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# A COMPREHENSIVE REVIEW OF MARKET DEMAND, ADVANCEMENTS AND APPLICATIONS OF MICROBIAL PIGMENTS

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## ABSTRACT
Natural pigments from bacterial, fungal, algal, and plant sources have gained significant attention due to their wide range of applications in food, pharmaceutical, textile and aquaculture industries. These pigments not only provide appealing colours but also offer additional beneficial properties that can be exploited to our advantage. These bioactive properties include antimicrobial, antioxidant, antiviral and antitumor effects. This review highlights the growing interest in microbial pigments apparent with its growing market demand. The pigments of high value presently include β- carotene, prodigiosin, violacein and fungal melanin. Some pigments have demonstrated more promising health effects and hence have opened new avenues for therapeutic applications. Besides, common applications such as textile and food colouring, the applications have been extended to improving the pigmentation and market value of ornamental fish and crustaceans using pigments such as astaxanthin, zeaxanthin, and bixin. Additionally, fungal spalting is another interesting application of biopigments that has emerged for decorating wood products, and further adding commercial and artistic value. Recent biotechnological studies are focused on optimizing pigment production and scaling up industrial applications, and thereby develop sustainable options to replace synthetic colorants. Overall, this review presents interdisciplinary research on natural microbial pigments and their advantages and multifunctional potential across different sectors.

**KEYWORDS**: Pigments, spalting, β- carotene, prodigiosin, applications, decoration, commercial.

## INTRODUCTION
Pigments are chemical compounds, produced by a variety of organisms including bacteria, fungi, algae and plants. These pigments absorb light in the visible spectrum (400–700 nm). The colour of the pigment is due to specific molecular group known as chromophore, which is responsible for their light absorbing properties.[1] The chromophores absorb energy causing an electron to move from a lower to a higher energy orbital. The portion of light that is not absorbed is reflected and/or refracted, and reaches the eye, where it turns into signals that the brain interprets as colour.[2]

Colour enhances the appearance and appeal of products. Additionally, they indicate the freshness of products and hence contribute to its aesthetic and sensory value. Since not all products are coloured, colour additives are used to enhance their appearance.[3] Some examples of pigments derived from natural sources are represented in Table 1.[4-8] Pigments can be classified based on their origin as natural, synthetic or inorganic. Natural and synthetic pigments are organic compounds. The natural pigments are obtained from nature, primarily using plants and microorganisms, whereas the synthetic pigments are chemically synthesized in the laboratories. The inorganic pigments can be found in nature or synthetically produced.[4] The natural pigments extracted from fruits, vegetables, seeds, roots and microorganisms are often referred as 'bio colours' and recognised as safe and edible colouring agents.[3] Among the natural sources, pigments from microbial sources are increasingly considered as promising alternatives to synthetic pigments.[1,3,4] However, compared to synthetic pigments, they show instability to light, heat, and pH variations, low water solubility, and limited availability throughout the year.[9] They are also economically less favourable.[10] Despite these shortcomings, there is a renewed interest in natural pigments driven by growing awareness of the potential health risks and environmental impacts of synthetic dyes and food colorants.[3, 11] For instance, pigments derived from heavy metals like lead chromate and copper sulphate can pose serious health hazards, and many synthetic colorants have been linked to carcinogenic and teratogenic effects.[9, 12] Most of the traditional pigments containing heavy metals like lead and mercury are banned since they are toxic, but few others (indicated in Table 2) containing chromium, zinc, and iron are still used.[9, 10, 12]

**Table 1: Pigments from natural sources.**

| Source | Pigment |
|---|---|
| **Plants** | |
| *Acacia catechu* (L.f) Wild (Bark) | Brown/black |
| *Acanthophonax trifoliatum L.* (Fruit) | Black (Acantrifoside, Nevadensin) |
| *Adhatoda vasica Nees* (Leaf) | Yellow (Adhatodic acid, Carotein, Quercetin) |
| *Capsicum annuum L.* (Fruits) | Red (Capsanthin, Capsorubin) |
| *Crocus sativus L.* (Flower) | Yellow-orange (Crocin, Picrocrocin) |
| *Carthamus tinctorious L.* (Flower) | Yellow, red (Carthamin Oil) |
| **Animals** | |
| Cochineal insects (*Dactylopius coccus* ) | Bright red |
| Kermes insects (*Coccus iticis*) | Crimson red |
| Murex predatory snails (*Bolinus brandaris*) | Tyrian purple |
| **Microorganisms** | |
| *Serratia marcescens* | Red (Prodigiosin) |
| *Corynebacterium insidiosum* | Blue (Indigoidine) |
| *Monascus roseus* | Orange Pink (Canthaxanthin) |
| *Staphylococcus aureus* | Yellow (Zeaxanthin) |
| *Rugamonas rubra* | Prodigiosin like red pigment |
| **Fungi** | |
| *Phycomyces blakesleeanus Blakeslea trispora* | Carotenoids of diverse colours |
| *Monascus spp.* | Yellow (Monascin) |
| *Penicillium oxalicum* | Pink Red (Anthraquinone derivatives) |
| **Algae** | |
| *Dunaliella salina* | Orange red (Beta carotene) |
| *Spirulina platensis* | Blue (Phycocyanin) |
| *Porphyridium cruentum* | Pink red (Phycoerythrin) |
| *Phaeophytes* spp., | Brown to orange green (Fucoxanthin) |

**Table 2: Synthetic Pigments Containing Heavy Metals.**

| Pigment Type | Colour | Chemical Name / Formula | Applications | Toxicity / Health Risks | Current Status |
|---|---|---|---|---|---|
| Aluminium pigment | Purple | Ultramarine violet (sodium aluminium silicate + sulfur) | Paints, cosmetics, plastics, coatings | Low toxicity, but dust inhalation can irritate lungs | Still used under controlled regulations |
| Iron pigment | Blue | Prussian blue (ferric hexacyanoferrate, $Fe_7(CN)_{18}$) | Textile dyeing, blueprints, paints, antidote for cesium poisoning | Low toxicity; cyanide bound in stable complex; non-toxic orally | Still used, some restrictions in textiles |
| Chromium pigment | Green | Chrome green (chromic oxide, $Cr_2O_3$) | Glass, ceramics, paints, roofing materials | Low toxicity in $Cr^{3+}$ form; $Cr^{6+}$ highly toxic and carcinogenic | $Cr^{3+}$ pigments still allowed; $Cr^{6+}$ banned |
| Arsenic pigment | Yellow | Orpiment (natural arsenic sulfide, $As_2S_3$) | Historical use in art and manuscripts | Highly toxic; arsenic is carcinogenic, neurotoxic, lethal | Banned in most countries |
| Lead chromate pigment | Yellow | Chrome yellow ($PbCrO_4$) | Paints, plastics, industrial coatings | Highly toxic; lead affects nervous system; $Cr^{6+}$ is carcinogenic | Banned or highly restricted worldwide |
| Cobalt pigment | Blue | Cobalt blue ($CoAl_2O_4$) | Ceramics, glass, artist pigments | Moderate toxicity; inhalation hazard; possible carcinogen | Still in use under regulation |

The microbial pigments offer solutions to many of these challenges. They can be produced under controlled conditions, independent of climate and season, and can be generated quickly using inexpensive culture media. In addition, minor modifications in chromophores of natural pigments can improve stability and hence expand their applications.[9,13] Moreover, some natural pigments, especially anthraquinones (a class of pigments), exhibit additional properties such as antimicrobial activity which further increases their industrial applications.[14]

Presently, there is a rising demand for eco-friendly, natural colorants for use in fabrics, foods, animal feeds, cosmetics and printing inks.[4, 9, 13]

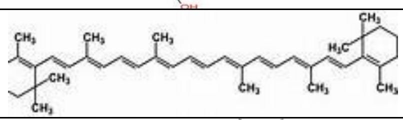
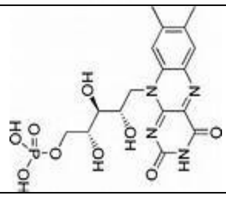
## TYPES OF MICROBIAL PIGMENTS

Microorganisms produce pigments for various reasons. Some bacteria such as cyanobacteria use phycobilins to carry out photosynthesis. Other examples for pigment-producing bacterial strains include *Serratia marcescens* (producing prodigiosin), *Streptomyces coelicolour* (prodigiosin and actinorhodin), *Chromobacterium violaceum* (violacein) and *Thialkalivibrio versutus* (natronochrome and chloronatronochrome). These bacteria can be isolated from various environmental sources such as water bodies, soil, plant surface, insects and even humans or animals.[5, 15] Gram-positive Micrococcus spp., produce a range of carotenoid pigments from yellow obtained from *M. luteus* and *M. varians*, orange from *M. nishinomiyaensis*, pink from *M. roseus* and red from *M. agilis*. The carotenoids are widespread in bacteria and play an important role in protecting them from light damage and oxidative stress in aerobic environments.[16]

Similarly, Gram-negative rods such as *Flavobacterium*, *Cytophaga*, *Chromobacteria*, *Serratia* and pseudomonads produce pigments of diverse colours. Prodigiosin, a red pigment, is characteristic of *Serratia marcescens*. Other bacteria that make prodigiosin or its derivatives include *S. rubidaea*, *Vibrio gazogenes*, *Alteromonas rubra*, *Rugamonas rubra*, and Gram positive actinomycetes, such as *Streptoverticillium rubrireticuli* and *Streptomyces longisporus*.[6] *Chromobacterium* spp. produces bright colour pigments such as violacein (a purple pigment). Bacteria producing violacein belong to at least three genera- *Chromobacterium*, *Janthinobacterium* and *Iodobacter*. Many of these organisms are isolated from soil and water. One possible role of violacein is to make the bacteria unpalatable to protozoa and nematodes.[17] *Pseudomonas aeruginosa* produces a water soluble, non-fluorescent blue-green pigment pyocyanin which may play a role in respiration.[18] The yellow water soluble fluorescent pigments produced by a number of *Pseudomonas* spp., especially under conditions of iron limitation, are known as pyoverdin, pyofluorescein or fluorescein. The pyoverdines are a large family of complex siderophores. They are able to bind metal ions, especially iron. Other pigments like pyorubins, pyomelanins are also produced by pseudomonads and are valuable natural products.[19]

The most commonly used natural pigments in industries are carotenoids, flavonoids and tetrapyrroles. Beta-carotenes are obtained from some microalgae and cyanobacteria.[1] Astaxanthin obtained from *Phaffia rhodozyma* and *Haematococcus pluvialis*, is a red pigment of great commercial value and is used in feed, pharmaceutical and aquaculture industries. Natural astaxanthin from microorganisms is mainly supplied by the red basidiomycetous yeast *Xanthophyllomyces dendrorhous* and the green algae *Heamatococcus pluvialis*. Astaxanthin production using *Agrobacterium aurantiacum* has also been investigated.[20] A fungus strain *Penicillium oxalicum* with the properties to produce a red colourant can be used in food and cosmetic industries. *Monascus* sp. have long been used in production of traditional East Asian foods such as red rice wine and red bean curd.[7] Micro-organisms which have the ability to produce pigments in high yields include species of *Monascus*, *Paecilomyces*, *Serratia*, *Cordyceps*, *Streptomyces*, *Penicillium herquei*, *Penicillium atrovenetum*, *Rhodotorula*, *Sarcina*, *Cryptococcus*, *Monascus purpureus*, *Bacillus* sp., *Achromobacter*, *Yarrowia* and *Phaffia*.[7, 16, 20] Few examples and colour characteristics of pigments of biological importance is indicated in Table 3.[21, 22]

**Table 3: Characteristics of selective pigments.**

| Group | Type (Example) | Colour | Biological Function | Source Organisms | Structure |
|---|---|---|---|---|---|
| Tetrapyrroles | Chlorophylls | Green | Light harvesting in photosynthesis | Plants, algae, cyanobacteria |  |
| Isoprenoid derivatives | Carotenes, Xanthophylls (e.g., Zeaxanthin) | Yellow | Antioxidant, photoprotection | Plants, microalgae, some bacteria |  |
| N-heterocyclic compounds | Flavins (e.g., Riboflavin) | Yellow | Electron transfer, redox reactions | Plants, bacteria, fungi |  |

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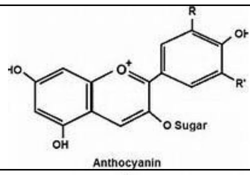
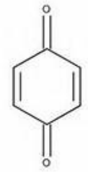
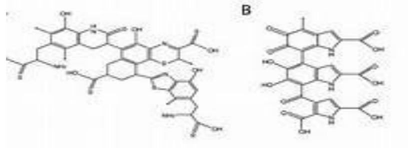
|---|---|---|---|---|---|
| Benzopyran derivatives | Flavonoids (e.g., Anthocyanins) | Blue–Red | UV protection, attraction of pollinators | Flowering plants, fruits |  |
| Quinones | Benzoquinones | Red | Electron transport, antimicrobial | Plants, fungi, bacteria |  |
| Melanins | Allomelanin | Yellow–Brown | Protection against UV, free radicals | Fungi, human skin, some bacteria |  |

## SAFETY, STABILITY AND DEMAND OF PIGMENTS

The global market for pigments was valued at around 24.13 billion USD in 2023, and is expected to grow with a compound annual growth rate (CAGR) of 6.0% between 2024 and 2032.[23] The natural colours market is currently estimated at 1.57 billion USD, and growing at a CAGR of 8.31%. Due to its high demand in several industries, the market size is expected to reach 3.49 billion USD by 2034.[24] The global market for beta-carotene alone was valued at USD 1.03 billion in 2023.[25] Many microbial pigments have been extracted and are at different developmental stages depending on their characteristic properties. The details of these pigments are described in Table 4. The use of natural colours and additives is subject to biosafety protocols and regulatory approvals, which vary across countries. Today all food colour additives are carefully regulated by federal authorities to ensure that foods are safe to consume, and they are accurately labelled. For instance, the European Union (EU) has given approval for fungal pigments as food colours. This reflects the expanding acceptance of microbial-derived pigments in mainstream markets.[26]

Pigment stability has been the focus of several important studies. Latha and Jeevaratnam[27] investigated the stability of crude pigments extracted from *Rhodotorula glutinis* DFR-PDY over a three-month storage period. They examined pigment stability in various solvents, including acetone and petroleum ether, across different temperature conditions (4°C, 25°C, and 40°C), under light and dark storage, and in the presence of antioxidants such as butylated hydroxytoluene and vegetable oils (sunflower, groundnut, sesame, palm, and coconut oils). The study reported that the pigment was notably less stable under light exposure compared to dark storage. Interestingly, the red yeast pigment showed excellent stability when stored in vegetable oils, suggesting its promising potential for incorporation into oil-based food products.[27] Similarly, Selim et al.[28] explored the stability of red anthocyanin under different conditions of pH and temperature. It demonstrated high stability in acidic environments. To assess their thermal stability, the pigments were heated for 30 mins at 60°C, 70°C, 80°C, 90°C, and 100°C, and they retained 99.87%, 99.24%, 94.49%, 86.35%, and 78.59% of their anthocyanin content, respectively. When the heating duration was extended to 60 mins, the colour retention was slightly decreased, with values of 96.99%, 86.75%, 82.10%, 76.72%, and 57.69%, respectively. These findings highlight the suitability of microbial pigments under acidic conditions and moderate heating, supporting their application as natural food colorants.

**Table 4: Recent progress on microbial pigments.**

| Molecule | Colour | Microorganism(s) | Applications | Recent Progress |
|---|---|---|---|---|
| **Ankaflavin** | Yellow | *Monascus purpureus* | Food, Cosmetics | Recent studies have explored its biosynthesis and pharmacological properties, highlighting its potential in various industries.[29] |
| **Anthraquinone** | Red | *Penicillium oxalicum* | Textile, Cosmetics | Ongoing research focuses on optimizing production methods and exploring its applications in different sectors.[7] |
| **Astaxanthin** | Pink-red | *Xanthophyllomyces dendrorhous* | Nutraceuticals, Cosmetics | High-yield production achieved using cane molasses and two-stage pH strategies, marking significant progress in sustainable production methods.[30] |
| | | *Agrobacterium aurantiacum* | Feed, Pharmaceuticals | Research is ongoing to enhance production efficiency and explore its applications in various industries.[31] |

| | | | | |
|---|---|---|---|---|
| **Canthaxanthin** | Dark red | *Bradyrhizobium spp.* | Food, Feed | Studies are investigating its biosynthetic pathways and potential industrial applications.[32] |
| | | *Haloferax alexandrinus* | Cosmetics, Pharmaceuticals | Research is focused on optimizing cultivation conditions for improved pigment yield.[33] |
| | | *Gordonia jacobea* | Food, Cosmetics | Efforts are being made to enhance pigment production through genetic and fermentation techniques.[33] |
| **Lycopene** | Red | *Blakeslea trispora* | Food, Nutraceuticals | Recent findings indicate that photoinduced synthesis in *B. trispora* is dependent on specific photoreceptors, offering insights into production optimization.[34] |
| | | *Fusarium sporotrichioides* | Food, Cosmetics | Research is ongoing to improve yield and stability of lycopene production.[7] |
| **Melanin** | Black | *Cryptococcus neoformans* | Pharmaceutical, Cosmetics | Advances in understanding its structure, biosynthesis, and regulation have opened avenues for metabolic engineering to enhance production.[35] |
| **Monascorubramin** | Red | *Monascus spp.* | Food, Cosmetics | Studies are exploring its biosynthetic pathways and potential health benefits.[36] |
| **Naphtoquinone** | Deep red | *Cordyceps unilateralis* | Pharmaceuticals | Research is focused on isolating and characterizing its bioactive compounds for therapeutic applications.[7] |
| **Riboflavin** | Yellow | *Ashbya gossypii* | Food, Feed, Pharmaceuticals | Efforts are being made to enhance production through metabolic engineering and fermentation optimization.[7] |
| **Rubropunctatin** | Orange | *Monascus spp.* | Food, Cosmetics | Research is ongoing to improve yield and explore its applications in various industries.[36] |
| **Torularhodin** | Orange-red | *Rhodotorula spp.* | Food, Cosmetics | Efforts are focused on optimizing cultivation conditions for enhanced pigment production.[37] |
| **Zeaxanthin** | Yellow | *Flavobacterium spp.*, *Paracoccus zeaxanthinifaciens*, *Sphingobacterium multivorum* | Food, Nutraceuticals | Studies are exploring genetic and fermentation strategies to increase production efficiency.[38] |
| **β-Carotene** | Yellow-orange | *Blakeslea trispora*, *Mucor circinelloides*, *Neurospora crassa* | Food, Feed, Pharmaceuticals | Research is focused on optimizing light conditions and fermentation parameters to enhance β-carotene synthesis.[7] |

## APPLICATIONS OF MICROBIAL PIGMENTS

Microorganisms have been used for a long time for production of molecules as diverse as antibiotics, enzymes, vitamins and texturizing agents. There is growing interest in the food industry for the use of natural ingredients derived from biological sources like plants or microorganisms. For instance, microbial pigments are in used in the fish feed industry to enhance the colour of ornamental fish. Some natural colorants have commercial potential for use as antioxidants. The industry is able to produce some microbial pigments for applications in food, cosmetics or textiles.[7, 29-38] This section describes the common and interesting applications of microbial pigments.

### Textile colouring

Synthetic colorants face several challenges such as dependence on non-renewable oil resources, sustainability of current operation, environmental toxicity and health risks. In contrast, during the biosynthesis of pigments by fermentation, they can be chemically modified to improve stability and used in textile industries.[9] Gulani et al.[39] evaluated the textile application of the red pigment prodigiosin from *Serratia marcescens*. In their study, the pigment showed additional properties such as antimicrobial activity, and was stable in acid, alkali and detergents. Fabrics such as wool, silk, nylon, cotton and polyester were dyed with methanolic extracts of the pigment and then treated with acid, alkali and detergents to investigate the stability of dyed fabrics. The results showed that the colour was retained completely in acids and detergents. However, little discolouration was observed in alkaline conditions. Thus, the study suggested the suitability of prodigiosin for textile colouring. In another study, Poorniammal et al.[40] explored the use of fungal pigments derived from Thermomyces sp. for dyeing wool fabrics. The dyed materials were tested for antimicrobial activity using standardized methods. Regression analyses were done to evaluate colour intensity, colour fastness and antibacterial effectiveness. By using bleached 100% wool along with various synthetic and natural mordants, the study concluded that the process offers an eco-friendly method for producing antimicrobial textile pigments. Raisanen et al.[41] reported one of the first applications of anthraquinone carboxylic compounds, dermorubin and 5-chlorodermorubin, isolated from the fungus *Dermocybe sanguinea*, for dyeing wool. The dyed wool exhibited good colour intensity and fastness. These properties were comparable to ISO standards and the result was an attractive orange-red hue. FTIR analysis suggested the presence of ionic interactions between the dye and wool fibers, contributing to the observed colour fastness. However, the pigment's colour was sensitive to alkaline conditions. Sharma et al.[42] studied three fungal species, *Trichoderma virens*, *Alternaria alternata* and *Curvularia lunata*, to optimize pigment production and evaluate their application in dyeing silk and wool fibres. Metallic mordants were employed during the dyeing process.

**Fish feed and colorant**

Feed colorants are substances added in trace amounts to diets or feed mixtures of fish. They primarily help to enhance feed visibility thereby improving ingestion. It also imparts desirable pigmentation to the flesh or body of cultured fish or shrimp. Besides, they can also serve as valuable sources of vitamins.[43] In their review on natural carotenoids for fish pigmentation, Gupta[44] compared the limitations of synthetic carotenoids and emphasized the benefits of using natural carotenoids in aqua feeds. The review further highlighted the potential of carotenoid rich natural ingredients such as microalgal pigments (from *Chlorella vulgaris*, *Haematococcus pluvialis* and *Dunaliella salina*), yeast (*Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous*), marigold, capsicum, and others. In addition, they listed common fish carotenoids and their associated colors, including tunaxanthin (yellow), lutein (greenish yellow), beta carotene (orange), alpha- and beta- doradexanthins (yellow), zeaxanthin (yellow orange), canthaxanthin (orange red), astaxanthin (red), echinenone (red), and taraxanthin (yellow).

One notable commercial product, NatuRose, contains astaxanthin derived from *Haematococcus pluvialis*. The main component is astaxanthin (15%), along with smaller amounts of canthaxanthin, lutein and beta carotene. NatuRose has been successfully used to produce colourful shrimp (*Penaeus monodon*, *P. japonicus*), rainbow trout, coho salmon, Atlantic salmon, poultry eggs, and sea bream. It has also shown good results in enhancing the coloration of koi and tropical fishes.[45] Ako et al.[46] observed that fish raised under intensive culture conditions often lose their coloration. To prevent this, they conducted experiments in which ornamental fish diets were supplemented with top coated algae. Their findings showed that freshwater red velvet swordtails (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topaz cichlids (*Cichlasoma myrnae*) exhibited significantly enhanced coloration after being fed diets containing 1.5–2.0% of a carotenoid rich strain of *Spirulina platensis* and 1% culture of *Haematococcus pluvialis* for three weeks. Interestingly, the observed colour enhancement appeared to act through natural carotenoid receptors. This is because the intensity diminished under stress. Also, only male fish showed colour changes in species with male specific coloration. Notably, the colour enhancement was also influenced by environmental factors. The study concluded that ornamental fishes serve as excellent models for dietary colour enhancement. Taufik et al.[47] studied the effect of zeaxanthin extracted from red paprika (*Capsicum annuum* L.) on the red skin coloration of Kohaku Koi fish (*Cyprinus carpio*). Zeaxanthin was extracted using acetone and petroleum ether (2:8), and diets were prepared using doses of 0, 4, 8, and 12 mg/kg, administered over 60 days. Measurements using a colorimeter showed an increase in skin color intensity from 198 to 205, 232, 242, and 253, respectively. Dananjaya et al.[48] evaluated the effects of natural bixin (a carotenoid extracted from achiote seeds) on the pigmentation and color enhancement of goldfish (*Carassius auratus*). Diets containing varying concentrations (0.05, 0.1, 0.2, and 0.6 g/kg) were formulated with 420 g/kg crude protein and 120 g/kg lipid. Results showed that bixin-enriched diets significantly enhanced skin and fin pigmentation, with 0.2 g/kg concentration providing the best balance for strong pigmentation as well as growth of fish.

**Wood spalting**

There is a rising popularity of wood spalting (adding bio pigments to woods through infection with fungal strains) due to increasing consumer demand for unique and customized products. *Polyporus brumalis* and *Trametes versicolor* have been reported to give maximum spalting within 10 weeks. In particular, sugar maple blocks treated with sub lethal levels of copper sulphate and inoculated with known spalting fungal strains produced good results, especially when incubated in copper sulphate treated vermiculite. Additionally, blocks incubated in soil have shown more weight loss compared to those in vermiculite.[49] Robinson and colleagues[50] investigated the scalability of spalting for commercial applications. For this purpose, they inoculated large logs of *Acer saccharum* (sugar maple), *Fagus grandifolia* (American beech) and *Populus tremuloides* (quaking aspen) using liquid spray cultures and live dowel pin cultures. The results showed that fungi successful in small scale spalting also produced significant spalting patterns in large logs. Notably, combinations such as *Trametes versicolor* with *Scytalidium cuboideum* or *Xylaria polymorpha* generated distinct zone lines. The dowel plugs produced more zone lines, and liquid sprays yielded more surface stain. These findings suggest that standard spalting fungi are viable for large-scale production.

Spalting occurs when white-rot fungi colonize hardwoods such as maple, birch and beech, creating striking zone lines where competing fungal territories meet. The resulting black, pink, gray and multicolored streaks arise from interactions among the wood, fungi and even insect deposits. However, excessive fungal activity can weaken the wood's structural integrity. Hence, controlled induction along with optimization of temperature, moisture, wood type and fungal species is essential for reliably standardizing spalting on a commercial scale.[51] Robinson et al.[52] further explored methods to enhance spalting and improve its commercial viability by comparing fungal induced spalting to fungal dyeing. Using species such as *Chlorociboria aeruginosa*, *Scytalidium cuboideum* and *Scytalidium ganodermophthorum*, they successfully induced spalting in shorter time frames. When treating wood with dichloromethane extracted fungal pigments, they achieved approximately 30% internal colouring, with cotton wood showing consistent patterns in all three cases evaluated. Tudor et al.[53] examined how the moisture content can affect fungal pigmentation. For this purpose, they used eight fungal species and two hardwood species. The study found that *Trametes versicolor* and *Xylaria polymorpha* were stimulated at lower moisture levels in both sugar maple and American beech, while *Inonotus hispidus* and *Polyporus squamosus* required higher moisture (22 – 28% in beech and 34 – 38% in maple) levels. *Fomes fomentarius* and *Polyporus brumalis* achieved peak pigmentation in beech at 26 – 41% moisture and in maple at 59 – 96%. The pink staining *Scytalidium cuboideum* required moisture levels above 35% for effective pigmentation.

**Pharmaceutical agent**

Many of the coloured compounds are produced by the organisms so that they have a selective advantage over the other bacteria, fungi, viruses and protozoans. These properties are exploited in the pharmaceutical industries to develop antibacterial, antifungal, antiviral and antiprotozoal agents.[15, 54-57] The microbial pigments have also been evaluated for use as anticarcinogenic and antitumorial agents, and their dose dependent efficacies have been reported in some studies.[55, 58-61]

**Antioxidant Potential**

One of the promising antioxidant compounds is the lipid-soluble carotenoid astaxanthin (a xanthophyll). A study demonstrated the antioxidant potential of carotenoids isolated from *Phaffia rhodozyma* using DPPH and ABTS assays. The carotenoid extract was first concentrated in 0.1 mL of methanol, followed by the addition of 3.9 mL of DPPH solution. A decrease in absorbance at $\lambda = 515$ nm was measured. In these assays, the faster the absorbance decreases, the stronger is the antioxidant activity of the test compounds, and indicates a higher hydrogen donating ability. For the ABTS assay, antioxidant activity was assessed in emulsified and bulk linoleic acid and sunflower oil. The percentage of radical scavenging ability was calculated using standard methods, and they indicated good antioxidant potential of carotenoids which was comparable to ascorbic acid.[62] Gulani et al.[39] investigated the total antioxidant capacity of the red pigment prodigiosin from *Serratia marcescens*. Using the phosphomolybdenum spectroscopic method, they measured absorbance at 695 nm, with ascorbic acid as the reference standard. This method relies on the principle of reduction of Molybdenum (VI) to Molybdenum (V) by antioxidant compounds. The antioxidant capacity of prodigiosin was reported to be 22.05 µg ascorbic acid equivalents per mL of extract. Overall, the study suggested good potential applications of prodigiosin as a therapeutic agent, besides being a natural food colorant. Dharmik et al.[63] explored the antioxidant potential of melanin pigment produced by actinomycetes. Using the DPPH method, they mixed 2 mL of the pigment sample with 2.5% linoleic acid, buffer and distilled water. After heating at 40°C, the mixture was treated with 75% ethanol and ammonium thiocyanate, followed by incubation. To this mixture, 0.02 M ferrous chloride was added along with 3.5% hydrochloric acid. Absorbance was measured at 500 nm and analyses were repeated over 24 h intervals for seven days. Their results indicated that the melanin pigment from *Streptomyces* strains exhibits antioxidant properties which were comparable to standard antioxidants like trolox and tocopherol.

Patel and Patel,[64] investigated the antioxidant activity of plant-derived coumarin pigments to investigate the development of coumarin scaffolds, and evaluate their potential in drug discovery. Using the DPPH assay for analysis, with ascorbic acid as a standard, the study evaluated the potential of three coumarin pigments (I, II, III). Notably, coumarin III showed stronger antioxidant activity than ascorbic acid, while compounds I and II demonstrated notable antibacterial properties. The study proposed that these compounds were promising therapeutic agents for anti aging and oxidative stress related disorders. Amalya and Sumathy[65] extracted carotenoids from the leaves and flowers of *Peltophorum pterocarpum* using column chromatography and assessed their antioxidant activities. They used five different assays for their analysis using DPPH radical scavenging, reducing power, nitric oxide scavenging, phosphomolybdenum, and hydrogen peroxide scavenging methods. In all cases, the carotenoid pigments demonstrated significantly higher antioxidant activity compared to crude ethanolic leaf and flower extracts, emphasizing their potential as powerful natural antioxidants.

**Cytotoxic Properties**

Deorukhkar et al.[66] investigated the antitumor activity of a N-alkylated prodigiosin analogue, specifically 2'-[3-methoxy–1' amyl–5'-methyl-4-(1-pyrryl)] dipyrrylmethane (MAMP-DM), produced by a strain of *Serratia marcescens*. The study was conducted in vitro on both malignant and non-malignant cell lines, including mouse lympho sarcoma ascites (LS- A), mouse fibroblast cell line L929, and human promonocytic leukemia cells (U- 937). Cytotoxicity was evaluated using the MTT assay to measure cell viability. Results showed that the red pigment exhibited selective cytotoxicity. They were significantly more toxic to cancer cells while showing minimal toxicity toward non-malignant cells. Kavitha et al.[67] reported the isolation of prodigiosin from *Serratia marcescens* that was screened and isolated from marine crustacean waste. Its anticancer activity was tested against the human cervical carcinoma (HeLa 229) cell line. The cell viability was assessed using both the MTT assay and the neutral red uptake assay. Prodigiosin demonstrated a dose dependent inhibition of cell proliferation, and indicated strong anticancer and apoptotic effects against cervical carcinoma cells.

Prashanthi et al.[68] screened the anticancer activities of a yellow pigment produced by *Streptomyces griseoaurantiacus* against human cervical cancer (HeLa) and human liver cancer (Hep G2) cell lines. They evaluated the activity based on different assays, including the MTT cell viability assay, caspase activity assay, lactate dehydrogenase (LDH) activity assay, DNA fragmentation assay, flow cytometry and trypan blue exclusion assay. The pigment showed significant cytotoxicity, with $IC_{50}$ values as low as 1.5 µg/mL for HeLa cells and 1.8 µg/mL for HepG2 cells. Moreover, it exhibited non-toxic effects on healthy human lymphocytes. Treated cancer cells showed clear signs of apoptosis, including reduced viable cell numbers, presence of apoptotic bodies, nuclear condensation and DNA fragmentation.

**Antimicrobial Properties**

Gulani et al.[39] evaluated the in vitro antimicrobial activity of prodigiosin using paper disc diffusion assays against various clinical isolates, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida parapsilosis*, and *Cryptococcus* species. The study found that the pigment exhibited antibacterial activity against gram-positive bacteria but was ineffective against gram-negative organisms. Additionally, prodigiosin displayed notable antifungal activity, particularly against *C. parapsilosis* and *Cryptococcus* species, with a lesser effect on *C. albicans*. The authors concluded that the combined antimicrobial and antioxidant potential of prodigiosin makes it a promising candidate for therapeutic applications.

Nakamura et al.[69] investigated the antibacterial properties of a violet pigment mixture (violacein and deoxyviolacein) produced by the psychrotrophic bacterium RT102 strain. The pigment mixture was tested against several bacterial strains, including *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Flavobacterium balustinum*, *Escherichia coli*, and the yeast *Trichosporon cutaneum*. Their results showed a concentration dependent inhibitory effect on gram positive bacteria, where increasing pigment concentrations led to stronger and faster inhibition. However, the pigment mixture was largely ineffective against gram negative bacteria and yeasts. The authors suggested that the observed antibacterial activity could be due to an additive effect between violacein and deoxyviolacein. The study further highlighted the potential for industrial scale production of the violet pigment based on the encouraging results reported in the study. Visalakchi and Muthumary[70] conducted a screening study on the antimicrobial activity of a pigment produced by the endophytic fungus *Monodictys castaneae* SVJM139. This study represented the first report of antibacterial activity from pigments derived from this endophytic fungus. The pigment exhibited significant inhibition against several human pathogenic bacteria, including *Staphylococcus aureus* (21.4 ± 0.34 mm), *Klebsiella pneumoniae* (20.0 ± 0.32 mm), *Salmonella typhi* (20.3 ± 0.32 mm), and *Vibrio cholerae* (20.7 ± 0.33 mm), with inhibition zones comparable to the standard antibiotic streptomycin. Based on these findings, the authors proposed that the pigment could be developed into products for treating wound infections or gastrointestinal tract infections.

**Other applications**

Pigments have long been used in applications such as paper colouring, candle making, and nail enamel production.[9, 13] Since these pigments frequently come in contact with human skin, there is significant concern about the safety and type of colours that are used. Synthetic and inorganic pigments cause various health issues,[9, 10, 12] making natural pigments an attractive alternative. The challenges associated with synthetic pigment production such as high costs and health risks, highlight the potential of natural pigments, many of which are stable when combined with the binders and solvents used in paints.[7, 8, 27] Additionally, if these natural pigments exhibit antimicrobial properties, they could be valuable in producing longer lasting paints. Despite their wide applicability, the biosynthesis and production of pigments from microbial sources remain an un derexplored resource. Lopes et al.[71] explored the antimalarial potential of violacein extracted from *Chromobacterium violaceum*. The pigment was tested both in vitro and in vivo against *Plasmodium* species. In vitro toxicity and dose response assays revealed that violacein, at micromolar concentrations, effectively killed both chloroquinesensitive and chloroquine resistant *Plasmodium falciparum* strains, with calculated $IC_{50}$ values indicating strong efficacy. The study was further extended to mouse models, where violacein maintained its antimalarial activity.

Many natural pigments offer advantages over synthetic ones, as they are resistant to heat, oxidative stress, and light, making them suitable even as food colorants.[27, 28] Today, several fermentative food-grade pigments are available commercially, including Monascus pigments, astaxanthin from *Xanthophyllomyces dendrorhous*, Arpink Red from *Penicillium oxalicum*, riboflavin from *Ashbya gossypii*, and beta-carotene from *Blakeslea* *trispora*. The successful marketing of pigments derived from algae or plant extracts, both as food colorants and nutritional supplements, demonstrates their value in global markets, where consumers are willing to pay a premium amount for products containing all natural ingredients.[7, 29, 36, 61] Ambati,[72] provided an extensive review on astaxanthin, highlighting its dual role as a carotenoid and vitamin source for commercially farmed sea bream. Major sources of this carotenoid include krill, shrimp, crab, crawfish, the yeast *Phaffia rhodozyma*, and chemically synthesized astaxanthin. It also emphasized on the profitability of pigment extraction from fish waste. Carotenoids not only enhance pigmentation but also play crucial biological roles in supporting growth and reproduction.[48] For example, dietary carotenoids have been shown to increase the weight of Atlantic salmon fish.[73] They also contribute to vital processes such as fertilization, respiration, photoreactivity, and stress protection (including resistance to elevated temperatures and ammonia) and serve as an essential source of vitamin A.[48, 72, 73]

## CONCLUSION

Natural pigments represent nature's beauty and are promising bio resources with multifunctional applications. Through interdisciplinary studies in biology and chemistry, the application of microbial pigments can not only lead to industrial advancements but also contribute to sustainable, health driven industrial solutions. Advancements in microbial fermentation, genetic engineering, metabolic pathway optimization, and process scalability are important areas that presently require improvements to further enhance pigment yields, stability, and cost-effectiveness. At the same time, a deeper understanding of pigment biosynthesis mechanisms, environmental interactions, and safety profiles is required. These steps will further ensure successful commercial adoption and regulatory approval.

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