

Microbial Pigments-A Short Review

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Abstract: Color is the main feature of any food item as it enhances the appeal and acceptability of food. In the recent years, coloring of food with pigments produced from natural sources is of worldwide interest and is gaining importance. Several types of dyes are available in the market as colouring agents to food commodities but biocolorants are now gaining popularity and considerable significance due to consumer awareness because synthetic dyes cause severe health problems. Though many natural colors are available, microbial colorants play a significant role as food coloring agents, because of their production and easy down stream processing. Industrial production of natural food colorants by microbial fermentation has several advantages such as cheaper production, easier extraction, higher yields through strain improvement, no lack of raw materials and no seasonal variations.

Keywords: Biocolorants, Synthetic dyes, Downstream process, Fermentation, Strain improvement.

I. Introduction

There is worldwide interest in process development for the production of pigments from natural sources due to a serious safety problem with many artificial synthetic colourants, which have widely been used in foodstuff, cosmetic and pharmaceutical manufacturing processes^{1,2,3,4}. It is well known that a variety of plants, animals and micro-organisms produce pigments^{5,6,7}. Although there are a number of natural pigments, only a few are available in sufficient quantities to be useful for industry because they are usually extracted from plants⁸. Therefore, it is advantageous to produce natural pigments using micro-organisms.

Pigments come in a wide variety of colors, some of which are water-soluble⁹. Microbial pigments are of industrial interest because they are often more stable and soluble than those from plant or animal sources¹⁰. Microorganisms can grow rapidly, which can lead to high productivity, and can produce a product throughout the year¹¹. Most of the bacteria and fungi are widely studied for their potential as a source of food colorants. Natural pigments possess anticancer activity, contain pro-vitamin A and have some desirable properties like stability to light, heat and pH¹². Thus, the food industry has become increasingly interested in the use of microbial technology to produce colors for use in foods. Special attention has been focused on the strains belonging to the *Monascus* genus of filamentous fungi. Some authors refer to these fungi as potent producers of natural pigments^{13,14,15}. However, there are other microorganisms which have the ability to produce pigments in high quantities, such as those belonging to the genus *Paecilomyces*¹⁶, producing red, yellow, and violet pigments in quantities of up to 4.73 g/L. Microorganisms belonging to the genera *Aspergillus* and *Penicillium* have also been studied as potential producers of natural pigments^{17,18,19,20,21}. The production of *Monascus*-like pigments from *Penicillium* strains has recently been reported. These pigments have a potential use in the food industry because they are not associated with citrinin production. They are homologues of pigments of *Monascus* which have similar chromophore polyketides²² and of fungal strains of the species *Epicoccum nigrum* that produce yellow pigments²³. The three *Penicillium* sp. strains capable of producing red pigments were also isolated recently²⁴.

Microorganisms are known to produce a variety of pigments; therefore they are promising source of food colorants^{25,26}. Some characteristics of synthetic colorants such as detrimental effects on environment, the development of mental illness, allergies and diverse type of cancers associate with the use of artificial colorants reduced the number of synthetic colorants approved by federal regulatory agencies²⁷. Furthermore, there is a growing demand for ecofriendly/non-toxic colorants, specifically for health sensitive applications, such as the coloration of food and dyeing of children's fabrics and leather garments²⁸.

II. Significance Of Microbial Pigments As Natural Colorants

Some of the most significant natural pigments are carotenoids, flavonoids, tetrapyrroles and some xanthophylls as astaxanthin. The pigment most frequently used in industries is beta-carotene which is obtained from some microalgae and cyanobacteria. Astaxanthin produced from *Phaffia rhodozoa* and *Haematococcus pluvialis*, is a red pigment of great commercial value and is used in feed, pharmaceutical and aquaculture industries. Some of the natural colorants are used in baby foods, breakfast cereals, sauces, pastas, processed

cheese, fruit drinks, vitamin-enriched milk products, and some energy drinks. Thus, natural colors in addition to being eco-friendly, can also serve the dual need for visually appealing colors and probiotic health benefits in food products²⁹.

III. Microbial Pigments Of Commercial Importance

The success of any microbial pigment produced by biotechnological means (for example fermentation) depends upon its acceptability in the market, regulatory approval, and the size of the capital investment required in bringing the product to the market. A few years ago, some expressed doubts about the positive commercialization of fermentation derived food grade pigments because of the high capital investments requirements for fermentation facilities and the expensive and time-span toxicity studies required by regulatory agencies^{30,31}. In addition to the above public perception of biotechnology derived products should also be taken into account for the absolute production benefit. Table. 1 states the successful microbial pigments already in use as food grade and/or nutritional supplements in the market and are derived from various bacteria, yeast and fungi. Based on the extensive research reports, these pigments broadly can be categorized into pigments of industrial production (IP), developmental stage (DS) and research project (RP) phase (Table 1)^{31,32}. Microbial colorants are in use in the fish industry already, for instance, to enhance the pink color of farmed salmon. Further, some natural food colorants have commercial prospective for use as antioxidants³³. The successful marketing of pigments derived from microbes, both as a food color and a nutritional supplement, reflects the presence and significance of niche markets in which consumers are willing to pay a premium for ‘all natural ingredients’.

Table 1. Pigments from various microorganisms which are already in use as natural food colorants^{31,32}.

Pigment	Color	Microorganism	Status
Ankaflavin	Yellow	<i>Monascus sp.</i>	IP
Anthroquinone	Red	<i>Pencillium candidum</i>	IP
Monascorubramine	Red	<i>Monascus sp.</i>	IP
Riboflavin	Yellow	<i>Ashbya gossypi</i>	IP
Rubropanctatin	Orange	<i>Monascus sp.</i>	IP
β Carotene	Yellow-orange	<i>Blakeslea trisporia</i>	IP
Astaxanthin	Pink-red	<i>Agrobacterium aurantiacum</i>	RP
Astaxanthin	Pink-red	<i>Paracoccus carotinifaciens</i>	RP
Cathaxanthin	Dark red	<i>Bradirhizobium sp.</i>	RP
Lycopene	Red	<i>Fusarium sporotrichioides</i>	RP
Melanin	Black	<i>Saccharomyces neoformis</i>	RP
Napthoquinone	Deep blood red	<i>Cardyiceps unilateralis</i>	RP
Zeaxanthin	Yellow	<i>Paracoccus zeaxanthinifaciens</i>	RP
β Carotene	Yellow-orange	<i>Fusarium sporotrichioides</i>	RP
β Carotene	Yellow-orange	<i>Neurospora crassa</i>	RP
β Carotene	Yellow-orange	<i>Phycomyces blaksleeanus</i>	RP
Unknown	Red	<i>Paecilomyces sinclairii</i>	RP
Astaxanthin	Pink-red	<i>Xanthophyllomyces dendrohous</i>	DS
Lycopene	Red	<i>Blakeslea trisporia</i>	DS
Rubrolone	Red	<i>Streptomyces echinoruber</i>	DS
Torularhodin	Orange-red	<i>Rhodotorula sp.</i>	DS
Zeaxanthin	Yellow	<i>Flavobacterium sp.</i>	DS
β Carotene	Yellow-orange	<i>Mucor circinelloides</i>	DS
Unknown	Red	<i>Penicillium purpurogenum</i>	DS

Note : Industrial Production (IP),
 Developmental Stage (DS) and
 Research project Phase (RP)

IV. ILow-Cost Substrates

J. Tinoi, *et al.*, reported that agro-industrial by-products and surpluses that frequently create serious environmental problems may be possibly used as inexpensive carbohydrate sources for microbial fermentations, thus decreasing their initial high biological oxygen demand (BOD) while obtaining biochemical compounds like pigments suitable for pharmaceutical, chemical and food industries³⁴. Low cost by products and residues of agro-industrial origin have shown their potential in production of different pigments by diverse group microorganisms. Along this line, variety of substrates and microorganisms has been tested. beta-carotene

synthesis by citrus products³⁵, carotenoids production using whey ultrafiltrate³⁶, sauerkraut brine³⁷ and peat extract³⁸, riboflavin in concentrated rectified grape must³⁹, astaxanthin on grape juice⁴⁰ are some promising studies.

These by-products from industrial processes and other agro or domestic sources are pollutants to the environment and their treatment involves high costs. The conversion of these wastes to value added materials like pigments by microorganisms would provide economic benefits and reduce waste materials impact on environment as pollutants. Many investigations have been performed and are under investigation to diminish the costs and optimize the pigments production⁴¹⁻⁴⁸. Factors such as carbon and nitrogen source are very important to consider on the selection of wastes as substrates. And pigment production also depends on minerals and other components in some cases. Pigments produced on several wastes were shown in Table 2.

Table 2. Different microorganisms and various inexpensive substrates used for pigments production.

S.no	Substrate	Microorganism	Pigment type	References
1.	Whey	<i>R. glutinis</i>	β -carotene	[49]
2.	Potato medium	<i>R. mucilaginosa</i>	β -carotene	[49]
3.	Crude glycerol	<i>R. glutinis</i>	carotenoids	[50]
4.	Chicken feathers	<i>R. glutinis</i>	carotenoids	[51]
5.	Fermented radish brine	<i>R. glutinis</i>	β -carotene	[52]
6.	Mung bean waste flour and Sweet potato extract	<i>R. glutinis</i>	carotenoids	[53]
7.	Mustard waste	<i>X. dendrorhous</i>	Astaxanthin	[34]
8.	Plant extracts	<i>X. dendrorhous</i>	Astaxanthin	[54]
9.	Coconut milk	<i>X. dendrorhous</i>	Astaxanthin	[55]
10.	Enzymatic hydrolysates of prehydrolysed wood	<i>X. dendrorhous</i>	Astaxanthin	[56]
11.	Sugarcane waste marine	<i>Streptomyces</i> sp.	melanin	[57]
12.	Jack fruit seed	<i>M. ruber</i>	Monascorubramine	[58]
13.	Rice bran	<i>M. purpureus</i>	Monascorubramine	[59]
14.	Wheat bran	<i>M. purpureus</i>	Monascorubramine	[59]
15.	Sesame oil cake	<i>M. purpureus</i>	Monascorubramine	[59]
16.	Palm kernel cake	<i>M. purpureus</i>	Monascorubramine	[59]
17.	Groundnut Oil cake	<i>M. purpureus</i>	Monascorubramine	[59]
18.	Cassava powder	<i>M. purpureus</i>	Monascorubramine	[59]
19.	Corn cob Substrate	<i>M. purpureus</i>	Monascorubramine	[60]
20.	Grape waste	<i>M. purpureus</i>	Monascorubramine	[61]
21.	Prickly Pear Juice	<i>M. purpureus</i>	Monascorubramine	[62]
22.	Corn steep liquor	<i>M. ruber</i>	Red pigment	[63]
23.	Shrimp & Crab shell powder	<i>M. purpureus</i> CCRC31499	Monascorubramine	[64]
24.	Hairy roots of madder (<i>Rubia.tinctorum</i> L.)	<i>P. candidum</i>	Anthroquinone	[65]
25.	Marigold flower (<i>Tagetes.ereecta</i> L.)	<i>R. glutinis</i>	Lutein	[66]
26.	Apple pomace	<i>R. glutinis</i>	carotenoids	[67]
27.	Radish brine	<i>R. glutinis</i>	β -carotene	[68]
28.	Durian Seed	<i>Monascus.sp</i>	angkak moncolin	[69]
29.	Kinnow waste	<i>M. purpureus</i> MTCC369	Bio-pigment	[70]
30.	Sugarcane bagasse	<i>P.echinulatum</i> 9A02S1	Cellulase, Xylanase	[71]
31.	Date syrup	<i>R. glutinis</i> (PTCC 5256)	Carotenoids	[72]
32.	Liquid Pineapple	<i>Chromobacterium. violaceum</i> UTM5	Violet pigment	[73]
33.	Spoilt Onion	<i>P.purpurogenum</i>	Red Exopigment	[74]

V. Conclusion

The overall objective of this study is to explore the possibility of pigment production by different microbial isolates from numerous sources on various substrates. Furthermore, the enhanced pigment production capability of carotenoid producing microorganisms in future may lead to the production of nontoxic plant & microbial food colorants which will be beneficial to the mankind & livestock.

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