Applications of fungal pigments in biotechnology

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Abstract
The rapid development is leading the demand of colors in biotechnology related to food, textile, medicine and cosmetics. The health hazards of synthetic colors compel industries to adapt natural pigments. “Among them; microbial pigments most important and very diverse in nature.” In this group the fungal pigments are of high demand due to mostly being extracellular metabolites, easy to extract and have enormous biotechnological applications. “Almost all genus of fungi produce efficient amount of pigment which depend and vary from species to species. Yeast, basidiomycetes, Zygomycetes, filamentous fungi, endophytic fungi, marine fungi and halophiles are most important groups which produce useful pigments. The pigments mostly found in kingdom fungi include melanin, phenazines, flavins, carotenoids, violacein, indigo, ankaflavins, canthaxanthin, prodigiosin and moascins. These pigments are being extracted on industrial scale for many biotechnological purposes. This review is intended to highlight the importance of pigments produced by fungi. The research on pigments producing fungi can lead to the development of industries related to textile, food, medicine, and cosmetics.

Keywords: Biotechnology; Cosmetics; Fungi; Food; Medicine; Textile

Introduction
The chemical compounds which absorb light in the wavelength range of the visible region are known as pigment, which can be natural, synthetic, or inorganic [1, 2]. “Natural pigments are found in all kingdoms of algae, fungi, plants, animals” [3]. All advance animals including human communicate and contact surrounding by the help of pigments that is why they are of high utility for living organisms [3]. The previous studies show that microbes and plants have the ability to produce some natural products, their chemistry and composition depends on the organism excreting it. These kinds of natural products are mostly secondary metabolites. Fungi have the ability to produce different secondary metabolites including cephalosporin, penicillin, statins, ergotrate, pigments and other compounds [4]. Among microbes, fungi have the ability to produce potent pigments which can be used as dye or as a food colorant [5]. A wide range of pigments are produced by fungi, exhibiting many biological properties beside some of being cytotoxic [6]. The studies show that pigment production by filamentous fungi is gaining interest amongst food colorants, textiles and cosmetic industries due to their properties [7].
**History of pigments**

The art of dyeing and coloring has long history. It has been in use since the Bronze age. The earliest use of natural dyes was first recorded in China dated back 2600 before Christ (BC). In 2500 BC dyeing technology was adopted in Indian Subcontinent, being evident in ruins of Mohenjodaro and Harappa civilization of 3500 BC. Evidence of wrapping mummies in colored cloths has been found in Egypt. Different dyes such as madder, wood, weld, Brazil wood, Dark reddish purple and indigo was known by fourth century anno Domini (AD). Even Brazil has been named by the wood found in there [8]. The art and designing of coloring spread widely with development of civilization [9]. This was main reason for studies on bio-colorant which enhanced due to their multiple functions in daily life [10].

Pigments can be divided into many classes on the basis of their nature such as natural pigments, synthetic pigments and inorganic pigments. Synthetic pigments are sourced by laboratories, inorganic pigments occur naturally in ores and natural pigments are produced by plants, microorganisms, animals and fungi [2].

**Microbial pigment**

The natural pigment extracted from microorganisms is termed as microbial pigment. Food and textile colorants are being extracted by bacteria, algae and fungi. These microbes are promising species to produce natural colors [11].

**Fungal pigment**

The kingdom fungi are full of diverse and unique group of organisms which produce various kinds of biotechnologically important metabolites. Among fungal species *Monascus Purpureus* produce variety of secondary metabolites which is usually colored naturally. This fungal species is also called red-purple yeast [12], and is reported to produce red (azaphilones), yellow pigment called ankaflavine and monascine, purple pigment called rubropunctatine, and orange pigment called rubropunctatine and monascorubrine. These pigments have been used from centuries in oriental foods (red mold rice) in Southern China, Japan and Southeast Asia. These pigments are being extracted by *Monascus* have abundant utilities in food, textile, cosmetics industry and approximately 50 patents have been made by color extract of this particular species [13].

Among all classes of fungi, filamentous fungi produce wide variety of bio-colorants, which include melanins, phenazines, flavins, carotenoids, quinones, violacein, indigo, and monascins [14]. Carotene is most important metabolite, being extracted by fungal species. More than 200 fungal species are reported to produce carotene [13]. The highest amount of carotene production is reported in class Zygomycetes from order Mucorales. The *Mucor, Phycomyces* and *Blakeslea* are *Mucorales* genus which produce highest amount of carotene. The species of basidiomycetes such as *Ustilago, Sclerotinia, Sporidiobolus* and *Rhodosporidium* also produce carotene. The Ascomycetes genus such as *Aschersonia and Cercospora* are also reported to produce efficient amount of carotene [15]. Among fungi pigments like anthraquinone, melanin, naphthaquinones, anthraquinones, dihydroxy naphthalene, flavin, chrysophanol, cynodontin, erythroglaucan, helminthosporin and tritispina are widely extracted [16].

The marine fungi are also capable of producing variety of pigments [14], those are more or less similar to terrestrial pigments [17]. However, there are some pigments which are only extracted by marine fungi, such as anthracene-glycoside and asperflavin-ribofuranoside, being yellow pigments, are extracted by *Microsporum ssp* [18]. The marine endophytic fungi, such as *Halorosellinia* [19], *Hortaea, Eurotium*
rubrum [20], Phaeotheca, and Trimmatostroma are known to produce large amount of pigments. The marine fungi combined with algae and coral enhance the beauty of marine life [14].

**Halophilic fungal pigment**

Halophilic fungi are capable of producing numerous extreme extracellular metabolites, which possess great importance in biotechnological applications [21]. Melanin is one of those halophilic metabolites; it was first extracted by black yeast named *Hortaea wernecki*. This pigment is important in medicinal world, as it showed activity against *Salmonella typhi* and *Vibrio parahaemolyticus*. The halophilic pigments are new promising sources of melanin which can be used in pharmaceuticals, and cosmetology [22].

**Applications of natural pigments**

The synthetic pigments show a great hazard to health and environment, due to this reason the alternate source of pigments is historically being investigated. The natural pigments show great potential of colors for food, textile, pharmaceuticals and cosmetic industries [23]. The existing microbial pigment extractions process are expensive and require efforts on industrial scale. It is need of time to explore new resources and techniques to extract more eco-friendly, biodegradable colorants [16].

**Biotechnological importance of fungal pigments**

Among all microbes, fungi have ability to release pigments in large quantity. They are valuable sources of natural pigments after plants. Fungi release pigment in cheap culture medium, thus making it feasible to use on industrial level. They are eco-friendly way to produce and use bio-colorants [6]. Some of the fungal pigment applications are mentioned in (Table 1).

**Table 1. Some representative species of fungi and their biotechnological applications**

<table>
<thead>
<tr>
<th>Species</th>
<th>Pigment</th>
<th>Properties</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Monascus sp.</em></td>
<td>Canthaxanthin, Ankaflavine, Monascorubramine Rubropunctatine</td>
<td>Orange-pink, Yellow, Red, Orange</td>
<td>Antioxidants, Anti-inflammatory, Virucidal, Antifungal, anti-tumor, anti-cholestrol activity [13, 25, 26, 28]</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Prodigiosin</td>
<td>Red</td>
<td>Antibacterial, anticancerous activity [37, 43]</td>
</tr>
<tr>
<td><em>Xanthophyllomyces dendrorhous</em></td>
<td>Astaxanthin</td>
<td>Yellow, red, orange</td>
<td>Food industry [46]</td>
</tr>
<tr>
<td><em>Ashbya gossypii</em></td>
<td>Riboflavin</td>
<td>Yellow</td>
<td>Food industry [47]</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>Anthraquinone</td>
<td>Blue, violet</td>
<td>Textile industry [51]</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans, aspergillus fumigates</em></td>
<td>Melanin</td>
<td>Brown</td>
<td>Cosmetics, eye glasses, sunscreens, sunblocks, melanoma treatment [55, 57, 58]</td>
</tr>
<tr>
<td><em>Rhodosporidium, ustilago, sclerotium</em></td>
<td>Carotenoids</td>
<td>Red-orange</td>
<td>In chemicals, pharmaceutical, poultry, food, cosmetics, antioxidant [30, 15]</td>
</tr>
</tbody>
</table>
In medicine
Microorganisms have potential to produce variety of metabolites which show biological and pharmacological activities. Microbial pigments show great antioxidant, anticancer and antimicrobial activities [24]. Amongst microbes, fungi have great potential to release such metabolites which carry biological and pharmacological activities. The fungal genus Monascus are one of those which produce great number of pigments, such as yellow pigment called Ankaflavin, orange-pink pigment named Canthaxanthin, red pigment named Monascorubramin, and orange pigment named Rubropunctatin. All these bio-colorants carry great biotechnological applications such as anti-tumor, antioxidant and anti-inflammatory properties [25], antifungal and virucidal activity is reported in Penicillium oxalicum [26]. Medicinal significance of some of the important fungal pigments are discussed following.

Antioxidant activity
The serious diseases such as, cardiovascular, autoimmune disorders, cancer and diabetes are associated with presence of free radicals. The fungal pigments such as carotenoid, violacein and naphthaquinone have been reported to produce efficient amount of antioxidant activity due to the presence of such free radical scavenging compounds [27]. The yellow, orange and red pigments extracted by Monascus species showed great antioxidant and anti-cholesterol activity [28], due to this reason many fermented products of Monascus species are being used on industrial scale. These products carry pigment and are good source of antioxidants [29]. Yeast is good source of carotenoids; extraction of carotenoids is being used as commercial for the sake of biotechnological applications. This fungal pigment contains many medicinally important compounds, in which antioxidants are most valued [30].

The other pigments extracted by filamentous fungi such as melanins, flavins, violacein, indigo and others possess great potential of antioxidant activity, giving coloring and medicinal importance both same time [24].

Antimicrobial activity
Drug resistance in human disease-causing microorganisms is increasing day by day and multi drug resistance is major problem in red biotechnology. So, it is need of time to look more resources of antimicrobials, in order to overcome drug resistance [31, 32]. The fungal pigment showed great biotechnological applications which include antimicrobial activity as well. Violacein showed bacteriostatic and bactericidal activity as well as antifungal activity [33]. Violacein also showed antiviral [34], and antiprotozoal activity [35]. Endophytic fungal pigment showed great activity against Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi and Vibrio cholera. Their antimicrobial activities were superior to commercially available antibiotic such as streptomycin [36]. A red colored pigment, prodigiosin extracted by Serratia marcescens showed antibacterial activity against gram-positive and gram-negative bacteria [37]. Due to great drug resistance developed by these pathogenic microorganisms, new discoveries for such antibiotics are needed constantly. Microbes and their metabolites are good source for such biotechnologically important compounds [38].

Anti-cancerous activity
Cancer is an uncontrolled growth of abnormal cells and often metastasize to other parts of the body. It is a leading cause of death worldwide. Despite the advancement in the early detection methods and improved therapies. It is still serious health challenge [39]. The microbial pigment reported to possess cell cycle inhibition and induce apoptosis [40, 41]. Among microbes, fungi possess ability to produce such compounds which show antimicrobial activity, anticancer activity, anti-mutagenic activity and anti-
obesity properties. *Monascus* pigment, monascin showed great inhibitory activity against carcinogenesis against mouse skin, Hep G2 and A549 human cancer cell lines. Similarly, Monaphilone A and Monaphilone B, showed anti-proliferative effects against HEp-2 human laryngeal carcinoma cell lines [42]. It was also reported that Anthraquinone from mangrove endophytic fungus *Alternaria* sp. ZJ9-6B showed anti-cancer activity against human breast cancer cell lines [43]. It was also reported that Anthraquinone from mangrove endophytic fungus *Alternaria* sp. ZJ9-6B showed anti-cancer activity against human breast cancer cell lines [43]. Prodigiosin, a fungal pigment was tested against 60 different cancer cell lines, and it showed great anticancer activity [44]. All above fungal pigment properties showed that it might be useful and potential therapeutic approach for treatment of cancer.

**In food industry**

Natural or synthetic colors are very important in attractive appearance of industrial food making more attractive. The synthetic pigments showed worse effect on health and quality of food which is the main reason that food processors are turning from synthetic to natural colors [45, 46]. Among microorganisms; fungi produce safe and efficient pigments for food processing. Such as red pigment extracted from *Monascus* sp, pink-red pigment from *Penicillium oxalicum*, astaxanthin from *Xanthophyllomyces dendrorhous*, β-carotene from *Blakeslea trispora*, riboflavin from *Ashbya gossypii* and ycopene from *Erwinia uredovora* and *Fusarium sporotrichioides* are being widely used in food processing industries [47]. Some pigments are used for specific purposes such as carotenoids protect food and act as sunscreen. Canthaxanthin is used in; candy, fish, meat, cheese, snacks, beverages, beer, wine and riboflavin are widely used in ice cream, beverages and instant desserts [48].

**For textile**

Textile industry plays a backbone role in economy of any country [49]. In developed countries 1.3 million tons of synthetic dyes and dye precursors are being used per year [45]. In that about 2000000 tons of dyes are wasted as effluents during dyeing and finishing process. The effluents get mixed with waste water treatment process and persist in environment, being non-biodegradable and show resistance against detergents, water chemicals, soap, temperature, bleach and other remediation [50].

To assess such environmental issues, there is great interest to search for eco-friendly dyes. Natural pigments extracted from microbes are very good source of such eco-friendly dyes [51]. Amongst microorganism; fungal pigment show more feasibility for such applications. Such as *Fusarium oxysporum* release Anthraquinone which can be used to dye wool fabrics [52], *Penicillium minioluteum* pigment is being used to dye wet blue goat nappa skin [53], red pigment from *Talaromyces verruculosus* tone and dye cotton fabric without any cytotoxic effect [50].

Yellow pigment from *Thermomyces* is evaluated for 3 different fabrics, cotton, silk and wool. The results showed that silk fabric has high affinity for thermomyces pigment as compared with other fabrics [54].

**For cosmetics**

The kingdom fungi consist of numerous biologically important active compounds. Many species of basidiomycetes fruiting bodies are being used in traditional medicines and cosmetics. There are plentiful compounds from mushrooms that are being used in nutraceuticals. The products from such compounds are of many types (such as creams, lotions, applied topically and ointments).

The cosmetic industry is most costly and it is constantly looking for new and natural products. In ancient time, in China and Japan, such compounds that have been used for cosmetics, were reserved for royal families [55].
Amongst fungal pigments, melanin show resistance against UV light by absorbing a broad range of electromagnetic spectrum and also protect from photo induced damage [56]. Melanin is produced and extracted by many fungal species such as Cryptococcus neoformans, Colletotrichum lagenarium, Paracoccidioides brasiliensis, Magnaporthe grisea, Sporothrix schenckii and Aspergillus fumigates [57]. Melanin is widely used in cosmetics, eyeglasses, sunscreens, sun blocks, and immobilization of radioactive waste such as uranium. Melanin is being reported in treatment of human metastatic melanoma [58, 59]. Some organic pigments also found in fungi such as carotenoids [60], are widely used in cosmetics especially in sun lotions, anti-aging facial; lycopene is used in lycopene face cream and other products [61].

**Conclusion**
The kingdom fungi are diverse group of prokaryotes. It produces extracellular metabolites which carry enormous biotechnological applications. The fungal pigment is one of those bio-active compounds. Almost all groups of fungi produce pigments of different colors and characteristics. The most important fungal pigments include carotenoids, melanin, phenazines, violacein, flavins, quinones, indigo etc. These pigments carry antibacterial, antifungal, anti-cancerous and antioxidant potential. Many fungal pigments show a great performance in textile, food and cosmetics industries. The health hazards and environmental concerns of synthetic pigments compel researchers to work and find substitute of such synthetic pigments. To overcome hazards of such issues, extracting fungal pigment and its use could be best solution, as it takes less efforts and cost.

**Authors’ contributions**
Designed and analysed the data: S Sajid, Advised on research topic: N Akbar, Helped in writing and made critical comments on manuscript: N Akbar, Wrote the manuscript: S Sajid & N Akbar.

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