A Survey on Microbial Pigments: Production and Applications

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Abstract :*Pigments have become an essential part of our daily lives and have extensive applications in many areas such as food, cosmetics, agriculture, pharmaceuticals, textile. Since the 1980s synthetic pigments have been widely used in various applications. These synthetic pigments have adverse effects on environment and public health. The carcinogenicity or hyper allergenicity effects of synthetic dyes have led to increased research on natural pigments. Due to such drawbacks of synthetic pigments, the use of natural pigments are considered as the best alternative to synthetic pigments.Natural pigments from microorganisms are of great interest due to their significant properties and broader applications. The increasing demand among the consumers opting for natural pigments. To fulfill these market demand of natural pigments new sources should be explored. Among the natural resources, Microbial pigments represent an eco-friendly alternative as they can be produced in large amounts through biotechnological processes and do not present environmental risks, as they are easily decomposable. This review article highlights the various types of microbial pigments and the latest studies on the discovery of these pigments, the biosynthetic pathways and applications of these pigments which hopefully provides useful information, guidance and improvement in forthcoming studies.*

Keywords : Microbial pigment, Synthetic dye, Carotenoid, Violacein, Antimicrobial, Antioxidant

1.INTRODUCTION

Pigments are colorful secondary metabolites produced by microorganism. Since the prehistoric era pigments have been used as coloring agents. The first synthetic dye mauvine was prepared in 1856 by Sir William Henry Perkin. The historical revolution of synthetic dyes initiated by development of mauvine [1]. At first synthetic dyes get much attraction because of their different benefits like the development process for snthetic dyes is easy, they have good coloring properties, they required in very small amount for use. But most of the synthetic dyes that are used are never tested for their toxic effects[2]. Several studies show that synthetic dyes can cause adverse effects towards human health and have negative impact on environment. Some synthetic coloring agents which were originally approved by Food and Drug Administration (FDA) for use, were later found to cause cancer and hyperactive in children so they had been withdrawn from use due to their hazards impact on health [3]. The composition of synthetic dyes contain the chemical compounds like lead, copper, mercury, chromium, benzene, that have adverse effects on human being. The colorants that are obtained syntheticallyinclude ethyl acrylate, benzophenone, pyridin are banned by the FDA. In Washington the Center for Science in the Public Interest pleading to the FDA in 2008 to decline synthetic food colorants due to it's harmful effects among children [4]. The below (Table.1) shows some studies was done to investigate the ecological toxicity of some synthetic dyes to different organisms.

Table1 : Ecotoxicity of some synthetic dyes

Synthetic dyes	Ecotoxicity % / organism	Reference
Benzophenone	*Chlorella vulgaris* (44.10%)	[5]
Pyridine	Rabbit	[6]
Methyl eugenol	Rat, Mice	[7]
Benzophenone-3	*Scenedesmus obliquus* (23-29%)	[8]
Ethyl Acrylate	Rats and mice (31%)	[9]

Many natural pigments apart from fulfilling their function of giving colors are also known as interesting bioactive compounds with potential health benefits. These compounds have a wide range of application in medicine, food, pharmacology, agrochemical, cosmetics. Many microbial bioactive pigments have been discovered and lots of them show antioxidant, antimicrobial properties [10]. The natural bio colorantsobtained from plants and microorganismare alternative to synthetic pigments. The pigments that are naturally obtained from microbes are supreme over plants due to their several characteristics such as their great stability and solubility potential , they areavailable throughout the year, the fast growth rate of microorganism in inexpensive mediumand they are easilycultured [11,12]. Futhermore the use of plants on large scale for pigment production can cause damage to precious species of plants. The yearly growth rate of using natural dyes has been increased up to 5-10% as compared to synthetic dyes having a lower growth rate that is 3-5% [13]. Among microorganisms such as bacteria, fungi, microalgae and yeast are an alternative source for microbial pigments, they produced different types ofpigmentswith high potential to be used as natural colorants that are mention in (Table. 2)

Table 2 : Pigments produced by microorganisms their characterization and applications.

Types of microorganism	Pigment	Color	Applications	Reference
Bacteria				
Agrobacterium aurantiacum	Astaxanthin	Pink-red	Antioxidant, Anti-inflammatory	[14]
Chromobacterium sp.	Violacein	Violet	Anti-microbial, anti-tumor	[15]
Corynebacterium glutamicum	Carotenoid	Red and yellow	Photoprotection, light harvesting	[16]
Janthinobacterium sp.	Violacein	Violet	Adaptation to low temperature	[17]
Lysobacter oligotrophicus	Melanin	Dark-brown	Protection against UV radiation	[18]
Arthrobacter agilis	Carotenoid	Yellow	Membrane stabilization at low temperature	[19]
Staphylococcus aureus	Zeaxanthin	Yellow	Photo protectant, Antioxidant	[20]
Kocuria Polaris sp.	Carotenoid	Orange	Photoprotection	[21]
Pseudomonas pelagia, Algoriphagus ratkowskyi	Zeaxanthin	Yellow and Red	Cryoprotective agent in regulating membrane fluidity	[22]
Fungi				
Penicillium oxalicum	Anthraquinones	Red	Antifungal	[23]
Talaromyces verruculosus	Lycopene	Red	Dye textile having antimicrobial activity	[24]
Fusarium oxysporum	Anthraquinone	Blue, violet	Use in textile industry	[25]
Stemphylium lycopersici	Anthraquinone	Yellow	Antioxidant activity	[26]
Cryptococcus sp.	Mycosporine	Pink and cream	Protection against UV radiation	[27]
Microalgae				
Arthrospira sp.	Phycocyanin	Blue	Antioxidant	[28]
Haematococcus pluvialis	Astaxanthin	Red	Photoprotective	[29]
Dunaliella salina	β-carotene	Orange	Anticancer, Antioxidant suppression of cholesterol synthesis	[30]
Chlorella and others Microalgae	Lutein	Yellow	Antioxidant	[31]
Yeast				
Xanthophyllomyces dendrorhous	Astaxanthin	Red	Anti-inflammatory, anti-diabetic	[32]
Saccharomyces, Neoformans	Melanin	Black	Antimicrobial, Antioxidant	[33]

2.PIGMENTS PRODUCED BY MICROORGANISM

2.1 Carotenoid

Carotenoid are the most widely distributed pigment in nature and are present in photosynthetic bacteria, some species of fungi, algae and plants[34]. Carotenoids are tetraterpenoids pigments, which exhibit yellow, orange, red colors [35]. There are different types of carotenoids such as α-carotene, β-carotene, β- cryptoxanthin, lutein, zeaxanthin, lycopene, astaxanthin, canthaxanthin. In the early part of the 19th century carotenoids were found in paprika (1817), saffron (1818), carrots (1831). In the 1930s Karrer and khun elucidated the structures of β-carotene and lycopene, they found that β-carotene was a precursor of vitamin A, they won the Nobel prize for this work in chemistry. Carotenoid signify the highly diverse and largest known group of natural pigments, and 1183 carotenoid structures are accumulated from 702 source organisms by Carotenoid Database Japan (https://carotenoidb.jp). Theabsorption capacity of carotenoids is maximum that are ranged from 440 to 520 nm and they showed stronger absorption coefficient [36]. Carotenoids play a important role in cell cycles during their cell differentiation and regulation [37]. The carotenoid produced by *Planococcus faecalis* from stools of Antarctica penguins. Several polar and non-polar types of carotenoid pigments produced by different Antarctica strains such as antarcticus sp. and *Micrococcus roseus* [38]. The different carotenoids such as Zeaxanthin, β-cryptoxanthin and β-carotene were reported from Antarctic bacterium *Sphingobacterium antarcticus*. The pigment carotenoid obtained from Himalayas region of India that was isolatedfrom cold adapted *Penicillium* sp [39].The steps for carotenoid, astaxanthin biosynthesis generated via MEP pathway, in *Haematococcus pluvialis*. The different enzymes and corresponding genes are involved in each steps, are shown in (Fig. 1)

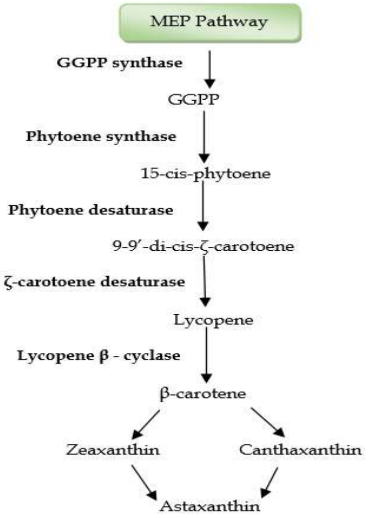

Fig1: Biosynthetic pathway of carotenoid by *Haematococcus pluvialis*. MEP: methylerythritol 4-phosphate; GGPP: geranylgeranyl pyrophosphate. [40]

2.2 Prodigiosin

The prodigiosin is a red color linear tripyrrole pigment. It was first characterized from *S.marcescens* and it is localized in vesicles that areextracellular and cell-associated and in intracellular granules [41]. Initially the pigment name as prodigiosin has been attributed to isolation from Bacillus prodigisus which was later renamed as serratia. The best known prodigiosin is a non-diffusible attached to the inner membrane. The biosynthesis of prodigiosin is controlled by the mechanism of quorum sensing [42]. Prodigiosin act as potent therapeuticmolecule, especially as an anticancer agent and immune suppresser. It also shown insecticidal, antifungal, anti-malarial and antibacterial activities [43]. It is best known for its capacity to trigger apoptosis of malignant cancer cells. Certain eco-physiological roles of prodigiosin are such as; light energy storage [44], ion exchange [45], energy discharge function in S.marcescens [46]. The prodigiosin protects *Vibrio* sp. from UV radiations [47]. The biosynthetic pathway of prodigiosin produced by Serratia sp. It involve separate pathways for production of bipyrrole,4-methoxy-2,2’-bipyrrole-5-carbaldehyde (MBC) and the monopyrrole, 2-methyl-3-n-amyl-pyrrole (MAP) which are coupled in final condensation step. The biosynthesis of MBC by using following Pig proteins: PigI, PigA, PigG, PigJ, PigH, PigM, PigF, PigN and pathway for biosynthesis of MAP, involving PigD, PigE, PigB, proteins are shown in (Fig. 2)

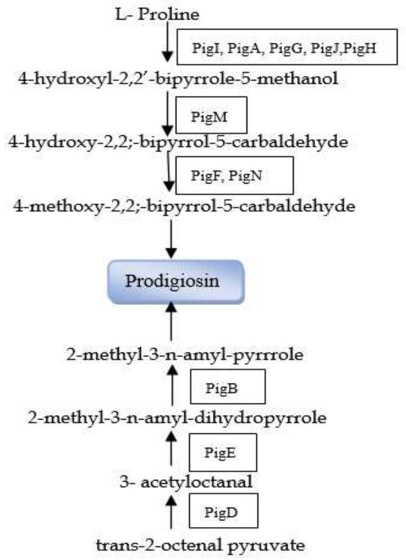

Fig 2 : Biosynthetic pathway of Prodigiosin by *Serratia* sp. [48].

2.3 Violacein

Violacein is a water insoluble pigment, it is violet in color, first obtained from gram negative bacteria *Chromobacterium violaceum* whichare isolated from amazon river which is situated in brazil. Apart from this bacterium Violacein production has been reported from various microorganisms such as *Collimonas* sp., *Pseudoalteromonas tunicate*, *Duganella* sp., *Microbulbifer* sp. And obtain from different environments like soil, marine [49], sea surface [50], glacier [51]. Violacein is known to have diverse biological activities including as an anti-viral, anti-tumor, anti-bacterial, anti-fungal and enzyme modulation properties [52]. The violacein having maximum UV absorption capacity nearly about 260nm, which shows its crucial role in the protection of cells fromUV radiation. The production process of violacein is regulated by quorum sensing thus act as marker of quorum sensing molecules. Violacein plays an important role in protection of bacterial cells from predation [53]. The (Fig. 3) represent the five-gene violacein biosynthetic pathway (Vio ABEDC) from *Chromobacterium violaceum*.

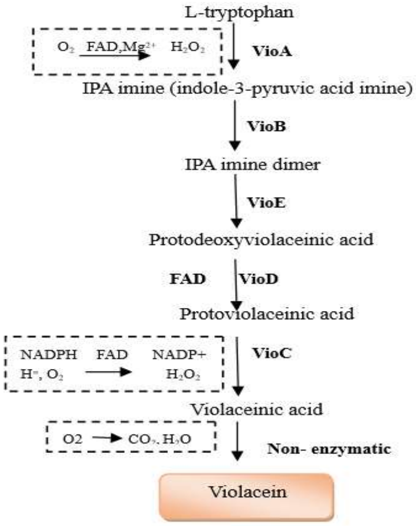

Fig 3: Biosynthetic pathway of Violacein by *Chromobacterium violaceum* [54]

2.4 Melanin

The pigment melanin shows a wide range of colors from red to yellow (pheomelanin) and brown to black (eumelanin). The molecular weight of melanin is high that are found in skin, eyes, scales, hair, feather. Melanin is chemically polymerized and it is product of phenolic and/or indolic compounds [55]. The presence of melanin in almost every large taxon suggests evolutionary importance and it is commonly found in all living systems [56]. Melanin is produced by a different variety of microorganisms such as *Cryptococcus neoformans*, *Sporothrix* sp. [57], and different species of Streptomyces [58]. Melanin gives protection against high temperature and chemical stresses. Melanin is used in some products such as cosmetics, photoprotective creams, eyeglasses and it is also used in immobilization of radioactive waste such as uranium. Melanin having great resistance capacity against UV light by absorbing a broad range of the electromagnetic spectrum and by preventing a photoinduced damage [59]. The melanin genes of some bacteria are used as reporter genes to screen recombination of bacterial strains.Melanin is also useful in development of monoclonal antibodies for the treatment of human [60]. Melanin is also strong absorber of UV radiation and provide strong protective function to microbes that's why extreme low temperature ecosystem are suitable habitats for microbial biosynthesis of melanin [61]. *A. fumigatus* are often grey or black due to the presence of pigment melanin in their cell wall, which is synthesized from acetate with the participation of enzymatic products of six genes (Fig. 4).

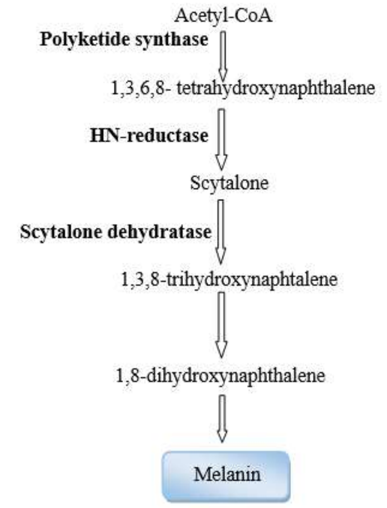

Fig 4: Biosynthetic pathway of Melanin by *Aspergillus fumigatus* [62]

3. APPLICATION OF MICROBIAL PIGMENTS

Microbial pigments are versatile with potential applications in textile, cosmetics, food, pharmaceutical industries as a promising natural sources of colorants. The diverse group of microorganisms under stress produces pigments that are non-carcinogenic and biodegradable with evident functional and biological properties. The worldwide trade of pigments that arenaturally derived has been increased upto 29% from 2007 to 2011 [63]. This means, now the microbial pigmets promptly lead the organic market & pigment industries[64]. (Fig. 5) illustrates the different types of applications of microbial pigments.

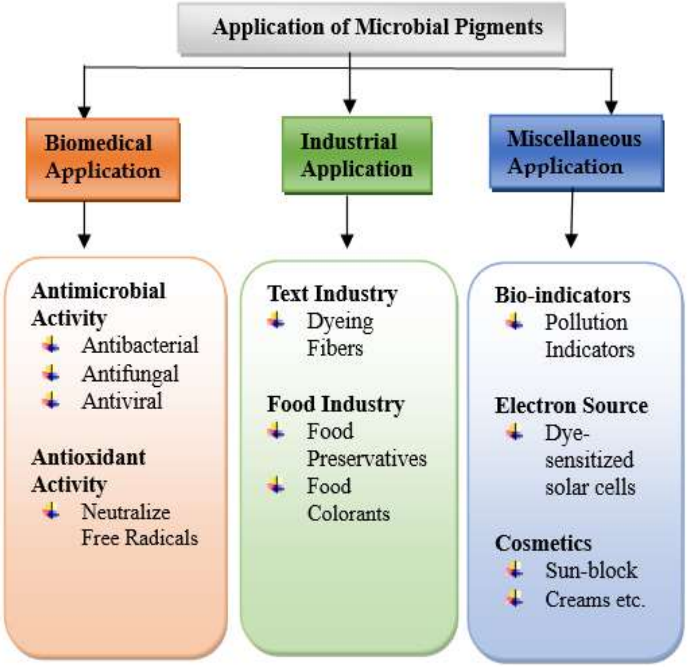

Fig 5: Different types of applications of Microbial Pigments

3.1 Biomedical application

3.1.1 Antimicrobial activity

Globally the major reasons for human deaths are infectious diseases and another is non contagious diseases in developed countries [65]. In recent few decades, microbial resistance against antibiotics is increasing this raised the demand for novel antimicrobial agents. As an alternative to antibiotics, several microbial pigments are assessed for antimicrobial activities. The pigment carotenoid obtained from *Halomonas* sp. having an antimicrobial potential against antibiotic-resistant *S. aureus, Klebsiella* sp., *Pseudomonas aeruginosa* [66]. The bacteria *Micrococcus luteus* showed inhibitory capacity against wound pathogens, *staphylococcus* sp. and *Pseudomonas* sp. [67]. The pigment violacein isolated from *C.violaceum* showed promising antibacterial activities. In past decades several viral eruptions occurred and still occurring such as Ebola virus epidemic and recent coronavirus pandemic with high mortality rates. As many viral infections lack effective treatments and vaccines, therefore there is need to discovered a novel antiviral drugs. Violacein exhibited antiviral activity against poliovirus, rotavirus, herpes simplex virus. Thus microbial pigments are alloted as a new source of medications against pathogens.

3.1.2 Antioxidant activity

In human body the rise of free radicalsincreases the risk of chronic diseases such as cancer, autoimmune disorder, diabetes. To elude this problem antioxidant compounds are used which donate electrons to free radicals and neutralize them and prevent the cellular damage [68]. Antioxidant are obtainfrom synthetic as well as natural sources, but now the demand for synthetic antioxidant are decreases because of their side effects on human health. Therefore, natural antioxidant are more demanding in pharmaceutical industry. Pigments from microorganisms such as carotenoid, naphthaquinone, xanthomonadin showed antioxidant potential. The pigment violacein which obtained from *C.violaceum* evinced antioxidant protection in gastric ulceration [69]. The pigment melanin which are obtained from *Pseudomonas* sp., act as antioxidant agent. The pigment carotenoids isolated from Antarctic bacterium Pedobacter which exhibited solid antioxidant activity with protection against oxidative harm [70]. The microbial pigments used as antioxidants may prevent the diseases such as heart diseases and cancer. According to the above studies we can uses the microbial pigments as antioxidant agents to prevent many chronic diseases.

3.2 Industrial application

3.2.1 Textile Industry

The textile industry is the largest industry by employment generation and economic contribution. These industries use around 1.3 million tons of artificial dyes out of which 15% leak as pollutent during their use [71]. Some portion of those dyes escape conventional wastewater treatment processes and cause adverse effects on human health and environment. It is important to exchanges synthetic dye in textile industry with environmentally friendly dye. Microbial pigments are eco-friendly colorants, they are applicable to dyeing textile fabric. Some microbial pigments were used to dye different types of fabric.Prodigiosin pigment from *Vibrio* sp. can dye nylon, silk, wool. The pigment that are obtained from *Serratia marcescens* can color up to five sorts of fabric including polyester, silk, cotton, acrylic, polyester microfiber [72]. Anthraquinones from Fusarium oxysporum might be used for dyeing wool with excellent color properties and high dye uptake. The pigment violacein having multiple functions which are extracted from *C.violaceum* is capable of dyeing both bacterial and synthetic fibers and has gained increasing importance in textile. The dyeing ability of two Strptomyces strains that are NP2 & NP4 shows red and deep blue color is depending upon fabric materials. The prodiginines obtained from *Vibrio* sp. act as dye in textile fabric exhibited antibacterial activity against *E. coli* and *Staphylococcus aureus* [73]. The extensive availability of the microbial pigments, their affinity towards different textile, their nontoxic nature, cost effectiveness due to these properties of microbial pigments may increase their market value and could replace such synthetic colors which are toxic to nature and human beings.

3.2.2 Food industry

The important goal in food industry is to make their apparence more attractive which have been done by using an synthetic dyes. After consumption of synthetic food colorants more health problems are occurs, so now food industries are prefer to used the natural food colorants. Nowadays the demand of natural food additives is increasing mainly in the food industry [74]. This demand fulfilled by providing a more natural, healthy and clean food colors. The researcher have isolated pigments from bacterial strains that might provide a natural food colorants that shows great stability, safety of health and they also acts as a preservatives [75]. The use of pigmented molecules various areas like in food, medicine, cosmetics and other medical devices is under control of the Federal Food, Drug, And Cosmetic Act. Give approval to Pigmentsas food color and nutritional supplements are more importantly depend upon the safety of consumers and product freshness. Natural food colorants play vital role in food industry because of its cheap production, high yield, easier extraction and seasonal variation. Pigments like riboflavin are used in beverages, instant desserts and ice creams. Carotinoids can act as sunscreen to maintain the quality of food by protecting them from intense light [76]. Pigments like canthaxanthin used in food products such as cheese, candy, meat, fish, snacks, beer and wine. Now several pigments obtained from microorganism are approved and are used in food for several purposes.

3.3 Bio-Indicator

Pigments are used as bio-indicators aside from antioxidant, antimicrobial agent, colorants. Fluorescent type pigments from bacteria can be used to check the progrees of specific reactions. A example is phycoerythrin, which is used during the prediction of rate of peroxy radical scavenging in human plasma, the pigment initially shows fluorescence, but, dark spots appear when pigment reacts with radicals [77]. Some pigments are used for the detection of heavy metals for example, the microbes *Vogesella indigofera* produce blue colored pigment under normal environmental growth condition; but when they exposed to heavy metal like hexavalent chromium, they not produced any kind of pigments [78]. The cynobacteriaact as goodbioindicators that are naturally present in water sources used for detection of heavy metals,when carotenoid content in these cyanobacteria get reduces, it indicates the presence of heavy metals in water bodies [79].The microbial pigments also used to monitor temperature variation.

3.4 Miscellaneous applications

Pigment producing microbes act as potential sources of electrons. These microbial pigments used in production of dye-sensitized solar cells (DSSC) that are the great alternative sources of conventional photovoltaic-silicon cells. So these DSSC play important role in solving the energy requirement problems at Antarctic regions.For biodegradable ink on plastic materials bacterial pigments can be used. Astaxanthin isolated from *Deinococcus* sp. exhibiting radio-protective and antioxidant activities that can be used in cosmetics like sunscreen and sunblock [80]. The pigments prodigiosin and violacein that are isolated from *S.marcescens* and *C.violaceum* shows antimicrobial and antioxidant activities that are used in sunscreens. Indigoidine used as an organic semiconductor with various application in electrochemical cells, carbon dioxide capture devices, batteries, super capacitors etc. [81].

4. CONCLUSION

Nowadays, the demand for microbial pigments are increasing in market and industries because of the adverse effects of synthetic dyes on environmentand human health. As compared to synthetic dyes microbial pigments are eco-friendly and used in varoius industries such as textile, food, and used as antioxidant, antimicrobial agents, as colorants and bio-indicators. The extensive research has been done to bring microbial pigments from the petri dish to market. The more efforts has been taken in finding of new microbial sources for pigments production and decreases in production optimization, strain improvement and genetic engineering have to be carried out to eradicate toxic synthetic dyes. The economic growth and vast applications of pigments increase the exploration of new sources of microbial pigments.

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