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Carotenoid pigment production from Yeast: Health benefits and their industrial applications

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Abstract
Carotenoids are natural pigments with important biological activities, such as antioxidant and provitamin A activity, that can be either extracted from plants and algae or synthesized by various microorganisms, including bacteria, yeasts, filamentous fungi, and microalgae. Advantages of microbial production include the ability of microorganisms to use a wide variety of low cost substrates, the better control of cultivation, and the minimized production time. Yeasts are more convenient than algae or molds for large scale production in fermenters, due to their unicellular nature and high growth rate. Carotenoids have good effect on human health, such as pro-vitamin A, antioxidant, anticancer, antiobesity effect and anabolic effect on bone components. Currently, carotenoids are used commercially as feed additives, animal feed supplements, natural food colorants, nutrient supplement and more recently, as nutraceuticals for cosmetic and pharmaceutical purposes.

Keywords: Natural pigments, microbial carotenoids, health benefits, industrial applications

Introduction

Carotenogenic yeasts are considered to be ubiquitious due to its world-wide distribution in terrestrial, freshwater and marine habitats and their ability to colonize a large variety of substrates.Carotenoids are biosynthesized by yeast, bacteria, algae, fungi, and plants, but not by animals, which must obtain them from their food. Carotenoid pigment production by yeasts namely, *Phaffia rhodozyma*, *Rhodotorula* sp., *Sporobolomyces roseus*, *Rhodosporidium* sp., *Sporidiobolus* sp. and *Candida utilis* is documented. Carotenoids such as β-carotene and xanthophylls like astaxanthin play central roles in the metabolism of the eye's macula and retina and in maintaining healthy vision. β-carotene play a role in the prevention of cancer. These carotenoid compounds are divided into two major classes based on their structural elements; carotenes, constituted by carbon and hydrogen (e.g. β -carotene, α -carotene and lycopene), and xanthophylls, constituted by carbon, hydrogen, and additionally oxygen (for example lutein, β -cryptoxanthin, zeaxanthin, astaxanthin and fucoxanthin) (Maldonade *et al.*, 2007) [14]. There is evidence that carotenoid pigments function as chemo-protectives. It may also act as nutraceuticals that prevent carcinogenesis through anti-oxidative, anti-free radical or other mechanisms. The beneficial nutraceutical functions of the carotenes and xanthophylls extend to the prevention of heart attacks and strokes. (Marova *et al.*, 2012) [15].

Carotenoids Diversity

Carotenoids are lipid-soluble pigments, colored from yellow to red, with a basic structure consisting in a tetraterpene with a series of conjugated double bonds. They can have only carbon and hydrogen in their structures or have one or more oxygen atoms, being classified as xanthophylls. The majority of carotenoids are C40 terpenoids, which act as membrane-protective antioxidants scavenging O2 and peroxyl radicals (Matagomez *et al.*, 2014) [16]. There are more than 700 types of carotenoids described and only about 50 are precursors of vitamin A. Carotenoids can reduce risks for degenerative diseases such as cancer, cardiovascular diseases, macular degeneration, and cataract. The biological activities, specially the antioxidant properties, depend on their chemical structure: number of conjugated double bonds, structural end-groups, and oxygen-containing substituents (Rodrigues *et al.*, 2012).

Carotenoids occur in photosynthetic systems of higher plants, algae, and phototrophic bacteria. In plants, carotenoids are embedded in the membranes of chloroplasts and chromoplasts. The colors of these pigments are masked by chlorophyll, but they contribute to the bright colors of many flowers and fruits (Bartley and Scolnik, 1995) [3].

In nonphotosynthetic organisms, as some bacteria and fungi have carotenoids which act as a protectors against photo-oxidative damage, a way of protection in growth conditions with light and abundant air.

The industrial production of carotenoids by plants is dependent on the season and geographic variability, and these cannot always be controlled. The chemical synthesis of carotenoids generates wastes that can cause damage to the environment and resistance by the consumers. Because of this, the biotechnological resources are becoming more interesting. The microbial production of carotenoids can be performed using low-cost substrates or substrates that are residues from industrial processes, like molasses, resulting in lower costs of production (Marova *et al.*, 2012) [15]. All conditions of this kind of production can be controlled and optimized, particularly know about the metabolic route of each microorganism utilized.

Main carotenoid biosynthesis pathways

Carotenoids are usually produced from the building blocks geranyl geranyl diphosphate (GGPP) and farnesyl diphosphate (FPP), like other secondary metabolites such as sesquiterpenoids and steroids. The most common pathway is the condensation of 2 GGPP units into prephytoene diphosphate and then to phytoene, a 40-carbon polyunsaturated precursor which is colorless. This precursor is converted into lycopene and then into several derived carotenoids such as β-carotene and oxidized derivatives such as lutein. The condensation of two units of FPP leads to 30-carbon precursors that are converted to steroids or apocarotenoids such as staphyloxanthin (Farre *et al.*, 2010) [8]. Apocarotenoids can also be produced by oxidative cleavage of carotenoids.

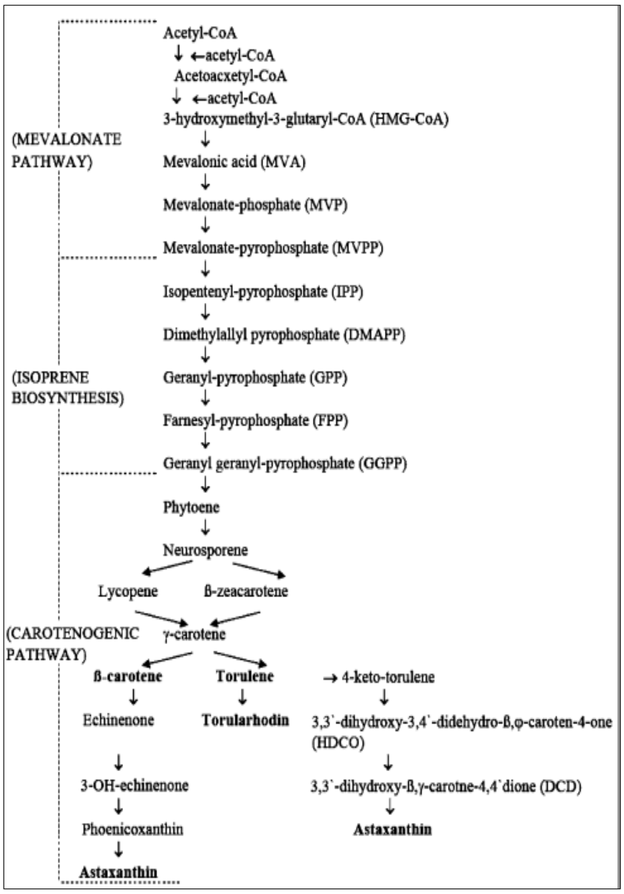

Fig 1: Isoprenoid pathway of carotenoid biosynthesis. (Frengova and Beshkova, 2009).

***Phaffia rhodozyma*:** The red yeast *X.dendrorhous* which was formerly known as *P. rhodozyma* was isolated by Herman phaff in the 1960's during his pioneer studies of yeast ecology. Initially, the yeast was isolated from limited geographical region, but isolates were subsequently obtained from Russia, Chile, Finland and United States. The biological diversity of the yeast is more extensive than originally envisioned by Phaff and his collaborators, and atleast two species appear to exist including the anamorph *P. rhodozyma* and the teleomorph *X. dendrorhous*. The Yeast has attracted considerable biotechnological interest because of its ability to synthesize the economically important carotenoid astaxanthin (3, 3'-dihydroxy-beta, beta-carotene-4, 4'dione) as its major pigment. This property has stimulated research on the biology of the yeast as well as development of the yeast as an industrial microorganism for astaxanthin production by fermentation.

Red yeasts can use diverse sources of carbon, including low cost raw materials, such as molasses, whey and agricultural industrial by products, such as crude glycerol. The red yeast is able to grow under a wide range of initial pH conditions from 2.5 to 9.5 and over a wide range of temperatures from 5 to 26 ^{0}C. It is the only known red yeast that produces astaxanthin. In fact, astaxanthin contributes to 80-90% of its total carotenoids. Carotenoids are produced during late log phase or stationary phase by the mavolanate isoprenoid pathway. The red pigmented fermenting yeast *P. rhodozyma* contains astaxanthin as the principal carotenoid pigment, Echinenone, 3-hydroxyechinenone and phoenicoxanthin were also isolated and identified; isocryptoxanthin and canthaxanthin were absent (Frengova and Beshkova,2009) [7, 9].

***Rhodotorula* sp:** Yeast in the genus *Rhodotorula* synthesize carotenoid pigments. Feed supplement with a *Rhodotorula* cell mass has been found to be safe and non toxic in animals. Its use in the nutrition of laying hens has also been documented (Abd El-Rahman *et al*., 2012) [1]. Various species of *Rhodotorula* were isolated from plant leaves, flowers, fruits, slime fluxes of deciduous trees, soil, refinery waste water, air and yoghurt (Aksu and Eren, 2007) [2]. The production of carotenoids by genus *Rhodotorula* is affected by species, medium constituents and environmental conditions. Besides carotenoids, *Rhodotorula* is a rich source of fats and vitamins and can be incorporated in feeds to enhance the nutritional value and prevent fungal contamination (Joshi *et al*., 2003) [10].

***Sporobolomyces* sp:** In *Sporobolomyces* species, primarily produced pigment is β-carotene. Production of torulene was not clearly confirmed, nevertheless, some oxidized carotenoid derivatives are produced in *Sporobolomyces* strains. In most of red yeast osmotic and oxidative stress was reported to induce carotenoid production probably as a part of stress response (Marova *et al*., 2012) [15]. The genus *Sporobolomyces* contains about 20 species. The most common one is *S. roseus* and *S. salmonicolor*. *Sporobolomyces* colonies grow rapidly and mature in about 5 days. They produce yeast like cells, pseudohyphae and ballistoconidia. The yeast like cell is the most common type of conidia and oval to elongate in shape (Nanjundasamy and Praveen, 2010) [18]. The optimal growth temperature is 25-30 ºC. The colonies are smooth, often wrinkled and glistening to dull. *S. roseus* produces other carotenoid pigments such as torulene and torularhodin (Maldonade *et al*., 2007) [14].

***Cryptococcus* sp**: *Cryptococcus neoformans* is the only yeast known that consistently produces a melanin-like pigment from many p-diphenols. Pigment production from diphenols by other members of the genus is very rare. However, pigment production by some *Cryptococcus* species can occur when hydroquinone is present in the substrate. *Cryptococcus* may also produce small amount of pigment from diphenols after prolonged incubation (10 days or more).The strains of *C. neoformans* produced brown cell-associated intracellular pigments while others produced pink extracellular pigments from l- or- dl-tryptophan. Additionally, various other *Cryptococcus* species produced brown or pink pigments when cultured on tryptophan.

Functions of carotenoid pigments

Being an important flavonoid compound, β- carotene has powerful antioxidant functions, helps the body scavenge free radicals, thereby limiting the damage to cell membranes, DNA and protein structures in the cell. Research studies suggest that dietary intake of foods high in β-carotene has positive association with decreased risk of cardio-vascular disease as well as oral cavity, and lung cancers. When converted to vitamin A in the intestines it has all the functions of vitamin A such as visual cycle, reproduction (sperm production), maintenance of epithelial functions, growth and development (Davoli *et al*., 2004) [5].

The synthesis of different naturally and commercially important carotenoids (β-carotene, torulene, torularhodin and astaxanthin) by several yeast species, belonging to the genera *Rhodotorula* and *Phaffia,* has led to consider these microorganisms as a potential pigment sources (Frengova *et al*., 1994) [6]. Perrier *et al.* (1995) [19] reported that *R. glutinis* produce characteristic carotenoids (torulene, torularhodin and β-carotene) in various proportions (Fig.1), (Table 1).

β-carotene: It protect cells from the damaging effects of free radicals, provide a source of vitamin A, enhance the functioning of immune system and helps reproductive system function properly (Pratheeba *et al*., 2014) [20].

Torulene: Torulene is a typical carotenoid found in yeasts. It is a very interesting carotenoid due to its structure, half β-carotene and half lycopene, which is responsible for provitamin A activity and antioxidant property (Maldonade *et al*., 2007) [14].

Torularhodin: It has a very important antoxidant activity and provitamin A. (Sakaki *et al*., 2000) [25].

Table 1: Carotenoid pigment and their properties

Microbial source (Yeast)	Molecule	Colour	References
Xanthophyllomyces dendrorhous	Astaxanthin	Pink-red	Ramirez *et al.* (2000)
Rhodotorula sp.	Torularhodin, β-carotene, torulene	Orange, yellow, pink	Sakaki *et al*.(2000)
Sporodiobolus salmonicolor	β-carotene	Orange	Valduga *et al*.(2008)
Sporobolomyces roseus	βcarotene, torulene, torularhodin	Orange, pink	Maldonade *et al*.(2007)
Cryptococcus sp.	β-carotene	yellow	Libkind and Broock (2006)
Rhodospiridium babjevae	β-carotene	Orange	Sperstad *et al*.(2006)
Phaffia rhodozyma	Astaxanthin	pink	Yang *et al*.(2011)
Sporobolomyces ruberrimus	Torularhodin	Orange	Ravaghi *et al*.(2016)

Applications of carotenoid pigment

Food industry: Carotenoid pigments are one of the leading food colourants in the world. Carotenoids has been applied to a range of food and beverage products to improve their appearance to customers, including items such as margarine, cheese, fruit juices, baked goods, dairy products, canned goods, confectionary and health condiments. Carotenoids, mainly β-carotene, astaxanthin, α-carotene, fucoxanthin, halocynthaxanthin and peridinin, are used as food colorants owing to their antioxidant nature and provitamin A activity. Carotenoids are usually fortified in the food preparations (mainly in baby and infant food) to improve their vitamin content. Applicability of microbial pigment extracted from *R. glutinis* DFR-PDY for colouring foodstuff was studied by adding to puffed products like popcorn, biscuit creams, cake icing and ice creams (Latha and Jeevaratnam, 2010) [12].

Feed industry: Carotene is used in various pet foods as both a colourant and a precursor to viamin A. Carotenoid pigment can be applied to an array of animal foods designed for pets, including dogs, cats, fish and birds. The antioxidant and precursory vitamin A properties increase the appeal and application of carotenoid pigment in pet foods.

Pharmaceutical industry: Industrially, carotenoids are used in pharmaceuticals, neutraceuticals, and animal feed additives, as well as colorants in cosmetics and foods (Mortensen, 2006) [17]. Canthaxanthin and astaxanthin also have considerable importance in aquaculture for salmonid and crustacean pigmentation, and also have commercial interest in the pharmaceutical and food industries (Bhosale and Bernstein, 2004) [4]. Basically carotenoids are precursors of vitamins, and they also present activities such as anti-inflammatory, antioxidant, immunomodulatory, anticancer, for cardiovascular therapy and neurodegenerative diseases (Shahidi and Ambigaipalan, 2015) [26] and anti-obesity (Lai *et al.*, 2015) [11]. Carotenoid contains provitamin A activity; vitamin A is an essentialnutrient for operation and maintenance of biological functions including vision, reproduction, and immunity (Revuelta *et al.,* 2016) [23]

Conclusion

The pigment production from microorganisms include easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades. Carotenoids are used in food industries as colourants, feed additive, vitamin A sources and are being preferred to synthetic pigments. The interest in the natural pigments has increased in recent years due to the consumer concern about the harmful effects of synthetic pigments on health and with the development of new food products based on natural ingredients

Reference

1. Abd El-Rahman A, El-Banna, Amal Mohamed Abd El-Rafik, El-Mahdy. Some Factors Affecting the Production of Carotenoids by *Rhodotorula glutinis* var. *Glutinis.* Food and nutrition sci. 2012; 3:627-633.

2. Aksu Z, Eren AT. Production of Carotenoids by Isolated Yeast of *Rhodotorula glutinis* Biochem. Eng. 2007; 35(2):107-113.
3. Bartley GE, Scolnik PA. Plant carotenoids: Pigments for photoprotection, visual attraction and human health. The Plant Cell. 1995; 7:1027-1038.
4. Bhosale P, Bernstein PS. Beta- carotene production by *Flavobacterium multivorum* in the presence of inorganic salts and urea. J Ind. Microbiol. Biotechnol. 2004; 43:112-125.
5. Davoli P, Mierau V, Weber RWS. Carotenoids and fatty acids in red yeasts *Sporobolomyces roseus* and *Rhodotorula glutinis*, Appl. Biochem. Microbiol. 2004; 40:392-397.
6. Frengova G, Simova E, Pavlova K, Beshkova D, Grigorora D. Formation of carotenoids by *Rhodotorula glutinis* in whey ultrafiltrate. Biotechnology and Bioengineering. 1994; 44:888-894.
7. Frengova G, Beshkova M. Carotenoids from *Rhodotorula* and *Phaffia*: yeasts of biotechnological importance. Journal of Industrial Microbiology and Biotechnology. 2009; 36(2):163-180.
8. Farré G, Sanahuja G, Naqvi S, Bai C, Capell T, Zhu C *et al*. Travel advice on the road to carotenoids in plants. Plant Science. 2010; 179:28-48.
9. Frengova G, Beshkova M. Carotenoids from *Rhodotorula* and *Phaffia*: yeasts of biotechnological importance. Journal of Industrial Microbiology and Biotechnology. 2009; 36(2):163-180.
10. Joshi VK, Attri D, Bala A, Bhushan S. Microbial pigments. Indian J Biotechnol. 2003; 2:362-369.
11. Lai CS, Wu JC, Pan MH. Molecular mechanism on functional food bioactives for antiobesity. Current Opinion in Food Science. 2015; 2:9-13.
12. Latha BV, Jeevaratnam K. Purification and Characterization of the Pigmentsfrom *Rhodotorula glutinis* DFR-PDY Isolated from Natural Source. Global J. Biotech and Biochem. 2010; 5(3):166-174.
13. Libkind D, Broock VM. Biomass and carotenoid pigment production by patagoniannative yeasts. Journal of Microbiology and Biotechnology. 2006; 22:687-692.
14. Maldonade R, Adilma R, Scamparini P, Rodriguez-Amaya B. Selection and characterization of carotenoid-producing yeasts from campinas region, brazil. Brazilian Journal of Microbiology. 2007; 38:65-70.
15. Marova I, Carnecka M, Halienova A, Certik M, Dvorakova T, Haronikova A. Use of several waste substrates for carotenoid-rich yeast biomass production.Journal of Environmental Management. 2012; 95:338-342.
16. Matagomez LC, Montañez JC, Méndez-Zavala A, Aguilar CN. Biotechnological production of carotenoids by yeasts: An overview. Microbial Cell Factories. 2014; 13:12.
17. Mortensen A. Carotenoids and other pigments as natural colorants. Pure Appl. Chem. 2006; 78:1477-1491.
18. Nanjundasamy A, Praveen VJ. Ind Microbiol Biotechnol. 2010; 37:1183-1192.
19. Perrier V, Dubreucq E, Galzy P. Fattyacid and Carotenoid composition of *Rhodotorula* strains. Archives of Microbiology. 1995; 164:173-179.
20. Pratheeba M, Umaa Rani K, Kotteeswaran R, Thamaraikannan H. Extraction and analysis of beta carotenoid from red yeast *Rhodotorula glutinis.* International Journal of Pharmacy and Integrated Life Sciences. 2014; 2(3):9-29.
21. Ramirez J, Nunez ML, Valdivia R. Increased astaxanthin production by a *Phaffia rhodozyma* mutant grown on date juice from *Yucca fillifera*. J Ind. Microbiol. Biotechnol. 2000; 24:187-190.
22. Ravaghi M, Razavi SH, Mousavi SM, Sinico C, Fadda AM. Stabilization of natural canthaxanthin produced by Dietzia natronolimnaea HS-1 by encapsulation in niosomes. Food Science and Technology. 2016; 73:498-504.
23. Revuelta JL, Buey RM, Ledesma-Amaro R, Vandamme EJ. Microbial biotechnology for the synthesis of (pro) vitamins, biopigments and antioxidants: Challenges and opportunities. Microbial Biotechnology. 2016; 9:564-567.
24. Rodrigues E, Mariutti LRB, Chisté RC, Mercadante AZ. Development of a novel microassay for evaluation of peroxyl radicalscavenger capacity: Application to carotenoids and structure-activity relationship. Food Chemistry. 2006; 135:2103-2111.
25. Sakaki H, Nakanishi T, Satonaka KY, Miki W, Fujita T, Komemushi S. Properties of a high-torularhodin mutant of *Rhodotorula glutinis* cultivated under oxidative stress. J. Biosci. Bioeng. 2000; 89:203-205.
26. Shahidi F, Ambigaipalan P. Novel functional food ingredients from marine sources. Current Opinion in Food Science. 2015; 2:123-129.
27. Sperstad S, Lutnaes BF, Stormo SK, Liaaen-Jensen S, Landfald B. Torularhodin and torulene are the major contributors to the carotenoid pool of marine *Rhodosporidium babjevae* (Golubev). J Ind. Microbiol. Biotechnol. 2006; 33:269-273.
28. Valduga E, Valerio A, Treichel H, Furigo A, Luccio MD. Study of the bio-production of carotenoids by *Sporidiobolus salmonicolor* (CBS 2636) using pre-treated agro industrial substrates. J. Chem. Technol. Biotechnol. 2008; 83:1267-1274.
29. Yang T, Haisheng Y, Rui S, Xiaohuan, Zhai Hairui, Kaimian Li. Astaxanthin production by *Phaffia rhodozyma* fermentation of cassava residues substrate. Agric Eng Int: CIGR Journal. 2011; 13(2):1-6.