers of ethanol making. Corn fibre is abundant (the USA ethanol industry generates four million tonnes annually) and costs about five cents a pound. Distiller's dry grains with solubles (DDGS) could also be used as a substrate. Using a novel, general method for the sequential, directional cloning of multiple DNA sequences, the isoprenoid pathway of the fungus was redirected toward the synthesis of carotenoids. Strong promoter and terminator sequences from the fungus were added to carotenoid biosynthetic genes from the bacterium *Erwinia uredovora*, and the chimeric genes were assembled, introduced in the fungus and expressed at levels comparable to those observed for endogenous biosynthetic genes. Cultures in lab flasks produced 0.5 mg(lycopene)/g of dry mass within 6 days and such a production will be increased within the next years (*25*).

Food Grade Pigments from Yeasts

Astaxanthin from Xanthophyllomyces dendrorhous, formerly known as Phaffia rhodozyma

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is widely distributed in nature and is the principal pigment in crustaceans and salmonids. The carotenoid imparts distinctive orange-red coloration to the animals and contributes to consumers' appeal in the market place.

Since animals cannot synthesize carotenoids, the pigments must be supplemented in the feeds of farmed species. Salmon and trout farming is now a huge business and feeding studies have shown that astaxanthin is very effective as a flesh pigmenter (*26*).

Among the few astaxanthin producing microorganisms, *Xanthophyllomyces dendrorhous* is one of the best candidates for commercial production. Therefore, many academic laboratories and several companies have developed processes which could reach an industrial level.

Several reports in the literature have shown that medium constituents, among other environmental factors, affect astaxanthin production in this yeast. The effects of different nutrients on *Xanthophyllomyces dendrorhous* have generally been studied in media containing complex sources of nutrients such as peptone, malt and yeast extracts. By-products from agriculture were also tested, such as molasses (*27*), enzymatic wood hydrolysates (*28*), corn wet milling coproducts (*29*), bagasse or raw sugarcane juice (*30*), and grape juice (*31*). Although such media are often convenient because they contain all nutrients, they suffer from the disadvantage of being undefined and sometimes variable in composition, which may mask important nutritional effects. For this reason, several studies are difficult to interpret in detail because of inadequate characterization of growth-limiting factors in the media. Thus, in order to elucidate the nature of nutritional effects as far as possible, chemically defined or synthetic media were used by some authors (*32*). For example, Palágyi *et al.* (*33*) assayed eleven strains for their ability to utilize 99 compounds as single carbon source. In a second study, carotenoid biosynthesis was increased at low ammonium or phosphate levels and stimulated by citrate. Factorial design and response surface methodology could be used to optimize the astaxanthin production (*34*). The optimal conditions stimulating the highest astaxanthin were: temperature 19.7 °C, carbon concentration 11.25 g/L, pH=6.0, inoculum 5 %, and nitrogen concentration 0.5 g/L. Under these conditions the astaxanthin content was 8.1 mg/L. Fermentation strategy also has an impact on growth and carotenoid production of *Xanthophyllomyces dendrorhous*, as shown with fed-batch (*35*) or pH-stat (*36*) cultures. The highest biomass obtained was 17.4 g/L.

A major drawback in the *Xanthophyllomyces dendrorhous* process is that disruption of the cell wall of yeast biomass is required prior to the addition to animal diet. This is essential for intestinal absorption of the pigment. Investigations have described several chemical, physical, autolytic, and enzymic methods for cell wall disruption. Fang and Wang (*37*) used a two-stage batch fermentation technique. The first stage was for red yeast cultivation. The second stage was the mixed fermentation of the yeast and *Bacillus circulans*, a bacterium with a high cell wall lytic activity.

Generation of mutants is also a starting point in optimization experiments (*38*), however time is now for metabolic engineering of the astaxanthin biosynthetic pathway (*39*). Authors should be able to manage carbon fluxes within the cell and resolve competition between enzymes such as phytoene desaturase and lycopene cyclase.

As a conclusion, the case of *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*) is very peculiar as hundreds of scientific papers and patents deal with astaxanthin production using this yeast (*40,41*) and the process has not been economically efficient up to now. New patents are filed almost each year, with improvement in astaxanthin yield, *e.g.* 3 g/g dry matter in the US patent 20030049241 (*42*).

Carotenoids from the genus Rhodotorula

Yeasts in the genus *Rhodotorula* synthesize carotenoid pigments. Most of the research is focused on the species *Rhodotorula glutinis*, however some papers deal with other species such as *R. gracilis*, *R. rubra* (*43*), and *R. graminis*.

The main compounds produced by these red yeasts are torulene and torularhodin, with minute quantity of β-carotene. Feed supplement with a *Rhodotorula* cell mass has been found to be safe and nontoxic in animals. Its use in the nutrition of laying hens has also been documented. As β-carotene content in wild strains of *R. glutinis* is quite low, efforts have been made to increase it through strain improvement, mutation (*44*), medium optimization and manipulation of culture conditions (temperature, pH, aeration, C:N ratio) (*45*). These studies resulted mainly in an increased yield of torulene and torularhodin, which are of minor interest. Some succeeded in increasing the target carotenoid and mutants derived from the wild type were able to produce 76-fold more β-carotene than the parent strain, *i.e.* 70 mg/L.

Food Grade Pigments from Bacteria

Zeaxanthin from Flavobacterium sp.

The yellow pigment known as zeaxanthin or 3,3′-dihydroxy-β-carotene can be used, for example, as an additive in poultry feeds to strengthen the yellow colour of the skin of animals of this kind or to accentuate the colour of the yolk of their eggs (*46*). This compound is also suitable for use as a colorant, for example in the cosmetics industry and in the food industry. Cultures of *Flavobacterium* sp. (*47*) in a nutrient medium containing glucose or sucrose, sulphur-containing amino acids such as methionine, cystine or cysteine, pyridoxine and bivalent metal ions selected from the group consisting of Fe^{2+}, Co^{2+}, Mo^{2+} or Mn^{2+} were able to produce up to 190 mg(zeaxanthin)/L, with a specific cell concentration of 16 mg/g dried cellular mass.

Canthaxanthin from the photosynthetic bacterium, Bradyrhizobium sp., or the extremely halophilic bacterium, Halobacterium sp.

The carotenoid pigment canthaxanthin has been used in aquafeed for many years in order to impart the desired flesh colour in farmed salmonids. A *Bradyrhizobium* sp. strain was described as a canthaxanthin (4,4′-diketo-β-carotene) producer (*48*) and the carotenoid gene cluster was fully sequenced (*49*). This keto carotenoid was also found in another microorganism, an extremely halophilic bacterium isolated from a salt farm, belonging to the genus *Halobacterium* (*50*). Interest in canthaxanthin is fading as a side effect of extreme overdosage of this molecule is the deposition of minute crystals in the eye, a fact leading to adverse media attention in the past, and some pressure to limit its use in aquafeeds. Debate is still open and some producers indicate that a real appraisal of the possible amount of canthaxanthin ingested by humans through the consumption of notable quantities of highly pigmented salmon reveals that it is extremely unlikely that consumers could be exposed to sufficient canthaxanthin to obtain the established acceptable daily intake (ADI) for this pigment (*51*).

Astaxanthin from Agrobacterium aurantiacum

Compared to the huge amount of research devoted to *Xanthophyllomyces dendrorhous*, astaxanthin production using *Agrobacterium aurantiacum* has been investigated to a lesser extent. First description of astaxanthin biosynthesis in this bacterium was published by Yokoyama *et al.* in 1994 (*52*). Astaxanthin is present among 10 kinds of carotenoids. The authors also described the biosynthetic pathway, the influence of growth conditions on carotenoid production and the occurrence of astaxanthin glucoside in two following papers (*53,54*).

As the C_{40} carotenoids represent a pigment group that has obtained increasing commercial interest in the recent years, numerous screenings have been conducted with the aim of characterizing new biological sources of astaxanthin, and positive targets were isolated such as *Paracoccus carotinifaciens* (*55*) or *Halobacterium salinarium* (*56*). Three interesting results were reported in the latter paper: (*i*) the extreme NaCl concentrations (about 20 %) used in the growth medium to prevent contamination with other organisms (no particular care has to be taken with sterilization); (*ii*) NaCl concentrations under 15 % induce bacterial lysis, so that no special cell breakage technique is necessary; and (*iii*) pigments may be extracted directly with sunflower oil instead of organic solvents, thus eliminating possible toxic reactions due to trace concentrations of acetone or hexane and hence facilitating pigment assimilation by animals.

Difficult-to-synthesize pigments, the case of aryl carotenoids

Among carotenoids under investigation for colouring or for biological properties, a very small number of molecules is available from natural extracts or chemical synthesis (*57*). The list is rather short compared to the long list of 700 entries in the Carotenoids Handbook (*58*). Biotechnology could be a solution for providing more pigments such as difficult-to-synthesize aryl carotenoids. Isorenieratene and hydroxyl derivatives were produced by our group using bacteria, *i.e. Brevibacterium linens*, *Streptomyces mediolani* or *Mycobacterium aurum* (*59,60*). Among these, the food grade *B. linens* (new name: *Brevibacterium aurantiacum* sp. nov.) is of particular interest as this microorganism is found in the rind of red-smear ripened soft cheeses. Its pigments have therefore been consumed by human beings for a long time (*61,62*).

Table 1. Microbial production of pigments (already in use as natural food colorants or with high potential in this field)

Molecule	Colour	Microorganism	Status*	References
Ankaflavin	yellow	*Monascus* sp. (fungus)	IP	(*12*)
Anthraquinone	red	*Penicillium oxalicum* (fungus)	IP	(*14*)
Astaxanthin	pink-red	*Xanthophyllomyces dendrorhous* (yeast), formerly *Phaffia rhodozyma*	DS	(*63–67*)
Astaxanthin	pink-red	*Agrobacterium aurantiacum* (bacteria)	RP	(*52*)
Astaxanthin	pink-red	*Paracoccus carotinifaciens* (bacteria)	RP	(*55*)
Canthaxanthin	dark red	*Bradyrhizobium* sp. (bacteria)	RP	(*49*)
Lycopene	red	*Blakeslea trispora* (fungus)	DS	(*68*)
Lycopene	red	*Fusarium sporotrichioides* (fungus)	RP	(*24*)
Melanin	black	*Saccharomyces neoformans* var. *nigricans* (yeast)	RP	(*69*)
Monascorubramin	red	*Monascus* sp. (fungus)	IP	(*12*)
Naphtoquinone	deep blood-red	*Cordyceps unilateralis* (fungus)	RP	(*70*)
Riboflavin	yellow	*Ashbya gossypi* (fungus)	IP	(*18*)
Rubrolone	red	*Streptomyces echinoruber* (bacteria)	DS	(*71*)
Rubropunctatin	orange	*Monascus* sp. (fungus)	IP	(*12*)
Torularhodin	orange-red	*Rhodotorula* sp. (yeast)	DS	(*44*)
Zeaxanthin	yellow	*Flavobacterium* sp. (bacteria)	DS	(*47*)
Zeaxanthin	yellow	*Paracoccus zeaxanthinifaciens* (bacteria)	RP	(*72*)
β-carotene	yellow-orange	*Blakeslea trispora* (fungus)	IP	(*73*)
β-carotene	yellow-orange	*Fusarium sporotrichioides* (fungus)	RP	(*24*)
β-carotene	yellow-orange	*Mucor circinelloides* (fungus)	DS	(*10*)
β-carotene	yellow-orange	*Neurospora crassa* (fungus)	RP	(*74*)
β-carotene	yellow-orange	*Phycomyces blakesleeanus* (fungus)	RP	(*23*)
Unknown	red	*Penicillium purpurogenum* (fungus)	DS	(*75*)
Unknown	red	*Paecilomyces sinclairii* (fungus)	RP	(*76*)

*Industrial production (IP), development stage (DS), research project (RP)

Concluding Comments

Nature is rich in colour, and pigment producing microorganisms (fungi, yeasts, bacteria) are quite common. Among the molecules produced are carotenoids, melanins, flavins, quinones, and more specifically monascins, violacein or indigo.

The success of any pigment produced by fermentation depends upon its acceptability in the market, regulatory approval, and the size of the capital investment required to bring the product to market. A few years ago, some expressed doubts about the successful commercialization of fermentation-derived food grade pigments because of the high capital investment requirements for fermentation facilities and the extensive and lengthy toxicity studies required by regulatory agencies. Public perception of biotechnology-derived products should also be taken into account.

Nowadays some fermentative food grade pigments are on the market (Table 1) and the successful marketing of algae-derived or vegetable-extracted pigments, both as a food colour and a nutritional supplement, reflects the presence and importance of niche markets in which consumers are willing to pay a premium for »all natural ingredients«.

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