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MINI-REVIEW

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Microbial xanthophylls

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Abstract Xanthophylls are oxygenated carotenoids abundant in the human food supply. Lutein, zeaxanthin, and cryptoxanthin are major xanthophyll carotenoids in human plasma. The consumption of these xanthophylls is directly associated with reduction in the risk of cancers, cardiovascular disease, age-related macular degeneration, and cataract formation. Canthaxanthin and astaxanthin also have considerable importance in aquaculture for salmonid and crustacean pigmentation, and are of commercial interest for the pharmaceutical and food industries. Chemical synthesis is a major source for the heavy demand of xanthophylls in the consumer market; however, microbial producers also have potential as commercial sources. In this review, we discuss the biosynthesis, commercial utility, and major microbial sources of xanthophylls. We also present a critical review of current research and technologies involved in promoting microbes as potential commercial sources for mass production.

Introduction

In the year 1837, Berzelius named yellow pigments from autumn leaves as xanthophylls. They are the group of naturally occurring oxygenated derivatives of hydrocarbon carotenoids synthesized mainly by plants and microorganisms (Fig. 1; Johnson and Schroeder 1995). In the past 20 years, xanthophylls have been used extensively as dietary supplements and cosmetics ingredients (Sieiro et al. 2003). Xanthophylls also have applications as natural food colorants, feed additives, and as an active ingredient in medicinal pharmaceuticals due to their outstanding antioxidant properties. Epidemiological evidence suggests that dietary xanthophylls may inhibit the onset of many diseases such as arteriosclerosis, cataracts, age-related macular degeneration, multiple sclerosis, and cancers (Peto et al. 1981; Greenberg et al. 1990; Stahl and Sies 1996).

Recent studies have continued to refine our understanding of the functions of xanthophylls in human health. A new role for the xanthophylls lutein and zeaxanthin was identified when these carotenoids were found to constitute the macular pigment, the yellow spot at the center of the human retina (Bone et al. 1985). Canthaxanthin, a xanthophyll with no provitamin-A activity, has been proposed to reduce the risk of cancer (Mathews-Roth and Krinsky 1987; Mayne and Parker 1989), an anticancer effect that may be attributable to its antioxidant nature (Edge et al. 1997). The diketo carotenoid astaxanthin has also shown to be highly effective in stimulating immune defenses when compared with other carotenoids (Jyonouchi et al. 1994, 1996; Okai and Higashi-Okai 1996).

The earliest role established for carotenoids in animals was as a vitamin A precursor. Many different carotenoids can be metabolized to products with retinoid activity, which also affect gene expression and cell differentiation. However, because of the presence of oxygenated groups in terminal rings in the carotenoid structure, most xanthophylls do not fit the structural requirements for provitamin A activity (Simpson and Chichester 1981), a fact that probably constitutes the major reason that their potential relevance in human health had long been overlooked. The formation of vitamin A from exceptional xanthophylls might account for a portion of their activities as anticancer agents. Cryptoxanthin, a monohydroxy carotenoid, is well known to have provitamin A activity, and lutein has been reported to be a precursor of vitamin A_2 in fish (Goswami and Barua 1981).

Xanthophyll carotenoids behave as excellent antioxidants by quenching singlet oxygen, reactive oxygen species, and free radicals that are by-products of metabolic processes in cells or from environmental pollutants (Edge et al. 1997). The ability to quench singlet oxygen resides not only on the triplet state, but also on the functional groups with increasing polarities, such as carbonyl and

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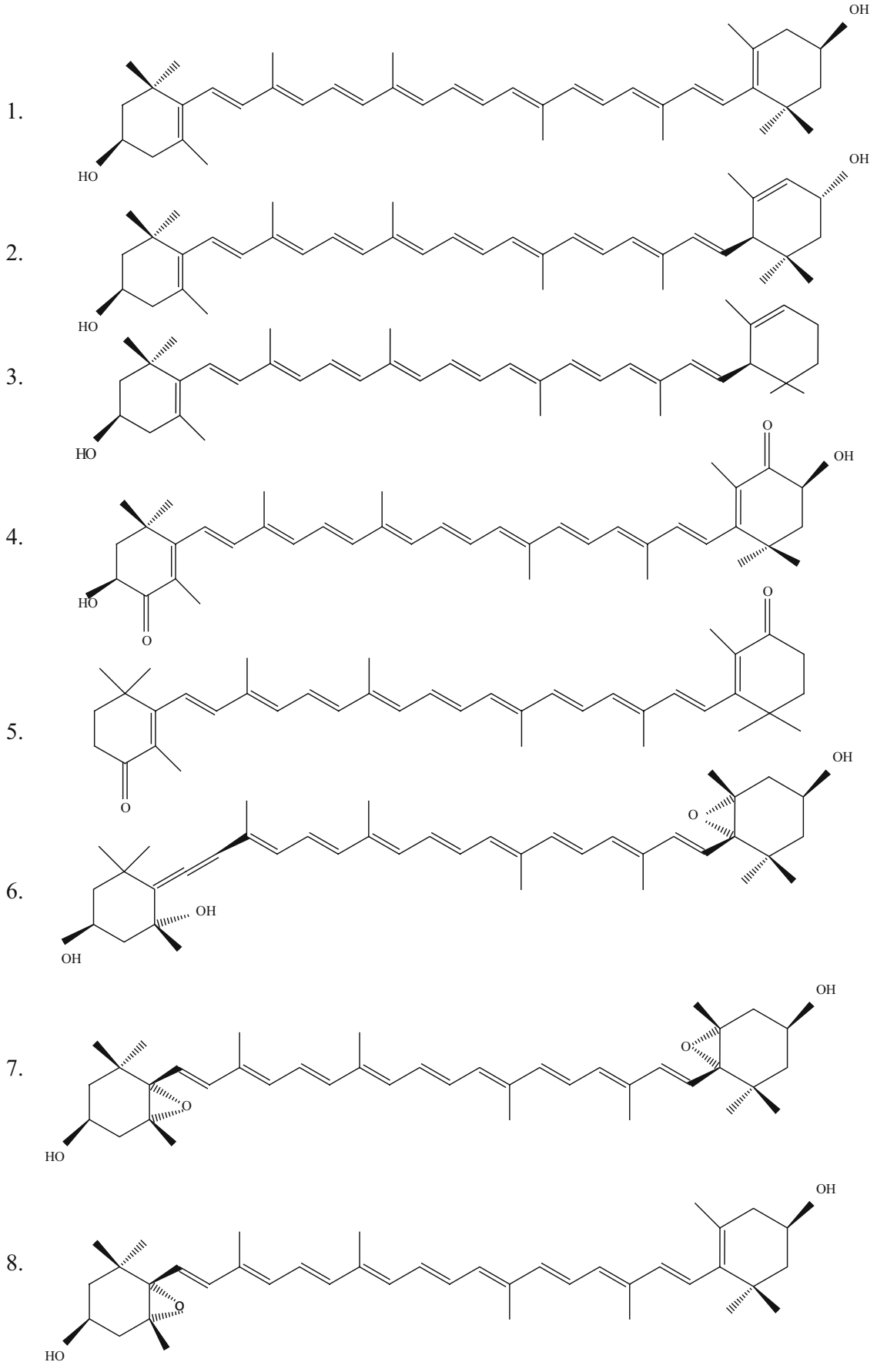

Fig. 1 Chemical structures of xanthophyll carotenoids: Zeaxanthin (**1**), Lutein (**2**), Cryptoxanthin (**3**), Astaxanthin (**4**), Canthaxanthin (**5**), Neoxanthin (**6**), Violaxanthin (**7**), Antheraxanthin (**8**)

hydroxyl groups, in the terminal rings (Miller et al. 1996; Di Mascio et al. 1990). Xanthophylls with keto groups in the β-ionone ring are known to retard hydroperoxide formation and radical-initiated lipid peroxidation more efficiently than β-carotene, which lacks oxo groups at 4 and 4′ positions (Terao 1989; Palozza and Krinsky 1992; Jorgensen and Skibsted 1993). Hydroxy xanthophylls such as zeaxanthin and β-cryptoxanthin were also observed to be more effective than β-carotene and lycopene against oxidation initiated in aqueous or lipid phases (Woodall et al. 1997).

Owing to their outstanding antioxidant properties and health-related functions, xanthophylls such as lutein, zeaxanthin, and astaxanthin constitute a multimillion-dollar market worldwide. Currently, xanthophylls are mainly produced by solvent-based extraction procedures from natural sources or by multistep chemical synthesis. Naturally occurring xanthophylls such as zeaxanthin, canthaxanthin, and astaxanthin are now produced synthetically on an industrial scale (Ernst 2002). Commercial production of carotenoids using microorganisms has been achieved in the case of astaxanthin, which is produced by fermentation using the red yeast *Xanthophyllomyces dendrohous* or in culturally manipulated open ponds using the alga *Haematococcus pluvialis* (Jacobson et al. 2000; Lorenz and Cysewski 2000; Olaizola 2000). Industrial and biotechnology researchers are currently exploring additional microbial resources that could provide pure and isomer-

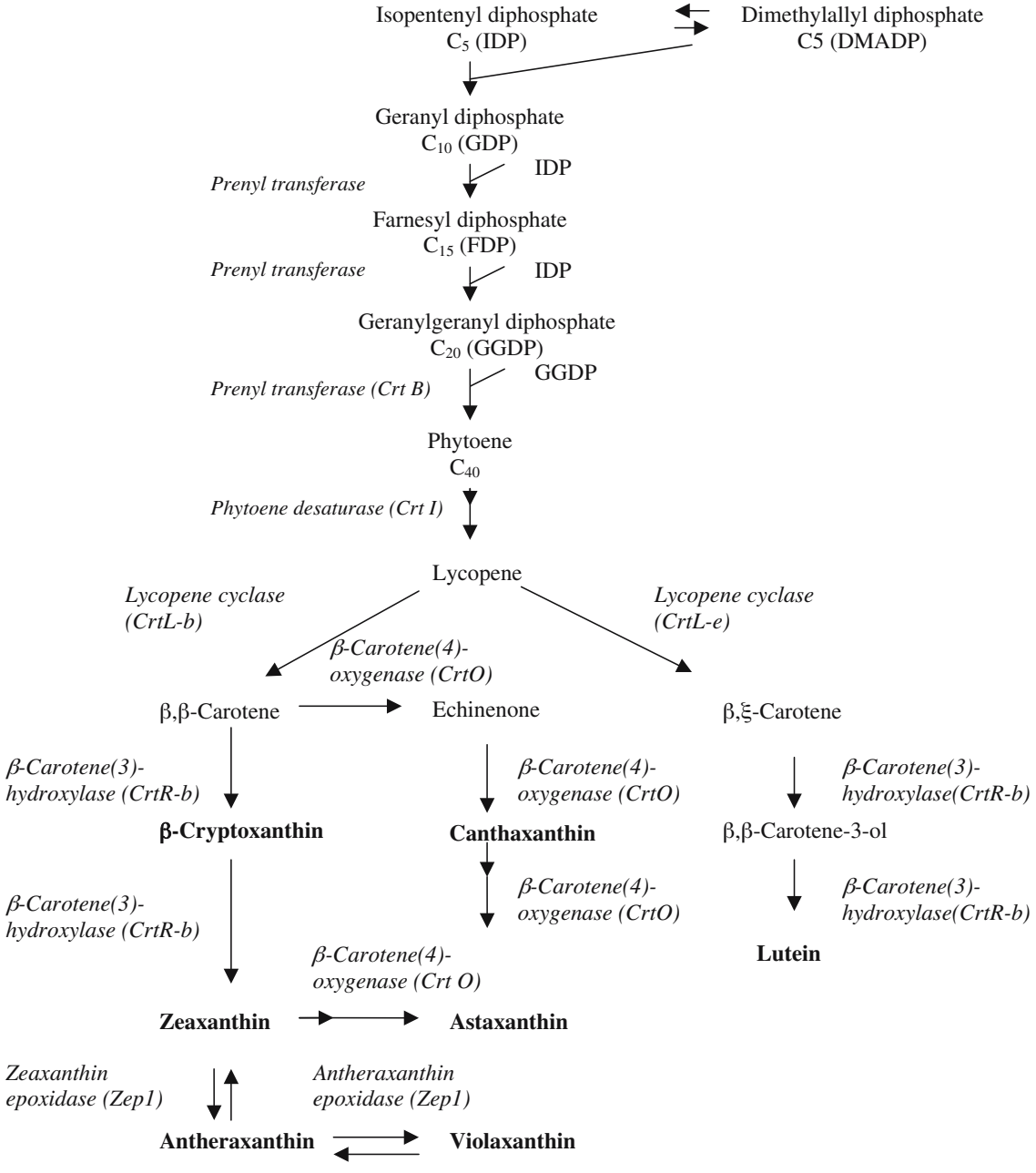

Fig. 2 Possible biosynthetic pathways for xanthophyll formation in bacteria, algae, and yeast

free preparations of beneficial xanthophylls. In this review, we explore several microbial sources of xanthophylls.

Microbial biosynthesis

Promotion of microbial biotechnology for xanthophyll production requires a detailed understanding of the biosynthesis of xanthophylls. All xanthophylls are synthesized from very basic C5 isoprenoid compounds. Isopentenyl diphosphate (C5) and dimethylallyl diphosphate (C5) are condensed into geranyl diphosphate (C10), and then elongated in five carbon steps to farnesyl diphosphate (C15), geranylgeranyl diphosphate (C20), and finally phytoene (C40) (Chichester 1967; Goodwin 1993). Phytoene desaturation leads to the formation of acyclic carotenoids such as zeta-carotene, neurosporene, and lycopene, which can be cyclized to α,β,γ-carotenes (C40).

Formation of xanthophylls involves sequential oxidations of postphytoene molecules yielding hydroxy, epoxy, and oxo groups. Many xanthophylls are oxygenated products of α- and β-carotenes. A common form of oxygenation is introduction of hydroxyl groups at the position of C3 and C3′ of the ionone rings which leads to the formation of zeaxanthin, and lutein which are C3,C3′-dihydroxy derivatives of β,β-carotene and β,ε-carotene, respectively (Fig. 2) (Britton 1998). A monohydroxy carotenoid, β-cryptoxanthin, acts as an intermediate in the biosynthesis of the dihydroxy carotenoid zeaxanthin. Metabolic labeling experiments coupled with selective inhibitors have demonstrated the cyclization and hydroxylation scheme in *Flavobacterium* species (McDermott et al. 1973).

Canthaxanthin and astaxanthin are formed via the introduction of keto groups at C4 and C4′ with or with out hydroxylation at C3 and C3′. The scheme of formation of keto carotenoids from β-carotene through echinenone, and canthaxanthin is well characterized in algae, yeast, and nonphotosynthetic bacteria (Fig. 2; Fraser et al. 1997; Britton 1998).

Epoxy groups may be subsequently added at the 5,6 and 5′,6′ positions. Epoxy xanthophylls such as violaxanthin, neoxanthin are mainly found in plants and green algae. Interconversion of violaxanthin, antheraxanthin, and zeaxanthin in green algae through epoxidation is a well-studied pathway (Fig. 2; Antia and Cheng 1983; Yamamoto et al. 1999). Varied biosynthetic pathways have been diagrammatically outlined in different microbial systems in recent reviews and reports (Sandmann 1991; Armstrong 1997; Ducrey Sanpietro and Kula 1998; Schmidt-Dannert et al. 2000; Lee and Schmidt 2002). Microbes often produce a spectrum of xanthophylls that is unique to their particular group.

Utilities and microbial sources of xanthophylls

Most of the xanthophylls detailed here are naturally occurring molecules, but there is enormous diversity. They are produced by a wide variety of microbes, which include oxygenic photosynthetic prokaryotes, anoxygenic phototropic bacteria, asporogenous yeasts and fungi, nonpathogenic as well as phytopathogenic bacteria, and halotolerant fresh water alga. Johnson and Schroeder (1995) predicted that xanthophyll biosynthesis might have evolved in conjunction with the emergence of anoxygenic phototropic bacteria millions of years ago because they are essential in protection of the photosynthetic apparatus from damage by excess light.

Naturally occurring carotenogenic microbes can fulfill only part of the demand for xanthophylls in the modern world (Johnson and Schroeder 1995). Trends in fermentation biotechnology toward the development of processes for high-value food additives have been recently reviewed, and they reveal strong interest of the food industry toward microbial sources of carotenoids (Hirschberg 1999). With the advancement of metabolic engineering protocols, xanthophyll pigment production by natural microbes has become competitive with chemical synthesis for a few selected xanthophylls. Recently, xanthophyll production has been engineered in noncarotenogenic microbes using recombinant DNA technologies (Ruther et al. 1997). In this review, we will also assess if microbial technology will be a major supplier in the future.

Hydroxy carotenoids: lutein, zeaxanthin, and β-cryptoxanthin

During the last decade, lutein (3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol) and zeaxanthin (3*R*,3′*R*)-β,β-carotene-3,3′diol) have attracted the attention of medicinal and biotechnological researchers due to their possible role in the prevention of age-related macular degeneration (AMD), the leading cause of legal blindness in western societies (Mares-Perlman et al. 2002; Beatty et al. 2004). Lutein and zeaxanthin are dihydroxy-carotenoids that accumulate in the macula of the human eye, where they, along with their oxidative metabolites, are collectively known as the macular pigments (Khachik et al. 2002). Oxidative damage of the retina may underlie the development of age-related macular degeneration and hence the presence of the antioxidants lutein and zeaxanthin may be protective (Seddon et al. 1994; Mares-Perlman et al. 2002). The xanthophylls may act to protect the eye from short wavelength visible light phototoxicity via quenching reactive oxygen species and/or by light screening mechanisms. Epidemiological studies have shown that dietary intakes of lutein and zeaxanthin, particularly from certain xanthophyll-rich foods such as spinach, broccoli, kale, and corn, are associated with a significant reduction in the risk for cataract and for age-related macular degeneration (Moeller et al. 2000). Other than ocular benefits, zeaxanthin may also play a critical role in the prevention of cancer (Nishino et al. 2002), and has been used as a colorant in the cosmetics and food industries (Hadden et al. 1999).

Lutein has also received attention due to many applications other than as ocular nutraceutical. Its anticancer activity (Chew et al. 1996) and its ability to enhance im-

mune function are well documented (Snodderly 1995). Lutein may also serve to protect skin from UV-induced damage, and may help reduce the risk of cardiovascular disease. Consumption of a lutein-dominated carotenoid composition is believed to help to maintain skin health by reducing UV-induced erythema (Stahl and Sies 2002). There is direct evidence of photoprotective effects displayed in mice, in which topically applied lutein leads to reduction of UVB-induced epidermal cell proliferation and erythema by 50%. Results show an inverse association between high lutein intake and/or higher serum levels and risk for lung cancer (Granado et al. 2003).

Commercial demand for zeaxanthin is mainly fulfilled by chemical synthesis because there are only a few microbes that synthesize zeaxanthin as their main carotenoid (Table 1). There is currently renewed interest in microbial sources of carotenoids (Nelis and DeLeenheer 1991; Johnson and Schroeder 1995; Ausich 1997; Bhosale 2004) due to public preference against synthetic chemical additives. Moreover, chemical synthesis of carotenoids involves a number of chemical conversion reactions (Ito et al. 1992). Among microbial sources, photosynthetic marine microalga *Dunaliella bardawil* produces lutein and zeaxanthin in considerable amounts under light stress (Salguero et al. 2005). Jin et al. (2003) reported excess production of zeaxanthin from a mutant (zea1) of the halotolerant unicellular green alga *Dunaliella salina* that has an impaired zeaxanthin epoxidation pathway. This alga therefore lacks a number of xanthophylls, such as neoxanthin, violaxanthin, and antheraxanthin, but constitutively accumulates zeaxanthin. Cyanobacteria (blue green algae) such as *Phormidium laminosum* (Fresnedo et al. 1991) are capable of carrying out oxygenic photosynthesis but will produce zeaxanthin under nitrogen starvation conditions.

Flavobacterium multivorum, a nonfastidious, nonpathogenic, and nonphotosynthetic bacterium, accumulates zeaxanthin through a simple sequence of reactions including desaturation, cyclization, and hydroxylation (Britton et al. 1977a). It is considered to be an important potential microbial source of zeaxanthin (Britton et al. 1977a,b; Pasamontes et al. 1997; Alcantara and Sanchez 1999; Masetto et al. 2001); however, low carotenoid yield and a lack of information about optimization of media for carotenoid production restricts the possibilities of its use (Nelis and Deleenheer 1991). Recent studies report statistical methods for optimization of media for hyperproduction, but the yield is still not suitable for commercialization (Bhosale et al. 2004). Several strains of *Flavobacterium* have been taxonomically reevaluated as *Paracoccus zeaxanthinifaciens* sp. (Berry et al. 2003). Humbelin et al. (2002) has detailed the genetics of isoprenoid biosynthesis in *Paracoccus zeaxanthinifaciens* which may help in the future to build a genetic base for a fermentation approach. Among yeast, asporogenous *Phaffia rhodozyma*, although best known as an astaxanthin producer, is also reported to be a zeaxanthin producer (Hoshino et al. 2004).

Like zeaxanthin, lutein is also predominantly extracted from nonmicrobial sources. Marigold flowers (*Tagetes erecta*) are by far the most abundant natural source of commercial lutein (Quackenbush and Miller 1972;

Table 1 Microbial procedures of xanthophylls with potential of commercialization

Microbes	Yield	Cultivation time	References
Lutein			
Chlorella zofingiensis (Chlorophyta)	21 μg/ml	360 h	Del Campo et al. (2004)
Chlorella protothecoides CS-41	225 μg/ml	240 h	Shi et al. (1999), Shi et al. (2000)
Muriellopsis sp.	35 μg/ml	240 h	Del Campo et al. (2001)
Zeaxanthin			
Dunaliella salina	6 mg/g	NG	Jin et al. (2003)
Flavobacterium multivorum	1.6 mg/g	32 h	Alcantara and Sanchez (1999), Bhosale et al. (2004)
β-Cryptoxanthin			
Brevibacterium linens	0.3 μg/ml	60 h	Guyomarch et al. 2004
Astaxanthin			
Phaffia rhodozyma[a]	4 mg/g	4–7 days	Jacobson et al. (2000)
Haematococcus pluvialis[a]	30 mg/g	10–15 days	Lorenz and Cysewski (2000)
Chlorella zofingiensis	1 mg/g	10 days	Ip and Chen (2005)
Canthaxanthin			
Micrococcus roseus	1.7 μg/ml	96 h	Cooney and Berry (1981)
Gordonia jacobaea MV-1 sp. nov.	13,373 μg/ml	168 h	De Miguel et al. (2001), Veiga-Crespo et al. (2005)
Brevibacterium sp. strain KY-4313	9.3 μg/ml	96 h	Nelis and De Leenheer (1989)
Chlorella emersonii	0.6 μg/ml	264 h	Arad et al. (1993)

NG Not given
[a]There are several strains reported in the review quoted

Khachik 1995; Delgado-Vargas and Paredes-Lopez 1997); however, marigold flowers contain several esters of lutein with similar polarity, and thus they are very difficult to separate from each other (Tsao et al. 2004). Among microbial sources, lutein production by microalga *Chlamydomonas reinhardii* is well studied (Francis et al. 1975). The precursor carotenoids such as violaxanthin, neoxanthin, and cryptoxanthin vary in a proportional fashion during the life cycle of the algae. In recent years several strains of the chlorophycean microalga *Chlorella* have been reported to produce high levels of lutein. *Chlorella* is preferred over other microalga due to its faster growth rate (Del Campo et al. 2004) and general acceptability as food and feed supplement. In *Chlorella*, xanthophylls can be synthesized in darkness as their primary carotenoid. Halotolerant *Chlorella zofingiensis* accumulate lutein as a primary compound of the photosystem and as an accessory pigment in light harvesting complexes. In *Chlorella zofingiensis*, lutein and astaxanthin formation takes place from divergent pathways originating from a common lycopene precursor, which means efficient blocking of astaxanthin can lean to further increases in lutein biosynthesis (Del Campo et al. 2004). *Chlorella protothecoides* grown in heterotrophic, nitrogen-limited fed-batch culture yields high levels of lutein. It is currently the most promising microbe for lutein production (Shi et al. 2002). Another chlorophycean microalga, *Muriellopsis* sp. has also shown promise as a commercial source (Del Campo et al. 2000; Del Campo et al. 2001). Several species of photosynthetic marine microalga such as *Dunaliella bardawil*, and *Dunaliella salina*, which commonly produce β-carotene as the main carotenoid are also reported to accumulate high amounts of lutein under specified conditions. However, the accumulation is not commercially viable. Along with lutein and zeaxanthin, there are also reports of the potential health benefits of the monohydroxy xanthophyll β-cryptoxanthin (3*R*-β,β-carotene-3-ol).

β-Cryptoxanthin is reported to have mild provitamin A activity (Melendez-Martinez et al. 2004) and potent antioxidant activity. High prediagnostic levels of β-cryptoxanthin in the serum have been found to be associated with reduced risk of lung cancer in a recent cohort study (Yuan et al. 2001, 2003). In addition to lung cancer, experimental and epidemiological data support the utility of dietary β-cryptoxanthin as a chemopreventive agent against risk of prostate cancer risk (Binns et al. 2004), colon cancer (Sumida 2002), and rheumatoid arthritis (Pattison et al. 2004). They are known to promote osteogenesis and thus may prevent bone diseases (Yamaguchi 2004). This finding is well supported by animal studies (Uchiyama et al. 2004). Khachik (2003) recently patented a method for production of β-cryptoxanthin from commercial lutein. Microbial biotechnology for β-cryptoxanthin production has so far been unsuccessful due to the lack of a major microbial source that produces cryptoxanthin as its final end product, although the carotenogenic *Brevibacterium linens* that belongs to the cheese ripening flora has been reported to produce β-cryptoxanthin (Guyomarch et al. 2000).

Table 1 summarizes major microbial producers for hydroxycarotenoids with potential commercial utilities. In general, algae are slow-growing microbes, and maintenance of stress conditions adds to production cost. Yeasts are not yet commercially viable for hydroxy-carotenoid production, and bacteria still need strain improvement and improved fermentation media to compete with chemical synthesis.

Keto carotenoids: astaxanthin and canthaxanthin

Astaxanthin (3*R*,3′R dihydroxy-ββ-carotene-4, 4′-dione) is a major carotenoid extensively used as a feed additive in animal diets including salmonids, lobsters, and egg yolks of chickens and quail. This not only increases their organoleptic properties, but it also is beneficial for improved consumer acceptance of products. Furthermore, astaxanthin is also used as a nutraceutical and a medicinal ingredient against degenerative diseases such as cancer (Mayne 1996; Chew et al. 1999), skin related illness, and heart disease (Guerin et al. 2003). Owing to successful experimentation in mice, astaxanthin-rich microalga offered a new strategy for treating *Helicobacter pylori* infection in humans (Wang et al. 2000). The antioxidant properties of astaxanthin are believed to have a key role in the medicinal, pharmaceutical, and food industries (Guerin et al. 2003).

The biosynthesis of astaxanthin is limited exclusively to microorganisms. So far, the green microalga *Haematococcus pluvialis* and the heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous*, the perfect state of *Phaffia rhodozyma*, are the only microbial systems with commercial potential for the production of astaxanthin. Along with these two, the *Brevibaterium*, *Mycobacterium lacticola* (Nelis and DeLeenheer 1991), marine bacteria *Agrobacterium aurantiacum*, and *Alcaligenes* sp. strain PC-1 also accumulate astaxanthin in considerable amounts (Yokoyama et al. 1994).

The red heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous* is widely used as an astaxanthin source in aquaculture industries. Biotechnology companies active in the business of pigment production have devoted considerable research and development efforts on this organism (Nelis and DeLeenheer 1991). In the last two decades, *Xanthophyllomyces dendrorhous* has been extensively studied for the effect of nutrients, cultural conditions, and supplementation with stimulants and inexpensive media sources on overproduction of astaxanthin (An and Johnson 1990; Meyer and Du Preez 1994; Vazquez 2001). Johnson (2003) recently reviewed the developmental journey from laboratory to industry of *Xanthophyllomyces dendrorhous*. However, the tough cell wall of yeast posed a problem of accessibility of astaxanthin to fish and salmonoids. In addition, with the advent of hyperproducing algal sources, the market for carotenogenic yeast has declined recently. Quantities of astaxanthin are low (0.40% dry weight) in yeast as compared to *Haematococcus* sp., which contains up to 3.0% dry weight astaxanthin.

Astaxanthin production from *Haematococcus pluvialis* has received tremendous attention due to its health benefits in humans (Guerin et al. 2003). The unicellular freshwater green alga *Haematococcus pluvialis* accumulates large amounts of astaxanthin in their aplanospores when exposed to unfavorable growth conditions or following different environmental stresses such as phosphate or nitrogen starvation, high concentration of salt in the growth medium, or high light intensity (Boussiba and Vonshak 1991; Kobayashi et al. 1992; Margalith 1999; Boussiba 2000). During this process, the vegetative cells of the alga form cysts and change their color from green to red. The high cell density cultivation of *Haematococcus pluvialis* for astaxanthin production in fed-batch modes results in fairly good yields (up to 64.36 mg/l; Zhang et al. 1999); however, this microalga grows slowly and yields low cell densities when cultured with traditional media. In order to compete with synthetic astaxanthin, suitable scaling-up is required. Olaizola (2000) reported expanded commercial production from *Haematococcus pluvialis* by using 25,000-l outdoor photo bioreactors; however, large-scale production in open ponds has proved unsatisfactory because of severe contamination problems. A selective medium might overcome this difficulty, and further research for the development of suitable strains is thus warranted. More recently, *Chlorella zofingiensis* was proposed as a promising producer of astaxanthin (Ip et al. 2004; Ip and Chen 2005). However, the yield is relatively lower than *Haematococcus pluvialis* (Table 1).

Canthaxanthin (4,4′diketo-β-carotene) is a keto-carotenoid with no provitamin A activity but strong antioxidant activity. There are several hypotheses proposed for the beneficial antioxidant behavior of canthaxanthin. It has been reported to be a more effective antioxidant than β-carotene due to the presence of keto groups at the 4 and 4′ in the β-ionone ring (Palozza and Krinsky 1992). Canthaxanthin is also known to increase resistance to lipid peroxidation primarily by enhancing membrane α-tocopherol levels, and secondarily by providing direct antioxidant activity (Mayne and Parker 1989). Canthaxanthin has been known to be effective in the treatment of polymorphous light eruptions, the most common of the idiopathic photodermatosis (Suhonen and Plosila 1981). It was marketed in Canada and Europe in the late 1970s as skin-tanning agent but was discontinued when crystallization was noted in the retina (Boudreault et al. 1983). Gensler and Holladay (1990) reported that canthaxanthin, along with retinyl palmitate, could prevent light-induced tumor and UV-induced immune suppression in mice. Canthaxantin has also been reported to offer protection against skin cancers in experimental animals (Santamaria et al. 1988). Canthaxanthin was evaluated for its efficacy in the prevention of chemically induced mammary cancers and was observed to be active in preventing cancer initiation but not promotion. In addition to medicinal applications, commercially synthesized canthaxanthin is widely used as colorant in food and feed additives, mainly in the farming of rainbow trout (Malak et al. 1976; Gordon and Bauernfeind 1982) and salmonids (Baker 2001). The Food and Drug Administration (FDA) has amended the color additive regulations to promote the safe use of canthaxanthin as a color additive in the feed of salmonid fish to enhance the color of their flesh (Food and Drug Administration and Department of Health Human Services 1998).

The complete process for chemical synthesis of canthaxanthin is well studied (Rosenberger et al. 1982), and it is thought to be an intermediate for the astaxanthin formation (Rodriguez et al. 1973). Only a few reports of microbial producers of canthaxanthin as an end product are available. Mutant strains of the bacterial phytopathogen *Corynebacterium michiganense* (Saperstein and Starr 1954), *Micrococcus roseus* (Cooney et al. 1966; Goodwin 1980), *Brevibacterium* sp. strain KY 4313 (Nelis and DeLeenheer 1991), and *Gordonia jacobaea* MV-1 (De Miguel et al. 2001; Veiga-Crespo et al. 2005) are known to produce canthaxanthin as their primary carotenoid. Among microalga, Czygan (1964) reported *Chlorella pyrenoidosa* to be a canthaxanthin producer. *Chlorella zofingiensis* growing under conditions of salt stress and low light accumulated higher amounts of canthaxanthin than astaxanthin (Pelah et al. 2004). The market for canthaxanthin is still growing with time, but the lack of promising of microbial sources remains a major obstacle.

Epoxy xanthophylls: violaxanthin, neoxanthin, and antheraxanthin

Violaxanthin (5,6,5′,6-tetrahydro-5,6,5′,6′-diepoxy-β,β-carotene-3,3′-diol), neoxanthin (5′,6′-epoxy, 6,7-didehydro-5,6, 5′,6-tetrahydro-β-carotene-3,5,3′-triol), and antheraxanthin (5,6-epoxy, 5,6-dihydro-β,β-carotene-3,3′-diol) are biochemical intermediates in the carotenoid biosynthetic pathways in green algae and plants. Along with other popular carotenoids, they are vital components of dietary foods rich in antioxidants (Czeczuga-Semeniuk et al. 2003). Neoxanthin was recently reported as protective against human prostrate cancer (Kotake-Nara et al. 2001) and chemically induced carcinogenesis in animals (Chang et al. 1995); however, its potential still needs to be fully explored.

Plants are the main source of the epoxy xanthophylls and only a few microbial sources have been reported, and even then, levels are affected by the light conditions during growth, especially in the case of green algae. *Chlamydomonas reinhardii* and *Dunaliella parva* have uneven accumulation of violaxanthin, neoxanthin, zeaxanthin, and β-carotene during light and dark phases of growth. High light conditions generally lead to the deepoxidation of violaxanthin to antheraxanthin and eventually zeaxanthin, but during dark incubation epoxy xanthophylls accumulate in higher amounts. Considerable redistribution among the carotenoids occurs during the dark period, notably the amount of β-carotene (Francis et al. 1975) and zeaxanthin (Young and Britton 1990) are increased relative to total xanthophylls. *Chlorella pyrenoidosa* and *Chlorella vulgaris* also accumulate violaxanthin as major pigments under dark conditions of cultivation (Goodwin 1980). Un-

der similar conditions, the principal carotenoid of *Euglena gracilis* is antheraxanthin, which accounts for over 80% of the carotenoid present (Krinsky and Goldsmith 1960).

Strategies for improving xanthophyll production

Developments in genetics, biochemistry, physiology, and process engineering provide the basis of rapid development in biotechnology of xanthophylls, but amplification of the recombinant DNA technique is the most significant landmark in this field. Identification of genes of enzymes from the xanthophyll biosynthetic pathway and their expression in a noncarotenogenic heterologous host have led to overproduction of astaxanthin (Fraser et al. 1997; Grunewald et al. 2001; Sandmann 2001a,b) and canthaxanthin (Lotan and Hirschberg 1995). Genes for carotenoid biosynthetic enzymes have been cloned from several microbes and expressed in several noncarotenogenic yet rapid growing bacteria such as *Escherichia coli* (Misawa and Shimada 1997; Schmidt-Dannert 2000) and in yeast (Shimada et al. 1998). Several reviews have summarized the work done on genetic approaches for overproduction of xanthophylls (Sandmann 1994; Armstrong 1997; Schmidt-Dannert et al. 2000).

Metabolic engineering is an alternative to classical strain improvement in the optimization of xanthophyll production. Directed genetic modifications can be introduced to improve the production properties of the microbial strains. The development of high-throughput DNA sequencing will enable researchers to identify the regulatory mechanisms for the overproduction of xanthophylls.

In addition to that, designing fermentation media that can significantly increase product concentration, yield, and volumetric productivity will be crucial for developing industrial fermentation processes for xanthophyll carotenoids. Adequate manipulation of carbon, nitrogen, and mineral sources, and the inclusion of precursors and stimulators in the medium represent the best strategy for improving medium development for microbial processes. The development of cost-effective medium supplies for microbial production of xanthophylls is another key aspect for a commercially successful process. These media manipulations can be done via systematic statistical approaches which are less time consuming and more accurate.

Conclusions

Xanthophylls are playing an ever-increasing role in human health in the developed world. Consequently, a multimillion dollar market has been established in the last 20 years. However, it is also the well-established fact that chemical synthesis is fulfilling most of this demand. Among xanthophylls, only astaxanthin is currently marketed as a microbial product. Lower yield, longer production time, lack of indoor mass production facilities for microbial sources, and poor exploitation of biosynthetic pathways are the major obstacles in the implementation of microbial technology, but recent identification of the genes for the complex biosynthetic pathways of xanthophyll biosynthesis have made it likely that these obstacles can be overcome in the near future.

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