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A review on microbial-pigment: A good source of biocolour

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Abstract
In today's world there is a growing demand for healthy food products has lead to the development of "Nutraceutical Food Products". Development of natural food colours or bio-colours is a growing research area to shift the synthetic food colours. Bio-colours have many health benefits properties such as anti oxidant activity, food products stabilization, anti cancer property, etc. The main challenge faced by the industry is the production of natural food pigments in bulk to replace the synthetic colours and also this extraction process from plant origin is time consuming, expensive and the product yield is low. The sources are non-renewable. In this regard, the micro organisms are less expensive and renewable alternative source. In this review paper the competitive study of different microbial pigment is done as well as use of microbial pigment as an alternative of synthetic food pigment and the nutraceuticals properties of those pigments are discussed. This paper also highlights the challenges faced and advanced technologies used in this developing the pigment extracted from microbial sources.

Keywords: Bio-colour, micro-organism, nutraceutical, food pigments

1. Introduction
Colours are a main property of sensory evaluation o any food materials. Since ancient times, human have added food colours to the food products to make the food look more presentable, to make up for the loss of colours during processing and to improve the quality. Customers have since long associated different characteristics of food with colour such as freshness, taste etc. Hence colours have for long served as the sensory evaluating tool. Due to the ease of production in bulk and the cheapness of production, artificial food colours are being used in large scale. However the knowledge about the toxic effect of these artificial food colours among the population is coming to the fore front. Thus a market shift towards development and production of Biocolours has been observed. This shift is more so catalyzed by the recent development of Nutraceutical Food Products as the bio colours are known to contain many beneficial properties and also natural colours are eco friendly. Thus if they replace artificial colour, the production of harmful chemicals polluting the environment will also stop. Now biocolours are generally extracted from plant and vegetable sources which are non renewable sources. However it has been discovered that pigments extracted from micro organisms can be an alternate means of production of natural food colours. Hence a Seemingly vast number of micro organisms found in nature, ease of cultivation of these micro organisms and their medicinal properties adds to the advantage in extraction and production of natural colours from them. Bacteria, Fungi, Algae etc. all produces pigments which can be used for production of bio colours. Among the molecules produced by microorganisms are carotenoids, melanins, flavins, phenazines, quinines (Joshi *et al*., 2003; Pankaj *et al*, 2016) [6, 13].

2. Microbial pigments as food colours
In this earth, uncountable numbers of types of Micro organism are found. Many microbes such as micro algae, bacteria, fungi etc all are known to produce a variety of pigments. As colour extraction from microbial sources have some benefits like, cheaper production, easier extraction, higher yields through strain improvement, less lack of raw materials and no seasonal variations, microbial pigment has become a major source of bio colours (Kamla *et al*., 2012; Dufosse *et al*., 2009) [7, 3]. The health benefits of the microbial pigment such as anti oxidant, non carcinogenic, anti microbial etc adds to the use of microbes. *Flavobacterium sp.* (yellow pigment: zeaxanthin), *Agrobacterium aurantiacum* (pink- red pigment: astaxanthin), *Micrococcus sp.* (different colored pigments, carotenoids), *Serratia marcescens* (red pigment),

Chromobacterium sp. (violet pigment) and *Rheinheimera sp*. (blue pigment) (Venil *et al*., 2013) [20] are some of the examples of microbes producing pigments. Now major microbial pigments among carotenoids are Zeaxanthin, Astaxanthin, canthaxanthin etc. While other pigments includes Violacein, Indigoidine, Prodigiosin and more. Hence these pigments if extracted successfully can be easily utilized in colouring of different food products like cake, pastry, milk etc.

2.1 Classification of microbial pigment
Microbial Pigment can be classified on the basis of source, colour and solubility.

- On basis of Source (Algal Sources, Fungal Sources, Bacterial Source)
- On basis of Colour
  - Yellow Pigment (Riboflavin, Carotenoids)
  - Red Pigment (Prodigiosin, Carotenoids, Porphyrins)
  - Blue Pigment (Indigoidine, Violacein)
- On basis of Solubility (Water Soluble, Fat Soluble)

2.2 Carotenoids
Carotenoids are a group of pigments which are widely distributed classes of from red to yellow present in a wide variety of bacteria, algae, fungi, and plants (Kushwaha *et al*., 2014) [8]. Carotenoids can be found widely in nature. Carotenoids are generally lipid soluble compounds and can be easily found in tomatoes, pumpkins, carrots etc. Carotenoids are a very important colouring additives used in food industry. It has been found a large number of microbes produce Zeaxanthin, astaxanthin, canthaxanthin, Beta carotene**,** which are types of carotenoids. So these compounds if extracted can be used as a food grade Biocolourant.

2.3 Zeaxanthin
Zeaxanthin generally is a carotenoid alcohol which is found in nature. It is basically a type of Xanthophylls. This pigment gives paprika, corn, wolf berries its yellow orange characteristics colour. Zeaxanthin breaks down to form picrocrocin and safranal (Yeum *et al*., 2002) [21]. These two pigments give Saffron its taste, aroma and colour. Zeaxanthin is generally a bacterial pigment. It can be found in bacteria such as *Staphylococcus aureus*, *Bacillus*, *Corynebacterium michigannise*, *Flavobacterium sp* etc. Hence on extraction can be easily used as a biocolourants. In the market, Zeaxanthin is code named E161h as a food additive (Van *et al*., 2000) [19].

2.4 Astaxanthin
Astaxanthin, classified as Xanthophylls, is a keto Carotenoid. It is a lipid soluble pigment. Astaxanthin is a reddish-orange pigment. Synthetically developed Astaxanthin have been in use for developing the pink hue in farm cultivated Salmon and poultry eggs for a long time. But now days, Astaxanthin produced by microorganisms have been commercialized and applied in the colouration of beverages, dairy products, and meats (Del Campo *et al*., 2000) [1]. Astaxanthin can be used as a food colour is a main carotenoid pigment found in red yeast, *Xanthophyllomyces dendrorhous* (*Zheng et al.,* 2006). The yeast, *Xanthophyllomyces dendrorhous,* and microalga, *Haematococcus pluvialis* are currently used for the large-scale cultivation of astaxanthin. Some bacterial Sources include *Paracoccus marcusii*, *Paracoccus carotinifaciens*, *Agrobacterium aurantiacum* etc. Although till now bacterial sources are not used for production of Astaxanthin.

2.5 Beta carotene
β-Carotene belongs to a group of more than 600 compounds, jointly called as carotenoids (Ludmila *et al*., 2018) [10]. It is basically organic a red orange pigment found in nature. It can be generally regarded as Isoprenoids, a derivative of Carotenoids. When used as food additive, it is code named E160a. Beta Carotene is found in carrot, tomatoes, spinach etc. Beta Carotene is used in ice cream, pastry, crème cheese etc. Some of the microbial sources are *Erythrotrichia carnea* **(**Algae), *Dunaliella tertiolecta* (Algae), *Phaffia rhodozyma* (Fungi), *Escherichia coli* (bacteria), *Serratia marcescens (*bacteria), *Agrobacterium tumefaciens (bacteria)*, etc.

2.6 Prodigiosin
Prodigiosin is a secondary metabolite alkaloid with a unique tripyrrole chemical structure produced by bacteria mainly (Shaikh *et al*., 2016) [16]. It is a red pigment. Prodigiosin can be a replacement of natural pigments to synthetic colorants such as FD&C Red No3, No 40 which is used for colouration in beverages, cereals, ice cream cones etc. The main bacterial strain which produces this pigment is *Serratia marcescens*, other Gram-negative, gamma proteo bacteria such as *Vibrio psychroerythrus* and *Hahella chejuensis*. Although this pigment has some draw back such as sensitivity, solubility, short stability to pH, high temperature, but spray-dried microcapsules containing Prodigiosin was produced. The particles were successfully applied to yogurt, milk and carbonated drinks (Namazkar *et al*., 2013) [12].

2.7 Violacein
Violacein is a naturally-occurring bis-indole pigment with anti biotic properties. It is basically violet-blue colour pigment. Due to its hue, it has the ability to replace several lethal synthetic colours such as FD&C Blue No2 which is extensively used in baked goods, cereals, snack foods, ice cream, confections, and yogurt. Violacein is generally regarded as bacteria pigment as it is produced by huge number of bacteria. Some of the sources are *Chromobacterium violaceum*, *Collimonas sp.*, *Pseudoalteromonas sp.* etc. Research and development formass production as a food grade biocolourant is underway. As of now, Violacein is only used in textile industry for dying.

2.8 Indigoidine
Indigoidine is an organic compound of the group of Azaquinones. It is a blue colour pigment which is formed by some bacterial stains. It has the ability to replace artificial food colourant such as FD&C Blue NO 1 and 2 Is used extensively baked goods, cereals, snack foods, ice cream, confections an yogurt. Some of the sources of bacteria from which Indigoidine is extracted are *Corynebacterium insidiosum*, *Eschericha coli*, *Streptomyces chromofuscus* etc.

2.9 Anthocyanins
As a member of the flavonoid group of polyphenols, anthocyanins are important chemicals in the plant kingdom as pigments, antioxidants, and antimicrobials (Zha *et al*., 2017) [22]. Anthocyanins are found in many food plants such as blueberry, raspberry, black rice, black soybean etc. Anthocyanins till now mainly are produced by extraction and purification from fruits, flowers, and other tissues of plants. But Anthocyanins extracted from plant sources exist as heterogeneous mixture of multiple types of molecules with

diverse chemical structures. The production of this pigment is neither stable nor sustainable due to varied productivity of plants. But pigment from micro organism sources provides sustainable ways for meeting the industry demand. It has been shown that the metabolically engineered strain of *E. coli* is able to produce Anthocyanin. Research is still going on for production of this pigment from other modified bacteria.

3. Challenges faced

Pigments extracted from micro organisms can be a very good source of bio colur. Now the raw material i.e. the micro organisms can be found in uncountable numbers. Even these microbes can be easily cultivated and the growth time is very less. But the real challenge lies in extraction of pigments from these microbes. Different extraction techniques that are used have high cost. Stabilization of these pigments along with the side effect of these pigments is a great challenge. Microbial pigments have a weaker tinctorial strength and may react on different food matrices, causing undesirable flavors and odors (Durán *et al*., 2016) [4]. It has been observed that biocolourants are sensitive to factors such as pH, temperature, UV, oxygen, heat etc. which can cause colour loss and lessen the shelf life. Some pigments are sensitive to metal ions, proteins or other organic compound. One example is that of vitamin C which will enhance the stability of beverage products, which are colored with carotenoids like beta-carotene and paprika oleoresin, but the same vitamin will cause the degradation of anthocyanin. Many fungal pigments are prohibited as natural colorants due to the presence of mycotoxins (Sen *et al*., 2019) [15]. Thus there are many hurdles in development of biocolours from micro organism.

4. Advance Technologies used & solutions to the challenges faced

Biocolours which are developed from plant sources are expensive due to both the method of extraction and the limited raw resources. Micro organisms can serve as the alternative resource effectively due to their availability, ease of cultivation and growth time thus bringing down the production cost. So the basic idea is to bring microbial pigments out of petri-plates and on to the market (Frisvad *et al*., 2004; Dufossé *et al*., 2018) [5, 2]. Many new technologies such as Strain Development and Fermentation which is basically using a fermentation tank for large scale pigment production and strain development by random mutagenesis and multiple selection rounds for cost effective and industrially viable production process is a good production technique. The main challenge faced is the extraction of these pigments from microbes. The traditional method of separation and recovery of pigment is costly. Extraction using organic solvents is complicated and time consuming process. More over the yield of pigment is low. The new technique of using non-ionic adsorption resins for extraction is very much cost effective and the product yield is more. Smarter Screening Methods is a newly advanced method developed for quick detection of microbial pigment. HPLC, mass spectrometry, LCMS, nuclear magnetic resonance (NMR), and UV-VIS spectra, handheld Raman Spectrophotometer are the instruments applied in this technique. Metabolic engineering of microbes i.e. the cloning of genes responsible for pigment biosynthesis has enabled overproduction of these pigments by gene manipulations. Pigment biosynthetic pathways have been extensively studied and engineered to overproduce a pigment and to change the pigments' molecular structure and color (Rao *et al*., 2017) [14]. *E. coli*, *Bacillus subtilis*, *Pseudomonas putida*, *Corynebacterium glutamicum*, *Pichia pastoris* are some of the organisms used for this technique. Thus this technique has made genetically engineering the production of pigments very cost effective and industrially viable. Thus the development and using tailor-made recombinants for making food grade pigment is a reality. More research is being conducted for developing more cost effective techniques and developing and modifying microbes to make Biocolour extracted from microbial sources commercially successful. For stability new techniques such as micro encapsulation, Nano encapsulation and nano formulation can be applied.

5. Health benefits of using Biocolours extracted from micro organisms

Biocolours boasts of many bio logical properties which are good for body's health. Most pigments contain polyphenols which are antioxidants. Microbial pigments like violacein, carotenoids, anthocyanins, and Naphthoquinone have been shown to be potent antioxidants agents (Sen *et al*., 2019) [15]. Many pigments showed anti microbial properties. A pigment obtained from an endophytic fungus was shown to be more potent than the commercially available antibiotic Streptomycin agents (Sen *et al*., 2019) [15]. Bacteria such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholera* can be countered using this pigment. Violacein has also shown anti microbial tendencies such as inhibition and even being able to kill bacteria. Aditionally these microbial pigments which also has the anti microbial property can be used as novel antibiotic compounds against multi drug resistant microbial stains. In number of studies, Anti cancer properties of biocolours extracted from microbes are being reported. These pigments can destroy cancer cells. Scytonemin which is a green-yellow pigment, produced by the aquatic cyano bacteria, inhibits the action of the cell cycle regulatory protein kinase, thereby showing an anti proliferative effect (Stevenson *et al*., 2002) [17]. Anthocyanins help to suppress neuro inflammation, neuro degradation and brain aging by blocking interleukin-1b, tumor necrosis factor a, and nuclear factor-kb (Tsuda *et al*., 2012) [18]. Many other pigments such as Astaxanthin, Canthaxanthin, Lycopene, Monascorubramin, Riboflavin, Rubropunctatin, β-carotene, Torularhodin, etc extracted from microbes are anti cancer, anti microbial in nature and show's hosts of many different biological properties beneficial to human. Prodigiosin is known to have antibacterial, anti-malarial, antineoplastic and antibiotic activity (Shaikh *et al*., 2016; Venil *et al*., 2013) [16, 20].

6. Conclusion and future perspective

Nature is rich in colour and also the source of colour. Micro organisms being one of the sources and the innumerable microbes are found. Food Grade biocolours extracted from, in coming years, has the potential to commercially replace artificial colour with shifting of market. The impact of them will not only be beneficial to us human but also for the environment. The pollution level by harmful chemicals will decrease. Thus more research and development is underway to fully utilize these microbes for the benefit of the human. Current range of bio colours are small but research is underway for expanding the range with the aim of replacing artificial colour from the market as a whole while at the same time exploiting the beneficial properties of these pigments.

Table 1: List of pigment producing microorganisms (Malik *et al.,* 2012; Lebeau *et al.,* 2017)

Microorganism(s)	Pigments/Molecule	Colour/appearance
Bacteria		
Agrobacterium aurantiacum	Astaxanthin	Pink-red
Paracoccus carotinifaciens	Astaxanthin	Pink-red
Bradyrhizobium sp.	Canthaxanthin	Dark- red
Flavobacterium sp., *Paracoccus zeaxanthinifaciens*	Zeaxanthin	yellow
Corynebacterium insidiosum	Indigoidine	Blue
Rugamonas rubra, Streptoverticillium rubrireticuli, Vibrio gaogenes, Alteromonas rubra	Prodigiosin	Red
Rhodococcus maris		Bluish- red
Xanthophyllomyces dendrorhous	Astaxanthin	Pink -red
Haloferax alexandrinus	Canthaxanthin	Dark Red
Staphylococcus aureus	Staphyloxanthin Zeaxanthin	Golden Yellow
Chromobacterium violaceum	Violacein	Purple
Serratia marcescens, Serratia rubidaea,	Prodigiosin	Red
Pseudomonas aeruginosa	Pyocyanin	Blue-green
Xanthomonas oryzae	Xanthomonadin	Yellow
Janthinobacterium lividum	Violacein	Purple
Algae		
Dunaliella salina	β-carotene	Red
Chlorococcum	Lutein	
Hematococcus	Canthaxanthin	
Fungi		
Blakeslea trispora	β -carotene	Cream
Fusarium sporotrichioides	Lycopene	Red
Haematococcus pluvialis	Astaxanthin	Red
Monascus sp.	Monascorubramin Rubropunctatin	Red Orange
Monascus purpureus	Monascin Ankaflavin	Red-yellow
Monascus roseus	Canthaxanthin	Orange-Pink
Monascus sp.	Ankaflavin	Yellow
Penicillium oxalicum	Anthraquinone	Red
Blakeslea trispora	Lycopene	Red
Cordyceps unilateralis	Naphtoquinone	Deep blood-red
Ashbya gossypi	Riboflavin	Yellow
Mucor circinelloides, Neurospora crassa and Phycomyces blakesleeanus	β-carotene	Yellow-orange
Pacilomyces farinosus	Anthraquinone	Red
Yeast		
Cryptococus sp.		Red
Saccharomyces neoformans var. *nigricans*		Melanin black
Phaffia rhodozyma	Astaxanthin	Pink-red
Rhodotorula sp. *Rhodotorula glutinis*	Torularhodin	Orange-red

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8. Reference

1. Del Campo JA, José MF, Herminia R. Carotenoid content of Chlorophycean microalgae: factors determining lutein accumulation in *Muriellopsis* sp. (Chlorophyta). Journal of Biotechnology. 2000; 76(1):51-9.
2. Dufossé L. Microbial Pigments from Bacteria, Yeasts, Fungi, and Microalgae for the Food and Feed Industries. Natural and Artificial Flavoring Agents and Food Dyes. Handbook of Food Bioengineering, Amsterdam: Elsevier, 2018, 7.
3. Dufosse L. Pigments. Encyclopedia of Microbiology. 2009; 4:457-471.
4. Duran N, Giselle Z, Durán M, Brocchi M. Advances in Chromobacterium Violaceum and properties of violacein-Its main secondary metabolite: A review. Biotechnology Advances. 2016; 34(5):1030-1045.
5. Frisvad JC, Smedsgaard J, Larsen TO, Samson RA. Mycotoxins, drugs and other Extrolites produced by species in *Penicillium* subgenus *Penicillium*. Stud Mycol. 2004; 49:201-41.
6. Joshi VK, Attri D, Bala A, Bhushan S. Microbial pigments. Indian J Biotech, 2003, 362-369.
7. Kamla M, Jayanti T, Sneh G. A review on microbial pigment. International Journal of Microbial Resource Technology. 2012; 1(4):361-365.
8. Kushwaha K, Saini A, Saraswat P, Agarwal MK, Saxena J. Colorful World of Microbes: Carotenoids and Their Applications. Advances in Biology, 2014, 13.
9. Lebeau J, Venkatachalam M, Fouillaud M, Petit T, Vinale F, Dufosse L *et al*. Production and new extraction method of Polyketides red pigments produced by ascomycetous fungi from terrestrial and marine habitats. J Fungi. 2017; 3:34.
10. Ludmila BR, Joanna H. β-Carotene-properties and production methods. Food Quality and Safety. 2018; 2(2):69-74.
11. Malik K, Tokkas J, Goyal S. Microbial pigments: a review. Int. J Microb. Res. Technol. 2012; 1:361-365.
12. Namazkar S, Ahmad WA. Spray-dried Prodigiosin from *Serratia marcescens* as a colorant. Biosci Biotechnol Res

Asia. 2013; 10(69)-76.
13. Pankaj VR, Kumar R. Microbial pigment as a potential natural colorant for contributing to mankind. Res Trends Mol Biol, 2016, 85-98.
14. Rao NMP, Xiao M, Li WJ. Fungal and Bacterial Pigments: Secondary Metabolites with Wide Applications. Front Microbial, 2017.
15. Sen T, Barrow CJ, Deshmukh SK. Microbial Pigments in the Food Industry-Challenges and the Way Forward. Front Nutr. 2019; 6:7.
16. Shaikh Z. Biosynthesis of Prodigiosin and its applications. J. Pharm. Biol. Sci. 2016; 11:1-28.
17. Stevenson CS, Capper EA, Roshak AK. Scytonemin a marine natural product inhibitor of kinases key in hyper proliferative inflammatory diseases. Inflamm Res, 2002.
18. Tsuda T. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. Mol Nutr Food Research, 2012.
19. Van Het Hof KH, West CE, Weststrate JA, Hautvast JG. Dietary factors that affect the bioavailability of carotenoids. J Nutr. 2000; 130(3):503-506.
20. Venil CK, Zakaria ZA, Ahmad WA. Bacterial pigments and their applications. Process Biochem. 2013; 48(7):1065-1079.
21. Yeum KJ, Russell RM. Carotenoid bioavailability and bioconversion. Annu Rev Nutr. 2002; 22:483-504.
22. Zha J, Koffas M. Production of anthocyanins in metabolically engineered microorganisms: current status and perspectives. Synth Syst Biotechnol. 2017; 2(4):259-266.