



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(9): 3200-3205
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www.thepharmajournal.com
Received: 21-07-2022
Accepted: 24-08-2022

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A review on potential microbial sourced bio-colours from fungi

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Abstract

Now a days synthetic colour production is technically advanced and economically efficient but most of the artificially synthesized colours used in foodstuff, dyestuff, cosmetics and pharmaceutical manufacturing processes, causing various hazardous effects like toxic diseases includes cancer and other behavioural disorders in children. And children are more prone to these types of diseases because they are pulled to foods with varied colours, especially sweets like pastries and confectionaries that come in a variety of colours. Thus to counteract the ill effects of the synthetic colorants, there is an increased awareness about eco-friendly and safe colours, there is worldwide interest in process development for the production of pigments and colours from natural sources. Bio-colorants are mostly isolated from different sources such as Plants (Flowers, fruits, seeds, roots etc.), Animals (Cochineal, lac etc.), Microorganisms. Most of the bio colorants are extracted from plants and which as disadvantage of low yield and low eco-efficiency. Thus microbially sourced bio colors are gaining importance to overcome this problems. Few organisms like Bacteria, Fungi, Yeasts and Algae can be used to extract most useful synthesized bicolor. Extraction of colours from natural source is simple and with effective protocols. Especially the bio colors sourced from Fungi provides reliable and scalable technology in extraction. Some microbial sourced pigments include Astaxanthin, Ankaflavin, Anthraquinone, β -carotenes, Canthaxanthin, Lycopene, Melanin, Naphtoquinone, Riboflavin, Rubropunctatin, Torularhodin are discussed here. And reviewed basic information about bio colors along with their nutraceutical values and health benefits and also microorganisms which produces the commercially important and effective colorants.

Keywords: Bio-colors, micro-organisms, fungi, pigments, nutraceutical functions

Introduction

Nevertheless the production of synthetic colours is now technologically and economically advanced, the majority of these colours, which are used in the manufacturing of foods, dyes, cosmetics, and pharmaceuticals, have serious health consequences, including cancer and other behavioural disorders in children. Children are more prone to these types of diseases because they are pulled to foods with varied colours, especially sweets like pastries, confectionaries and milk chocolates that come in a variety of colours. Thus, there is a greater interest in the development techniques for the synthesis of pigments and colours from natural sources on a global scale in order to counteract the negative impacts of these synthetic colorants^[1]. Since the name "bicolor" is made up of the words "bio" and "colour," which both refer to things that are derived naturally and are used to colour things, bio colorants are an alternative to artificial colouring agents in food products^[2, 4].

Many food manufacturers all across the world are expressing interest in producing natural colours in response to consumer demand, and they are making an effort to replace potential hazardous artificial colorings in the majority of foods and beverages. Natural colours make up 29% of the \$600 million global market for food colorants, which is already rising at a rate of 7% yearly. We need to switch to colours generated from microbes because the majority of natural colours are mostly extracted from plants, which have the drawback of instability and seasonal supply^[1, 5, 6].

History

The use of colour has been a heritage for mankind since the dawn of creation. According to sources, natural dyeing was originally practiced in India and China in 2600 BC, as evidenced by the discovery of madder dye traces in coloured clothing as well as in the excavations of the Harappa and Mohenjo Daro civilizations. Natural dyeing was also reportedly used in Europe during the Bronze Ages (3500 BC). Mummy wrappings added additional colour evidence in

Egypt, and records confirmed the existence of the natural dye Alizarin, an isolate of madder, in King Tutankhamen's tomb, supporting the utilization of natural dyes. Cochineal dye was produced by people in Central and North America throughout the Aztec and Maya cultures. Up to the fourth century AD, colours such as woad, madder, weld, Brazil wood, indigo, and reddish-purple have been used. The presence of the dye woad gave Brazil its name. Even though the Bible mentioned saffron, the use of henna dates back to before 2500 BC. The writing in the shosoin from the Nara period of the eighth century also mentions that people from some sections consume coloured processed food [7, 8].

Classification

Bio-colorants are mostly isolated from different sources, such as:

1. Plants (Flowers, fruits, seeds, roots etc.),
2. Animals (Cochineal, lac etc.)
3. Microorganisms (*Monascus*, *Rhodotorula*, *Bacillus*, *Achromobacter*, *Phaffia* etc.).

The majority of bio colorants are obtained by extracting them from plants, which has the limitations of low yield and low eco-efficiency. In order to solve these issues, microbially sourced bio colors are becoming much more important. A variety of microorganisms, including Bacteria, Fungi, Yeasts, and Algae, can be employed to extract the majority of bio

colors produced by microbes. Color extraction from natural sources can be done easily and with advanced techniques [6]. Safety and efficacy are important factors that should be taken into consideration when extracting colours from microorganisms. They ought to be safe because they play a vital role as food colourings. They are shown substantial advantages for pigment generation. Through the use of microbes, the limitations of plant-based pigments, such as their reliance on the weather, can be eliminated. Microbes can grow quickly and readily, and by using cheaper substrates, it is possible to synthesize pigments with a variety of hues [9]. The rapid rate of growth and mass multiplication capabilities of microorganisms used to create colours are two key advantages. The bio colors sourced from Fungi provides reliable and scalable technology in extraction of color [7, 8, 10, 11, 12].

The microbially sourced natural bio colour can be synthesized by using class of microorganisms mentioned below

1. Bacteria
2. Yeast
3. Molds

Bacteria

Bacterial species may synthesize a variety of diverse secondary metabolites, such as the flavours, colours (Table-1) and antibiotics [13, 14].

Table 1: Bio pigments produced by different strains of Bacteria

Pigment	Colour	Micro organism
Astaxanthin	Pink-red	<i>Agrobacterium aurantiacum</i> , <i>Paracoccus carotinifaciens</i>
Canthaxanthin	Dark- red	<i>Bradyrhizobium sp.</i> , <i>Haloferax Alexandrine</i>
Indigoidine	Blue	<i>Corynebacterium insidiosum</i>
Prodigiosin	Red	<i>Roseomonas rubra</i> , <i>Streptomyces luteireticuli</i> , <i>Vibrio aerogenes</i> , <i>Alteromonas rubra</i> , <i>Serratia marcescens</i> , <i>Serratia rubidaea</i>
Pyocyanin	Blue-green	<i>Pseudomonas aeruginosa</i>
Staphyloxanthin	Golden Yellow	<i>Staphylococcus aureus</i>
Violacein	Purple	<i>Janthinobacterium lividum</i> , <i>Chromobacterium violaceum</i>
Xanthomonadin	Yellow	<i>Xanthomonas oryzae</i>
Zeaxanthin	Yellow	<i>Flavobacterium sp.</i> , <i>Paracoccus zeaxanthinifaciens</i>

Fungi

Yeast: Several Yeast species are considered to be the best

sources of naturally occurring microbial pigment, and they may be used to synthesize various carotenoids (Table- 2).

Table 2: Bio colours produced by different Yeast [13-14]

Pigment	Colour	Microbial source
Astaxanthin	Pink-red	<i>Xanthophyllomyces dendrorhous</i> (<i>Phaffia rhodozyma</i>)
Melanin	Black	<i>Cryptococcus sp.</i> <i>Red Saccharomyces neoformans var. nigricans</i>
Torularhodin	Orange-red	<i>Rhodotorula sp.</i> <i>Rhodotorula glutinis</i>

Astaxanthin: The carotenoid pigment astaxanthin (3, 3'-dihydroxy-carotene-4, 4'-dione), which has the chemical formula C₄₀H₅₂O₄, does not exhibit pro vitamin A activity. In 1938, it was initially isolated from lobsters. It is being utilised commercially in the feed industries with canthaxanthin, the most expensive and important pigment in aquaculture for the colouring of salmon, trout, and shrimp flesh. Astaxanthin is an essential pigment found in the diet of big ornamental fish and aquarium fish. Numerous scientific studies have shown a beneficial effect of these pigments on the colour of egg yolks, skin, and meat tissue in broiler chicken carcasses. It is occasionally used in some nations, such as America, to fortify foods and beverages [15]. The Yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) is particularly concerned

with producing the natural colour astaxanthin. And is distributed widely across the biosphere and the most promising pigment in salmonids and crustaceans. When added to the feed of some farm animal species, this astaxanthin pigment can provide an orange-red colour. To increase the efficacy of the astaxanthin pigment when it is digested and metabolised by the animals, it is essential to rupture the cell wall of the Yeast (either chemically, physically, or enzymatically) before adding it to feed [13,16,17]. It can be produced by using molasses, date juice from *Yucca fillifera*, pineapple juice and grape juice for supplementation of molasses, coconut-milk, potato and carrot extracts we can expect an yield of 7-9 g/L [18]. Astaxanthin is beneficial to cardiovascular, immune, inflammatory, diabetes,

carcinogenic, and neurodegenerative diseases, and as an antiaging and sun proofing agent. And US-FDA considered this under GRAS (Generally recognised as safe) based on their intended use [17].

Torularhodin: *Rhodotorula*, a significant environmental habitant that may be grown from soil, water, and air samples, produces the carotenoid pigments to lutein, carotene, and torularhodin [19]. Carotenoids are organic terpenoid Molecules of 40 carbons. Which, based on the isoprene chain structure and the presence of oxygen in the molecule, is divided into two categories. Which includes carotenes are compounds that include carbon and hydrogen atoms, whereas xanthophyll's are Molecules that have at least one additional oxygen atom. Because of the presence of these functional groups, xanthophyll's are more polar Molecules than carotenes, of which -carotene and lutein are examples. Additionally, the xanthophyll family includes astaxanthin and canthaxanthin [20]. Torularhodin is categorised as a xanthophyll since the molecule contains a carboxyl group. The existence of torularhodin, which was derived from *Rhodotorula* genus of Yeast biomass, was first recognized in the 1930s. Additionally, lutein was noted in *Rhodotorula rubra* in 1946. However, the production and research into the dye have just increased in the last ten years. Some genera of fungus, such as *Cystofilobasidium*, *Dioszegia*, *Neurospora* (*Neurospora Crassa*), *Rhodotorula*, *Rhodospiridium*, *Sporidiobolus*, and *Sporobolomyces*, can be used to make lutein. *Cystofilobasidium* (*Cystofilobasidium infirmominium* and *Cystofilobasidium capitatum*), *Rhodotorula* (*Rhodotorula glutinis*, *Rhodotorula mucilaginosus*), *Rhodospiridium* (*Rhodospiridium babjevae*, *Rhodospiridium toruloides*, *Sporobolomyces*

(*Sporobolomyces ruberius*, and *Sporobolomyces salmonicolor*). There may be many species in these genera, but only a small number of these species have considerable levels of carotenoids [21, 23].

Melanin: Is a pigment that is found all around nature and serves an essential purpose in UV protection. Because of its insoluble property, many details about the chemical structure are still unknown. Most melanised fungus can thrive in severe environments like Antarctica and polluted nuclear reactors because to their radiation resistance [24, 25]. Some Fungi that have become brown have demonstrated their ability to survive in dishwashers and their resistance to heat and detergents [26]. In fungus, there are two routes that can produce Melanin. The DHN pathway is used by the majority of fungus to create Melanin. Additionally, Fungi use 1-3, 4-dihydroxyphenylalanine (L-dopa), a pathway that is similar to mammalian Melanin biosynthesis, to synthesise Melanin. However, *Cryptococcus neoformans* only produces Melanin through the L-dopa route and is completely dependent on external substrate, making it a crucial component of fungal melanisation. [3, 27].

Molds: Fungal carotenoids have recently been given approval by the European Union to be used as food colorants in the future to make polyketide azaphilone colours. The manufacturer is no longer dependent on the seasonal availability of raw materials, which minimizes batch-to-batch variability, which is one of the key benefits of employing colorants from fungal sources. Food coloring can be produced using non-toxic fungal strains like *Penicillium* and *Epicoccum* sp and some of them are mentioned in Table-3 [13, 14].

Table 3: Bio pigments produced by different moulds

Pigments	Colour	Micro organism
Ankaflavin	Yellow	<i>Monascus sp.</i>
Antraquinone	Red	<i>Paecilomyces farinosus</i> <i>Penicillium oxalicum</i>
β -carotene	Cream	<i>Blakeslea trispora</i> , <i>Mucor circinelloides</i> , <i>Neurospora Crassa</i> , <i>Phycomyces blakesleeanus</i>
Canthaxanthin	Orange-Pink	<i>Monascus roseus</i>
Lycopene	Red	<i>Blakeslea trispora</i> , <i>Fusarium sporotrichioides</i>
Naphthoquinone	Deep blood-red	<i>Cordyceps unilateralis</i>
Riboflavin	Yellow	<i>Ashbya gossypii</i>
Rubropunctatin	Red Orange	<i>Monascus sp. Monascorubrin</i>

Ankaflavin: Chinese people are highly familiar with *Monascus* fermented rice, also known as ang-kak or red rice, which is made by fermenting cooked rice [28]. *Monascus anke*, *Monascus ruler*, and *Monascus purpureous* are the most productive *Monascus* species used in Taiwan for the manufacturing of ang-kak, and these species primarily generate six colours when cooked and fermented as follows, the orange pigments-monascorubrin and rubropunctatin pigments-monascorubrin and rubropunctatin, and yellow pigments-Ankaflavin and monascorubrin become physiologically active [29]. This was originally used by the Chinese as a culinary colouring, preservative, and medicinal ingredient. When they are added to the diet, they lower blood cholesterol and lipoprotein levels in people, and the active ingredient monacolins K aids in this process and it has been proved that Ankaflavin is cytotoxic to Hep G2 cells, a kind of human cancer [30, 31]. US-FDA approved this as generally recognised as safe.

Antraquinone: also known by its chemical name 9, 10-dihydro-9, 10-dioxoanthrone, is a light yellow water soluble pigment that is often created by chemical synthesis for industrial use. It is said to have a significant production volume in the United States. It is employed in the paper industry as a pulping catalyst and as a raw material for the production of vat dyes, a type of water-insoluble dyes that are readily converted into water-soluble colours used in the fibre and textile industries and are primarily brilliant and well-fast. Several plants, including aloe, latex senna, rhubarb, and some fungus, like *Paecilomyces farinosus* and *Penicillium oxalicum*, naturally contain these colours and in some lichens and insects reports shows that they are not harmful to human health but there is no evidence of use of these pigments in food [34, 35].

Beta carotene: There are more than 600 different types of carotenoids, many of which are natural sources of vitamin A and colours as well as foreign foods for people. Beta carotene,

also known as β -Carotene, is a stable bright red-orange pigment. $C_{40}H_{56}$ is naturally present in fruits and vegetables including carrot, tomato, potato, and watermelon as well as in some birds and aquatic creatures as body colours. It is insoluble in water and just slightly soluble in ethanol and ether and readily soluble in chloroform. Regular consumption of beta-carotene in the diet reduces the risk of age-related macular degeneration (AMD), immune modulatory activities, cataract osteoclast genesis, cancer, and coronary heart diseases. Beta-carotene is a very potent anti-oxidant and a precursor to vitamin-A, which functions as retinol. Because of its importance in food, beverage, animal nutrition, pharma, and medicine, its extraction from natural sources (i.e., from fruits and vegetables) is limited by low yield and seasonal geographic variations; as a result, chemical synthesis offers sustainable technology but also has safety limitations; as a result, the trend is moving toward microbial sourced β -Carotene, and in recent years metabolic-engineering developed fermentation technology with fast speed, short fermentation cycle, single product high biological activities and non-geographic and environmental restriction along with which provides safety can greater improves yield and achieve sufficient industrial production of β -Carotene. The Food and Drug Administration (FDA) has categorised beta-carotene as generally recognised as safe (GRAS) and urged usage as a dietary supplement in adult and infant diets. It was initially allowed by the European Union with E number E-160a. By using industrial wastes like cheese whey, crude vegetable oil, beet, molasses, cabbage waste, watermelon husk, peach peel waste, and cooking oil, some mould species, such as *Blakeslea trispora*, *Mucor circinelloides*, *Neurospora Crassa*, and *Phycomyces blakesleeanus*, can help to produce beta carotene [3, 36, 37].

Canthaxanthin: The natural pigment canthaxanthin, which has the chemical formula $C_{40}H_{52}O_2$ and is an orange-red keto-carotenoid with an 8-isoprenoid group, can be produced by plants and some photosynthetic microorganisms by adding two keto-groups to the precursor form of beta carotene [38]. This process is made possible by the presence of the enzyme C-4 -oxygenase, also known as carotene-4,4'- ketolase the enzyme present in microorganism which is responsible for the formation of additional -ionone rings. This is anti-oxidant, anti-inflammatory, anti-tumour, and anti-carcinogenic and this pigment was first obtained from the edible mushroom chanterelle (*Cantharellus cinnabarinus*). It can also be obtained from some plants, birds, crayfish, sea trout, mould species like *Monascus roseus*, and Bacterial species like *Bradyrhizobium sp.*, *Haloferax alexandrines*. And as a result, it is utilised as a feed supplement, nutraceutical, prospective industrial, medicinal, and food colouring [3, 39, 40]. Both the Scientific Committee for Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) examined the current Acceptable Daily Intake (ADI), which is 0.03 mg/kg bw/day [41].

Lycopene: is a red, lipid-soluble carotenoid with the chemical formula $C_{40}H_{56}$ that is found in a variety of red fruits and vegetables, including tomatoes, pink grapes, guavas, apricots, and watermelons. It can also be produced chemically is economically efficient, though this may not always be safe, so it can also be produced biologically by *Fusarium sporotrichioides* and *Blakeslea trispora* in a process comparable to two-phase pathways of tomatoes, namely a fermentation phase followed by the extraction of the

biosynthesized compounds [42]. As it is fat soluble it is formulated into 20 percent or 5 percent sunflower oil suspension with alpha-tocopherol at 1% of lycopene levels, and is also available as cold water dispensible (CWD) formulations with alpha-tocopherol containing at 10 percent and 20 percent lycopene to address stability difficulties. Lycopene from microbiological sources is recovered, formed, and packaged exclusively in the dark, at a regulated temperature, and in a nitrogen environment. The European Union allows the use of lycopene in drinks at a rate of 30 mg/kg. 20 mg are included in cereal goods. Fish and fish products contain 25 mg. Lycopene from microorganisms is permitted in a range of 30 mg, which is 4-10% and 5% greater than synthetic and tomato, respectively, for regulatory purposes [43]. It is also permitted at a range of 15-25 mg in milk and milk products and 30 mg in sugar. 0.37-0.15 milligrams per kilogramme of body weight [44].

Naphthoquinones: Common metabolites of plants, animals, Fungi, and Bacteria are 1, 4-Naphthoquinones. Demonstrates effects that are cytotoxic, antitumor, antifungal, antiviral, antiprotozoal, and antimicrobial. Lawsone (2), one of the best-known natural Naphthoquinones, is extracted from the tropical shrub *Lawsonia inermis* (henna), which may be used to dye wool and silk an orange hue. There are three different forms of natural Naphthoquinones are juglone (3), lapachol (5), and lawsone (2). Women use the ground-up henna leaf paste to colour their hair a recognisable shade of red. From tropical plants in the Bignoniaceae family, 2-hydroxy-3-(3-methyl-2-butenyl)-1, 4-naphthoquinone lapachol (5) and its cyclic derivatives - and -lapachone (6-7) were identified. Traditional Brazilian medicine employs lapachol (5) and its cyclic derivatives, - and -lapachone (6-7), as antiparasitic, antimalarial, anti-inflammatory, antiseptic, and anticancer medicines. Juglone (5-hydroxy-1, 4-naphthoquinone, 3), which exhibits anti-Bacterial and Fungicidal activities, was extracted from the leaves and nuts of several plants (Juglandaceae)1, 6. The related 7-methyljuglone (8) and its monomer diospyrin (9) were shown to be the main antitubercular ingredients in root extracts of *Euclea natalensis* [44, 45].

Riboflavin: A water-soluble, yellowish pigment known as lactochrome, Riboflavin is a heterocyclic isoalloxazine derivative with a ribitol side chain and the parent precursor of the co-enzymes FMN or Riboflavin 5'-phosphate and FAD. It was initially isolated from milk and whey. Similar to how Riboflavin plays a crucial central role as a 2 electron donor/acceptor and 1 electron donor/acceptor complex in the electron transport chain, most B complex vitamins aid in body homeostasis by assisting metabolism. They also help in metabolism of lipids, drugs, and xenobiotic, and they prevent migraines, anaemia, cancer, hyperglycaemia, hypertension, oxidative stress, diabetes mellitus, and cardiovascular diseases. Because Riboflavin is produced in a single step as opposed to the several steps involved in chemical synthesis, it is economically, environmentally, and cost-effectively viable to employ microorganisms as a source of Riboflavin, which significantly reduces the risk of breast cancer [47]. The first bacterium to make Riboflavin is *Clostridium acetobutylicum*, and other species of fungus, including *Eremothecium ashbyii*, *Aspergillus gossypii*, *Pichia guilliermondii* (asporogenic *Candida guilliermondii*), *C. famata*, *Candida boidinii*, *Schwanniomyces occidentalis*, *Pichia caribbic* [48, 49].

Algae

Micro Algae serve as a storehouse for materials of economic worth. Exploiting their incredibly effective photosynthetic mechanism is the foundation of the production. A set of extremely light-absorbing pigments based on the bilin or tetrapyrrol skeleton are produced by red Algae (*Rhodophyta*) and blue-green Algae (*Cyanophyta*). The utilisation of these phycobilin proteins as natural colorants in food, cosmetics, and medicines, particularly as alternatives to synthetic dyes (Table-IV). Proteinase treatment is used to separate the protein-bound pigments, which are then removed into diluted alkali. Ice candies, frozen confections, sherbets, confectioneries, and chewing gum are all advised for use with pigment preparations that are either in water or alcohol ^[14].

Table 4: Commercial bio-colours in the market

Pigment	Colour	Micro organism
β -carotene	Red	<i>Dunaliella salina</i>
Canthaxanthin	Red	<i>Haematococcus</i>
Lutein	Red	<i>Chlorococccum</i>

Conclusion

Here we have reviewed basic information about Bio colors along with their nutraceutical values and health benefits and also microorganisms which produces the respective colours. And role of organic waste produced in food processing for bio color production. Since all this pigments got very good nutraceutical importance and most of the pigments have proven that that they are safe to most of them are approved by food safety authorities of different countries and generally recognised as safe (GRAS). And even most of this pigments from microbial sources are in use as feed supplement and in some food at regulatory doses as nutraceutical components and still more research should be conducted on stability to use this pigments as effective colorants at commercial scale.

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