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Review Article

Harnessing Industrial Potential of Microbial Pigments through Screening, Optimization and Characterization

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ABSTRACT

Microbial pigments are promising natural alternatives to synthetic dyes. The properties of natural pigments, such as bioactivity, biodegradability and broad industrial applicability in food, pharmaceuticals, textiles, and cosmetic sectors, have led to their increased demand over the years. Despite these advantages, only a few microbial pigments are commercially produced for industrial use. The primary barriers include issues of poor color stability, reduced vibrancy, and relatively low yield compared to synthetic pigments. However, compared to the negative environmental impact of synthetic dyes, these challenges are relatively minor and can be addressed with relevant research initiatives. Recent advancements in bioprocessing, fermentation technologies, and structural modification of pigments have already provided avenues to enhance pigment yield and stability. This review aims to present a comprehensive overview of the current literature in microbial pigment production. It emphasizes the critical need for continued exploration and screening of novel pigment-producing strains from diverse and extreme environments. This review especially focuses on the selection and refinement of media components, including the adoption of low-cost raw materials and solid-state fermentation techniques. These approaches are vital for economic scalability. Overall, this review highlights the progress made in microbial pigment production from natural isolates. This information will serve as a valuable resource for researchers aiming to isolate high-yielding and optimise the production of stable and commercially viable pigments, while following the global sustainability goals.

Keywords: Pigment, Dye, Optimise, Sustainable, High-yielding strain.

INTRODUCTION

Pigments contain chromophore groups that absorb light in the visible spectrum and impart distinct colours to compounds. These compounds have been used since ancient times to enhance the aesthetic appeal and acceptability of finished products.[1] Synthetic pigments, especially those derived from heavy metals such as lead chromate and copper sulphate, are associated with serious health risks such as cancer, respiratory irritation, allergies and metabolic disorders.[2,3] Moreover, their structural stability allows their persistence in nature, resulting in long-term ecological impact.[4] As a result, there is a shift in interest towards natural pigments that are derived from plants and microorganisms. These naturally occurring compounds are secondary metabolites which are safer, non-toxic and biodegradable alternatives to synthetic pigments.[5] These properties also make them ideal for application in food, pharmaceuticals, and cosmetics.[6]

The pigments derived from fruits, vegetables, seeds and roots hold significant value in terms of sustainability. However, their practical application is constrained by several limitations such as seasonal availability, susceptibility to degradation, low stability and poor solubility in different solvents.[7] At the same time, large-scale cultivation is required for pigment extraction, which may compete with food production.[8] Hence, plant pigments present both logistical and economic challenges. Though these limitations can be overcome by sourcing agricultural wastes, it will still limit the diversity of extracted pigments to a few colors.

Microbial pigments offer a potentially diverse color range which can be efficiently scaled up based on market demand.[6] Microorganisms such as bacteria, fungi, actinomycetes and yeasts can be cultivated in a controlled bioreactor, and hence their growth is independent of seasonal or climatic variations.[5] Many researchers have demonstrated the use of low-cost substrates, including agro-industrial waste, to cultivate pigment-producing bacteria; thus making the process economical and sustainable. Additionally, microorganisms make processes such as metabolic engineering and genetic manipulation possible, to enhance pigment yield, stability and functionality.[5,6] These factors, along with increasing consumer demand for natural and safe ingredients, have led to a surge in interest in microbial pigments.

Despite these benefits, microbial pigments presently face significant challenges in achieving color stability and vibrancy comparable to synthetic dyes. Hence, there is a need for more exploratory research on the screening of pigment-producing microbial strains from diverse habitats and the optimization of pigment yield.

This review provides an overview of the existing research on pigment production, fermentation parameters and metabolic engineering strategies. It also discusses the screening techniques reported for isolating high-yielding pigment-producing strains.

Screening and Isolation of Pigment Producers

In nature, pigments are produced in response to stress and provide competitive advantages in an ecological niche. Consequently, a wide range of natural environments has been explored by researchers for the screening and isolation of pigment-producing microorganisms. Also, pigment-producing organisms are known to exist in various types of symbiotic associations. For instance, Keneni and Gupta[9] isolated a red pigment-producing endophytic bacterium from the root nodules of Faba beans (*Vicia faba* L.). Gondinho and Bhosle[10] isolated an orange-pigmented alkaliphilic organism from the coastal sand dunes using polypeptone, yeast extract glucose broth. These organisms are prevalent in the rhizospheres of the sand dune creepers of *Ipomoea*, and hence, this site was selected for the study. In another study, Espinoza-Hernández *et al*.[11] isolated two strains of pigmented fungi from oak leaves (*Quercus* spp.) and one strain from the creosote bush (*Larrea tridentata*) in the Mexican semi-desert, on malt extract agar, potato dextrose agar, and Czapek-Dox medium, respectively. This study was the first to report pigmented *Penicillium* species from the Mexican semi-desert. Dharmik and Gomashe[12] isolated a *Streptomyces* strain producing a dark brown and diffusible pigment on starch casein nitrate agar and standard *Streptomyces* isolation agar. Screening was performed from botanical soil samples in Nagpur, India, using the serial dilution and standard plate technique. The isolates appeared chalky and tested positive in media with L-tyrosine substrates, indicating melanin production.

Schloss *et al*.[13] studied microbial communities in non-permafrost cold Alaskan soil using culture-dependent and culture-independent methods. They analysed the phylogeny and functional properties of over 1,000 isolates. During the screening, the study also reported a non-typical, red-pigmented prodigiosin-producing strain of *Janthinobacterium lividum* and a violacein-producing strain BP01. This was the first study reporting prodigiosin production among β-Proteobacteriain unexplored environments. Similarly, two rare strains of blue pigment-producing bacteria were isolated by Cardona *et al*.[14] from municipal water sources in Puerto Rico. One of these strains was isolated from the south (BPBW, accession HM236169) and another from the southwest (BPB-SW, accession HM236170) regions of Puerto Rico.

Ahmad *et al*.[15] collected 16 samples from diverse acidic environments of pH ranging between 5 and 7. These sites included brackish water, oil refineries, and aquaculture facilities in Port Dickson, Malaysia. The collected samples included the waste liquid from fish rearing, rotifer and fish breeding and organic waste collectors. The solid samples from shrimp ponds were also collected. The isolated organisms produced red, orange, yellow and grey violet pigments. Yokoyama *et al*.[16] isolated an orange-red pigment-producing *Flavobacterium* strain PC6 from seawater near Palau. Their work focused on carotenoid pigments in the marine environment. Brown Seward[17] also investigated the presence of carotenoids in lake sediments from Little Round Lake in Ontario. The pigment yield by different strains was compared to that of standard carotenoid-producing *Rhodopseudomonas spheroides*.

Unagul *et al*.[18] isolated a fungal strain of *Cordyceps unilateralis* BCC 1869. This isolate produced six distinct red extracellular naphthoquinone pigments, which were pathogenic to insects. Jagannathan *et al*.[19] isolated a red pigmented psychrotrophic bacterium, *Micrococcus roseus* MTCC 678, from soil samples at Schirmacher Oasis, Antarctica, and a psychrotrophic strain of *J. lividum* from cold Alaskan soil, specifically from the Tanana River's Bonanza Creek area. Palanichamy *et al*.[20] isolated pink pigmented facultative methylotrophic bacteria from the phyllosphere of Malvaceae family plants (*Hibiscus rosa-sinensis*, *Gossypium* and *Abelmoschus esculentus*) in Thorapadi, Vellore. These *Methylobacterium* species utilize methanol from plant leaves and produce carotenoids that protect them from light and radiation. Franks *et al*.[21] studied the marine environment and highlighted it as a potential source of novel compounds, including a yellow tambjamine pigment from *Pseudoalteromonas tunicata*, which is commonly found on submerged surfaces. These bacteria are typically found associated with living and inert surfaces in the marine environment. Vidyalakshmi *et al*.[22] used rice as a fermentation substrate to isolate Monascus molds, which produce edible pigments that can be used in food and feed. This method offered a cost-effective production approach. Natori *et al*.[23] used wheat grains from Minamikushiyama village, Japan, to isolate *Penicillium purpurogenum* NHL6124 for the production of rubratoxin B.

Gunasekaran *et al*.[24] described the methods used for isolating *Penicillium* sp., which produce red extracellular pigments, from the Western Ghats region of Maharashtra. Takahashi and Carvalho[25] isolated three xerophilic strains of *Penicillium* producing red pigments from the Mexican semi-desert using potato dextrose medium. One of the strains was identified as *P. purpurogenum* GH2. Sundaramoorthy *et al*.[26] collected soil samples from Yercaud hills and screened them for the presence of *Serratia marcescens*- a red pigmented bacterium. Latha *et al*.[27] isolated a red pigmented yeast strain *Rhodotorula glutinis* DFRPDY, using the particle sedimentation method.

Overall, these studies demonstrate the wide diversity of pigment-producing microbial strains that can be isolated from varied habitats.

Identification of the Cultures

Many studies in the past reported using standard biochemical methods for the identification of isolates in the laboratory. However, many a time, species confirmation can be challenging with standard biochemical tests. This is because microorganisms adapt to conditions prevalent in their habitat and can show atypical biochemical characteristics.[28] Hence, most recent studies rely on 16S rRNA gene sequencing techniques for culture identification. Vladimir *et al*.[29] identified *Roseococcus thiosulfatophilus*, *Erythromicrobium ramosum*, and strain T4T with the help of the 16S rRNA gene sequencing technique. The selected sequences were 1426, 1115, and 1114 nucleotides long, respectively. These were aligned with small subunit rRNA sequences from the alpha subclass of Proteobacteria using a sequence database for comparative analysis. Arai *et al*.,[30] on the other hand, extracted genomic DNA from fungal mycelium for sequencing, using the shotgun sequencing method. The 454 sequencing reads were assembled into contigs and converted into a BLAST database for local searches. Gene predictions were verified manually by comparing them with homologous genes and proteins in the GenBank database. Functional domains in the translated protein

sequences were predicted using the Conserved Domain Search tool on the NCBI site.

Yilmaz *et al*.[31] introduced a revised fungal classification system using a polyphasic approach. This method considered macro- as well as micromorphology, extrolite production, and multigene phylogeny characteristics for grouping fungal isolates. The phylogenetic relationships were studied using internal transcribed spacer barcodes. The macroscopic characteristics of isolates were observed on various media, including Czapek yeast extract agar with and without 5% NaCl, yeast extract sucrose agar, creatine sucrose agar, dichloran 18% glycerol agar, oatmeal agar and malt extract agar. They evaluated colony characteristics such as diameter, sporulation, color, colony morphology (obverse and reverse) and soluble pigment production after incubation to classify strains. The extrolites were collected by growing the organisms on Czapek yeast extract and malt extract agar plates, and were analysed by high-performance liquid chromatography with diode array detection (HPLC-DAD) and Ultra-HPLC-DAD. The compounds were eluted and detected by comparing retention time, retention index and UV spectra between 200 to 400 nm. The obtained UV spectra were compared to a standard UV spectra database. The ITS and the regions of beta tubulin, calmodulin and RPB1 and RPB2 genes were amplified and sequenced. The ITS sequences were used to study the phylogenetic relationships of the culture.

Cardona *et al*.[14] used a combination of biochemical, genetic, and molecular techniques to identify a blue-pigmented strain isolated in Puerto Rico. The preliminary identification was done based on Gram staining and colony morphology characteristics. The biochemical tests confirmed the isolate as *Vogesella indigofera*. Molecular identification was performed using PCR with universal bacterial primers Oligo 14-F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492-R (5'-GGTTACCTTGTTAGGACTT-3'). The results revealed 97% similarity with *V. indigofera*. Espinoza-Hernández *et al*. [11] also identified three xerophilic fungal strains using morphological, biochemical, as well as molecular techniques. Molecular tools included polymerase chain reaction, restriction fragment length polymorphism, random amplified polymorphic DNA, internal transcribed spacer and intergenic spacer analyses. The strains were identified as *P. purpurogenum* (GH2) and *P. pinophilum* (EH2 and EH3). Similarly, Keneni and Gupta[9] used a combination of methods for bacterial identification. They used phase contrast and scanning electron microscopy for detailed morphological analysis. Biochemical identification was done based on protocols described in Bergey's Manual of Determinative Bacteriology. Molecular identification was carried out by PCR amplification using 16S rRNA-specific primers; PigB16S-F (5'-CGGCAGGCTTAACACATGCA-3') and PigB16S-R (5'-TCTACGAATTTCACCTCTACACT-3'). On similar lines, Gondinho and Bhosle (2008) confirmed their isolated strain to be *Microbacterium arborescens* AGSB.

Measurement of Pigment Yield

For measurement of pigment yield, measuring wavelength and turbidity using a spectrophotometer is the standard method. Stahly *et al*.,[32] in their detailed study, suggested appropriate wavelengths for measuring diverse microbial pigments and turbidity. Their studies included sensitive organisms as well as normal soil flora such as *Chromobacterium violaceum*, *Flavobacterium suaveolens*, *F. arborescens*, *Serratia marcescens*, and *S. aureus*. The study specified corresponding wavelengths for both pigment absorption and turbidity assessments. For red pigment produced by *Monascus* sp., Carvalho *et al*.[33] measured pigment levels using a spectrophotometer at 500 nm. Absorbance values were converted into specific absorbance units (AU/g) by accounting for fungal dry mass. This method allowed pigment quantification relative to biomass. It also enabled comparative analysis across varying conditions. Takahashi and Carvalho[25] adopted the same approach to measure red pigment from *Monascus purpureus*. Carvalho *et al*.[34] further refined the method by expressing pigment levels in specific absorbance units (AU/g). It was calculated by multiplying absorbance by the dilution factor and dividing by the dry mass of the fermentation substrate. This standardization enabled consistent comparisons across different fermentation setups.

Vidyalakshmi *et al*.[22] extracted pigments from *Monascus* sp. using chloroform:ethanol:water mixture (65:25:4). Absorbance was measured at 510 and 410 nm to quantify red and yellow pigments, respectively. Cho *et al*.[35] employed a standard curve based on previously isolated red pigment from *Paecilomyces inclarii* to measure pigment concentration under various optimization conditions. Absorbance was recorded at 500 nm to estimate unknown pigment concentrations. Gunasekaran *et al*.[24] quantified red extracellular pigments by measuring the absorbance of culture filtrate at 530 nm using a spectrophotometer. Mycelial biomass was separated through centrifugation, filtered and dried at 50°C for 48 hours to determine dry weight. Kim *et al*.[36] monitored both cell growth and pigment production. The optical density of the broth was recorded at 600 nm in their study. Pigments attached to the mycelia were extracted using acidified methanol and absorbance was measured at 535 nm. Pigment concentration was calculated using a standard curve from purified pigment. Sundaramoorthy *et al*.[26] quantified prodigiosin pigment extracted from *Serratia marcescens* by measuring absorbance at 534 nm. Pigment concentrations in their study were also estimated based on a standard curve created using known prodigiosin concentrations. Unagul *et al*.[18] filtered red pigments produced by *Cordyceps unilateralis* BCC 1869, using Whatman No. 1 paper. The filtrate was analyzed for pigment concentration at 500 nm using a UV-Vis spectrophotometer. The total naphthoquinone content was calculated using the formula:

$$A = \varepsilon \times L \times C$$

Where:

A = absorbance at 500 nm; L = path length of the cuvette (1 cm); ε = average molar absorptivity of total naphthoquinones (6456 L/mol/cm²) and C = concentration of pigment (mol/L)

Selection and Optimisation of Media Parameters for Pigment Production

Cho *et al*.[35] optimized parameters such as carbon and nitrogen sources, pH and inoculum growth phase for pigment production by *Paecilomyces sinclairii*. Batch fermentation under optimized conditions showed promising results and yielded 44 g/L of pigment. They also observed that increasing the pH from acidic to alkaline altered the pigment color from red to violet. This characteristicis similar to anthocyanin pigments. Goswami *et al*.[37] examined the effect of

media type, pH and temperature on a yellowpigmented Gram positive bacterium. Optimum pigment production was observed at pH 7 and 30°C, highlighting the influence of both physical and chemical parameters. Choi *et al*.[38] studied a novelpsychrotrophic strain RT102, which was closely related to *J. lividum*. It produced a mixture of violacein and deoxyviolacein. Optimal pigment production was achieved at pH 6, 20°C, and in media with 1-mg/L dissolved oxygen. The obtained yield was 3.7 g/L of pigment. Peptoneglycerol broth, supplemented with maltose, peptone, iron salts, and inorganic phosphatesfurther enhanced pigment production. Their findings provide basic data for a study on large scale industrial production of the violet pigment. Similarly, Sundaramoorthy *et al*.[26] evaluated temperature, pH and nutrient sources for red pigment (prodigiosin) production by a soil isolate. Unagul *et al*.[18] optimized cultivation conditions for red naphthoquinone pigments from *C. unilateralis* BCC 1869. The maximum yield of naphthoquinone was 3 g/L. It was obtained from a 28 day culture grown in potato dextrose broth adjusted to pH of 7 and incubated ata temperature of 28°C with shakingconditionsset at 200 rpm. Kim *et al*.[36] implemented an integrated fermentationseparation process using polymeric adsorbent resin (HP20) for *S. marcescens*. Maximum prodigiosin yield (6.92 g/L) was achieved at 10% resin concentration. This yield was 31% more compared to that obtained from standard batch fermentation.

Tallapragada *et al*.[39] used *M. purpureus* to optimize pigment production in solidstate fermentation.The effect of growth pattern, pH and temperature were investigated on pigment production. The maximum yield was obtained on 16th day at a temperature of 30°C and pH 5.5. The study was primarily carried out to optimize the carbon and nitrogen sources for solid state fermentation. The substrates such as *Oryza* spp. (local unpolished rice), *Fagopyrum* spp. (Buckwheat) flour, *Colocasia* spp. (arbi), and *Manihot* spp. (tapioca) were used in the study. Palanichamy *et al*.[20] optimized pigment production by a methylotrophicbacterium isolated from the phyllosphere. The isolate produced pink color pigments. It was cultivated in the presence of different carbon sources (0.5%) such as methanol, dichloromethane, formaldehyde, glucose and fructose.Optimum production was achieved at pH 7.5 and 25°C using 0.5% methanol as the carbon source in ammonium mineral salt broth. Kumar *et al*.[40] summarized optimal media parameters for various pigment producing microorganisms. They reported that *Monascus* species require temperature between 25 and 28°C and pH between 5.5 and 6.5, while *Pseudomonas* prefers 35–36°C. Neutral to slightly alkaline pH promotes lycopene production, while acidic pH favors β-carotene production in *Pseudomonas* sp. In solid media, *Monascus* tolerated higher zinc concentrations and showed improved pigment yields compared to the yield obtained from submerged fermentation. Also, ammonium chloride, ammonium nitrate and glutamate supportedpigment production whereas potassium nitrate inhibited the same. Higher levels of pigment production by *M. ruber* was observed when 70% initial moisture level was maintained in substrate (rice). Hence, the bed of rice was continuously aerated by sparging with humidified air (95–97% relative humidity). Krairak *et al*.[41] extracted yellow pigment from *Monascus* in a 5 L fermenter with media composed of glucose, peptone, malt and yeast extract. Optimal conditions were 600 rpm agitation, 0.75 rpm aeration, 28°C and pH 6.5.

Gunasekaran *et al*.[24] optimized red pigment production by *Penicillium* under varying conditions of pH (3–9), temperatures (15–35°C) and C/N ratios. Maximum pigment yield occurred at pH 9.0, 30°C, and a C/N ratio of 1:1 using starchpeptone media with 200 rpm agitation.Their results indicated that biomass and pigment production was slightly affected by initial pH of the medium. The highest biomass and pigment production was observed when initial pH of culture medium was set at pH 9.0. Takahashi and Carvalho[25] studied the effect of combined pH and temperature on red pigment of *P. purpurogenum* GH2. The pigment production started at 120 h and the maximum yield was obtained in 240 hours. The optimum parameters were pH 5 and 24°C, followed by pH 9 and 34°C. They also noted the morphological and metabolic differences among *Monascus* and *Penicillium* strains. Their results suggested that pH and temperature can affect the activities of enzymes involved in the biosynthesis of pigments, but this effect depends on the characteristics of microbial strains.

Mekhael *et al*.[42] optimized *Serratia marcescens* for prodigiosin production. Peanut seed culture broth yielded the highest pigment yield of 255.21 units/cell. Optimal growth conditions were 28°C, pH 8 and a 72 hours incubation period with good aeration. Latha *et al*.[27] cultivated *Rhodotorula glutinis* DFR-PDY in a 10 L fermenter with modified CzapekDox medium. Fermentation occurred at 29–32°C for 48 hours under aeration (0.6–1.0 rpm) and agitation (400 rpm), with 10–50% dissolved oxygen maintained in the broth. Gondinho and Bhosle[10] optimized pigment production by *Microbacterium arborescens* AGSB using polypeptone yeast extract glucose broth (PPYG) at pH 10.5 and 28°C. Cultures were incubated on a rotary shaker at 160 rpm. Yamashita *et al*.[43] formulated a PG medium with 0.5% bactopeptone and 1% glycerol for *S. marcescens*, allowing separation of prodigiosin and serrawettin. The study also suggested that silica gel addition significantly supports cell growth.

Espinoza-Hernández *et al*.[11] used a Plan Puebla II matrix to optimize pH and temperature for three *Penicillium* strains. Fermentations were carried out at pH values from 4 to 10 and temperatures from 8 to 32°C using agar-malt extract (AME) medium .Dharmik and Gomashe[12] optimized melanin production by *Streptomyces* using ISP2 broth and solid-state fermentation on wheat and rice bran. Lian *et al*.[44] designed a multi-step fermentation strategy for *M. ruber* 102W involving various media formulations. Fermentations were carried out at 32°C with sequential cultivation in seed and production media. The media was composed of malt extract (5 g), yeast extract (3 g), glucose (5 g), and agar in 1L of ultrapure water. The initial pH of the medium was 6.0. The seeding culture medium contained rice powder (40 g), KH_2PO_4(2.5 g), $NaNO_3$(3 g), $MgSO_4.7H_2O$ (4g) in 1L of deionized tap water. The initial pH of the medium was adjusted to 4.5 with lactic acid. The fermentation medium contained rice powder (80 g) and hydrolytic bean powder (20 g) in 1 L of deionised tap water. The initial pH of the medium was adjusted to 3.5 with lactic acid. Arai *et al*.[30] selected a buffered medium (pH 5.0) for *Penicillium* strain TA85S-28-H2, containing glucose, malt extract, yeast extract and $MgSO_4$. Bulk production involved transferring seed cultures into a 5 L fermenter under dissolved oxygen levels of 2.5 ppm at 30°C. Ogihara *et al*.[45] and Ogihara *et al*.[46] studied pigment production by *Penicillium* sp. AZ. Though no pigmentation was observed during early cultivation,

subsequent modifications including addition of different nitrogen sources (yeast extract and tryptone) enhanced pigment production in citric acid buffer at pH 5.0 and 30°C.

The Use of Cheap Raw Materials and Solid-State Fermentation in Pigment Production

Solidstate fermentation (SSF) has been widely explored for pigment production using agroindustrial residues. Apple pomace, a byproduct constituting approximately 25–35% of apple juice processing waste, has been effectively utilized for microbial pigment production. In addition to pigments, pomace has also served as a substrate for ethanol and enzyme production.[47] Ahmad *et al*.[15] investigated the production of natural bacterial pigments such as prodigiosin from *S. marcescens* and violacein from *Chromobacterium violaceum*, using both solid and liquid pineapple waste. These pigments were successfully applied in textile dyeing, showcasing their practical industrial utility. Vidyalakshmi *et al*.[22] employed SSF to obtain red, orange and yellow pigments from *Monascusruber* MTCC 2326. These pigments were characterized and used in flavored milk preparation. The study emphasized the need for natural pigments, considering the potential carcinogenic effects associated with synthetic dyes. Tauk Tornisielo *et al*.[48] evaluated xylanase production in SSF using wheat bran as the substrate. Fungal isolates including *P. citrinum*, *P. fellutanum*, *P. rugulosum*, and *P. decumbens*, sourced from Brazilian soils, were tested in their study. Among these, *Trichoderma* spp. proved to be the most efficient xylanase producer, though all isolates were able to grow on wheat bran.The research was undertaken since there is a lot of demand for xylanases in many fields. Tallapragadaand Venkatesh[49] optimized xylanase production by *Aspergillus niger* using oat spelt xylan as the carbon source. Various parameters such as temperature, carbon and nitrogen sources, aeration, and agitation were systematically studied to enhance enzyme yield.

Gordillo *et al*.[50] reported the secretion of three acetyl xylanesterases (AXE I, II, and III) by *P. purpurogenum*, and also studied other xylanase-producing organisms like *Bacillus pumilus* and *Simplicillium* spp. Ravanal *et al*.[51] explored the catalytic role and 3D structure of xylanases, identifying a novel bifunctional enzyme, ABF3 which is an L-arabinofuranosidase/ xylo-biohydrolase obtained from *P. purpurogenum*. This was the first report from this fungal species. Its novelty was confirmed with the help of amino acid sequence and catalytic behavior. Steiner *et al*.[52] enhanced xylanase production in *P. purpurogenum* through UV and nitrosoguanidine induced mutagenesis. Mutants grown on oat spelt xylan and wheat bran exhibited elevated xylanase and β-xylosidase activities, showing promise for industrial applications.

Extraction Methods

Many methods have been used for the extraction of the pigments. Most of these, however, depend on solvent extraction processes. Latha *et al*.[27] studied *R. glutinis* DFR-PDY for carotenoid production. Cells were hydrolyzed in 1N HCl at 70°C, washed, and then extracted in acetone. The acetone extract was partitioned into petroleum ether, washed with distilled water, and analyzed spectrophotometrically. Carvalho *et al*.[33] compared solvents for extracting *Monascus* pigments and found that methanol was most effective, followed by DMSO and ethanol. Due to its low cost and safety, ethanol was selected for largescale extraction of pigments. They recommended a solvent tosubstrate ratio of 3:1, which improved efficiency over the previously used 5:1 ratio.For violacein extraction from *C. violaceum*, pigments were first recovered using ethyl acetate, followed by evaporation under reduced pressure or filtration. The dried extract was resuspended in alcohol. In another protocol, cell pellets were washed, resuspended in ethanol, and subjected to ultrasonication until completely bleached.[53]

Cho *et al*.[35] extracted red pigment from *P. sinclairii* by acidifying the culture filtrate to pH 3 with 2N HCl and recovering it with ethyl acetate. The solvent was evaporated under vacuum at 50°C. The pigment residue was dissolved in water, filtered, dried at 50°C, then re-dissolved in ethanol and filtered again. Crystallization was achieved using a chloroform:heptane (2:1 v/v) mixture. Rettori and Duran[54] extracted violacein from *C. violaceum* using ethanol, followed by soxhlet extraction with chloroform, diethyl ether, and ethanol. Crystallization was performed with methanol and water, under vacuum. HPLC was carried out to separate pigment fractions for further characterization.

Purification of Pigments

Pigments are typically composed of multiple subfractions. Hence, purification is a crucial step for the characterization of pigments and studying their potential applications. A wide range of techniques have been employed for pigment purification, with most methods relying on chromatographic separation of pigments extracted in solvents.

Latha *et al*.[27] extracted red pigments from *R. glutinis* DFR-PDY using acetone, followed by partitioning them in light petroleum. The crude extract was purified through column chromatography and further resolved by thin-layer chromatography (TLC). The results revealed the presence of three distinct fractions of yellow, orange, and red. These were further analyzed by HPLC and subjected to FTIR and NMR spectroscopy for structural elucidation. Vidyalakshmi *et al*.[22] purified food-grade pigments from *Monascus* grown on broken rice. A silica gel column (60–120 mesh) was eluted with chloroform:ethanol (9:1 v/v), and fractions were further purified using preparative TLC (chloroform:ethanol:water, 65:24:4). High-performance thin-layer chromatography (HPTLC) with benzene:methanol:chloroform (30:10:9) and gas chromatography mass spethinlayer chromatographytrosthinlayer chromatography analysis revealed the presence of compounds such as dehydromevalonic lactone, 5-hydroxymethylfurfural, palmitic acid, and oleic acid. Ahmad *et al*.[15] performed preliminary purification of violacein from *Chromobacterium violaceum* using TLC with n-hexane:ethyl acetate (4:6 v/v) as the solvent system. Silica gel column chromatography using methanol as the eluent allowed the separation of multiple pigment fractions, ranging from dark violet to bright pink, and effectively removed dark brown impurities. Dharmik and Gomashe[12] vacuum dried pigment obtained from actinomycetes, which were extracted using ethyl acetate. TLC was performed on silica gel plates with varying solvent systems including methanol, hexane, chloroform, and acetic acid. The optimal separation was achieved using n-hexane:chloroform (50:50) solvent system, which yielded three distinct spots under UV light, with the pigment at the center.

Kim *et al*.[36] extracted red pigments from *Monascus* cells using 70% ethanol. The cells were shaken at 180 rpm for 2 hours in a

water bath, followed by centrifugation at 10,000 rpm. Pigment concentrations were quantified using spectrophotometry at 510 nm. Ogihara[45] purified red pigment PP-R from *Penicillium* by extracting mycelia with methanol, followed by silica gel column and Sephadex LH-20 chromatography. Ogihara used another modified method for extracting the violet pigment (PP-V) of *Penicillium* AZ, where the culture broth was filtered and the mycelia obtained were washed twice with 50 mM citric acid/ sodium citrate buffer, followed by the addition of sodium acetate four times at an interval of 12 hours.[46]

Properties of the Pigments

Ahmad *et al*.[15] performed comprehensive characterization of violacein pigment extracted from *C. violaceum*. Spectral analysis methods used included UV–visible spectroscopy, FTIR, and ^{1}H-NMR. UV/Visible analysis (200–800 nm scan) of the crude violet pigment showed maximum absorption peaks at 623.67 nm for pH 5.4, 576.61 nm (pH 6.1), 589.81 nm (pH 6.4) and 573.17 nm (pH 3, 7, 9 and 13). FTIR analysis was performed on dried pigment (0.2–1%) using KBr pellets. Spectral bands indicated O–H and N–H stretching, consistent with secondary amides. The observed trans- and cis-amide bands suggested hydrogen bonding in dimers or polymeric forms, along with C=O and N–H bending vibrations. There were weak bonds seen in the sample, which were attributed to trans secondary amide or C=O stretching and N-H in-plane bending (cis secondary amide). The ^{1}H NMR spectrum revealed 13 protons, with distinct singlets corresponding to indole NH (δ 11.87 ppm) and lactam NH (δ 10.72 ppm). Aromatic protons in the indole skeleton were observed at δ 6.79, 7.23, and 7.35 ppm, while additional peaks at δ 8.06 and 9.32 ppm indicated hydroxyl and other functional groups.

Fang Shi-Ming *et al*.[55] compared wild and DES mutagenized strains of *P. purpurogenum* G59. Ethyl acetate extracts from mutant strain BD-1-6 revealed three unique HPLC peaks, which were absent in the wild type strain, suggesting novel metabolites. Bioassay-guided fractionation resulted in the isolation of purpurogemutantin and purpurogemutantidin, which were structurally characterized using spectroscopy. The study by Gonçalves *et al*.[56] was among the first studies to characterize melanin pigments from *Aspergillus niger*. They used mutant strains MEL-1 and MEL-2. The pigment melanin is not degraded in concentrated acids; it is insoluble in water and organic solvents, soluble in alkali solutions and tests positive for polyphenols. The pigment also gives the indole test positive. CHNS-O elemental analysis confirmed high nitrogen content, indicative of DOP Amelanin. Spectroscopic analyses (UV–Vis, ESR, and IR) confirmed structural similarity to synthetic DOP Amelanin. Harki *et al*.[57] studied black pigments from *Tuber melanosporum* and reported similar insolubility and bleaching properties. Positive polyphenol tests and UV–IR spectral analysis confirmed structural resemblance to DOP Amelanin. Suryanarayanan *et al*.[58] investigated pigment from *Phyllosticta capitalensis*, which resisted extraction by common organic solvents. The pigment showed positive reactions in standard fungal melanin tests, including brown precipitate with $FeCl_3$ and gray with $AgNO_3$. The pigment exhibited typical melanin-like absorption, with strong UV absorption that decreased linearly with increasing wavelength.

CONCLUSION

Microbial pigments have immense potential as sustainable and safe alternatives to synthetic dyes. However, in order to bridge the gap between laboratory production and industrial application, certain challenges need to be addressed. This review reports ample evidence of studies on screening and identification of pigment producers, media optimization, and cost-effective fermentation techniques. Advanced extraction and purification methods, along with metabolic engineering techniques, may further enhance pigment properties and production efficiency. Hence, while substantial progress has been made, more in-depth research is needed, particularly in the field of biochemical engineering and materials science, to unlock the full potential of natural pigments and improve their colour efficiency and stability.

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