I J R B A T, Issue (XIII) Volume (I) Jan 2025: 127-132

A Double-Blind Peer Reviewed & Refereed Journal

e-ISSN 2347 – 517X

Review Article

INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY

EXPLORING THE ANTIMICROBIAL EFFICACY OF PIGMENTS PRODUCED BY BACTERIA OBTAINED FROM SOIL SAMPLE

Pranjali Hedau and Charu Sukheja
Sevadal Mahila Mahavidyalaya, Nagpur, INDIA
Corresponding Email: - hedaupranjali@gmail.com

Communicated :19.11.2024 Revision: 23.11.2024 & 19.12.2024 Accepted: 27.12.2024 Published: 30.01.2025

ABSTRACT:
Microorganisms serve as a valuable source of pigments with distinct properties and wide-ranging applications, and recent studies have revealed new opportunities for their industrial use. In this research, different pigmented bacteria were isolated from various samples. The isolates were identified as *Serratia, Micrococcus, Erythrobacter, Azorhizobium, Xanthomonas sp., Sarcina sp., Rhodotorula sp., Roseomonas gilardii, K. turfanensis, Cytophaga spp., Micrococcus luteus, Micrococcus roseus, and Pseudomonas aeruginosa.* The growth of these pigment-producing bacteria was influenced by factors such as temperature, pH, and salt concentration. Pigments were extracted using solvents like chloroform, methanol, and ethanol. The extracted pigments were characterized through TLC and UV spectrophotometry, with most of them identified as carotenoids. These pigments demonstrated antimicrobial properties against human pathogens, making them potentially useful for various applications in industries such as textiles, cosmetics, food, and pharmaceuticals, etc.

Keywords: - Pigments, TLC, UV spectrophotometer, carotenoids, antimicrobial activities, applications.

INTRODUCTION :

Pigments derived from various natural sources around the world are gaining increasing recognition (Sinha et al., 2017). These natural pigments can be extracted from ores, insects, plants, animals, and microorganisms. Specifically, bacteria that produce pigments are known as *chromogenic bacteria* (Chaturwedi et al., 2024). Microorganisms are capable of producing a variety of pigments, including carotenoids, melanins, flavins, monascins, violacein, and indigo. Among these, carotenoids are the most commonly observed and extensively studied pigments (Samyuktha et al., 2016). Natural pigments possess key characteristics such as stability to light, heat, and ph variations. Since these pigments are sourced from plants and animals, they are considered safe for use, being non-toxic, non-carcinogenic, and biodegradable. Microbial pigments, in particular, are advantageous due to their rapid growth in inexpensive culture media, independence from environmental conditions, and the wide range of color variations they offer. These pigments also have important functions, such as protecting microbial cells from the damaging effects of visible and near-ultraviolet light. Often classified as secondary metabolites, these pigments are synthesized by various microorganisms under specific conditions (Sinha et al., 2017).

Some bacteria produce water-soluble pigments that spread throughout the growth medium, while others produce pigments that are soluble in fats. The study and application of microbial pigments has been extensively explored by researchers, making it a broad area of scientific inquiry (Siddharthan et al., 2020). Microorganisms are highly versatile in biotechnology, capable of producing a wide range of molecules such as enzymes, antibiotics, organic acids, and pigments. Recent research highlights that microorganisms are a valuable source of natural colors (Selvi et al., 2018). The industrial production of natural food colorants through microbial fermentation offers several advantages, including more cost-effective production, simpler extraction processes, increased yields through process optimization, and the elimination of raw material shortages or seasonal variations. Microbial pigments are utilized across various industries, including food, textiles, paper, and agriculture. Unlike certain synthetic dyes, whose manufacture is restricted due to carcinogenic risks and environmental disposal concerns, natural pigments not only enhance the market appeal of products but also offer additional health benefits, such as antioxidant and anticancer properties (Priyadharshini et al., 2021).

Using agricultural residues as substrates for pigment production can help reduce costs, making the process more economically viable. Microbial pigments can be extracted via solvent extraction and further characterized using various instrumental techniques such as NMR, GC-MS, TLC, UV-Vis, FTIR, HPLC, and gel permeation chromatography. Microbes generally produce two types of pigments: those that remain attached to the microbial cells and those that are released into the fermentation broth. Pigments that remain bound to the microbial cells can be recovered by filtering the mycelia with acetone, while secreted pigments are typically extracted from the broth using organic solvents like ethyl acetate (Priyadharshini et al., 2021).

Extracting and characterizing pigments from pigmented bacteria is generally easier than from non-pigmented strains, which are more challenging and time-consuming to identify and analyze. Furthermore, the collection of microorganisms is sustainable and does not harm the environment. The search for new antimicrobial agents from natural resources is particularly crucial in developing countries, where antibiotic resistance is a growing concern. In this context, bacterial pigments, as secondary metabolites, hold promise as potential antimicrobial agents (Chaturwedi et al., 2024). Therefore, studying microbial pigments is essential for discovering novel bacterial pigments from diverse sources and exploring their potential applications across various industries.

REVIEW OF LITERATURE :

S. Samyuktha and Sayali Naphade Mahajan,2016 studied on the Isolation and identification of pigment producing bacteria and characterization of extracted pigments in which they isolated. Three different pigmented bacteria were isolated. The production and intensity of pigment increases at low temperature(4°C). Chloroform was used as a solvent for extraction. Absorbance was taken by calorimeter indication for carotenoids. No inhibition was observed against bacteria and there was no special effect on the seed germination. These pigments were used in various industries.

Sinha S, Choubey S, Ajay Kumar A and Bhosale P, 2017 studied on Identification, Characterization of Pigment Producing Bacteria from Soil and Water and Testing of Antimicrobial Activity of Bacterial Pigments which focuses on the organisms from different geographical location and climatic conditions. Soil and water samples were collected from various locations, leading to the isolation of five pigmented microorganisms from different species, including *Serratia sp.*, *Micrococcus sp.*, *Erythrobacter sp.*, and *Azorhizobium sp.* Pigments were extracted using a solvent extraction method. These pigments exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as antifungal properties against certain fungi. As a result, these pigments have potential applications in various industries.

Megha Waghela and Shabib Khan, 2018, studied on Isolation, Characterization of Pigment Producing Bacteria from various food samples and testing of antimicrobial activity of bacterial Pigments. In this study, microorganisms were isolated from various food samples, and pigmented colonies were cultured. The pigments were extracted using a solvent extraction method, and the extracted pigments demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria.

P. Senthamil Selvi and Priya Iyer,2018, carry out the Isolation and Characterization of Pigments from Marine Soil Microorganisms. In this study, microorganisms were isolated from soil sample and characterized by various chemical tests. The pigments were extracted using solvent extraction, resulting in yellow pigment from *Xanthomonas sp.*, orange pigment from *Sarcina sp.*, and pink-red pigment from *Rhodotorula sp.* The pigments were then purified through column chromatography and identified using Thin Layer Chromatography (TLC). They were further characterized through spectrophotometry and GC/MS analysis. These pigments exhibited antimicrobial, antioxidant, and anticancer activities against the tested organisms. .They were used as a dye to dye cotton and fibres.

Minal. R. Dave and Rohini Shetty,2018, performed Screening and Extraction of Microbial Pigment from Organism Isolated from Marine Water. Which includes the isolation of as they want to isolated pigmented bacteria from water sample. That pigmented bacteria were characterised by gram staining and biochemical tests. Isolated colonies were isolated in 2% glycerol containing nutrient broth for screening. Yellow pigment was extract by extraction; absorption was observed by UV-visible Spectrophotometer. DPPH of pigment was done which shows antioxidant properties and this yellow pigment also show antibacterial and antifungal activity. So microbial pigment has various application in different industries.

Sapna Chaudhari, Mansi Mehta and Gaurav Shah,2019, studied on “Extraction of plant and microbial pigment, comparision and to check its stability”2019. Plants and microorganisms produce pigments which are secondary metabolites. The pig1607ment extracted from biological sources are called as “biocolors”. Microbial pigments are easily extracted as compared to plant pigment as plants requires extract preparation and various solvent for extraction. Different temperature and ph were provided to check the stability of both pigments. Antimicrobial activity of both extracted pigment was checked against test organism which show zone of inhibition. From this it concludes that both plants and microorganisms were good source of pigment.

Pratik P Joshi, Saikiran K Yewatkar, Ruchita R Ghumare and Trupti D Aglave studied on Isolation, Characterization of Yellow Pigments Producing Bacteria, 2019. The synthetic pigments create toxicity problem to decrease these problems natural pigments from plants and microorganisms are used. In this study yellow-coloured pigmented colonies were isolated. After gram-staining and biochemical test, organism was characterised from genus micrococcus and by TLC analysis yellow pigment was identified as carotenoid. These pigments have various application in industries.

Siddharthan N, Sandhiya R and Hemalatha N,2020, studied on Extraction and characterization of antibacterial pigment from Roseomonas Gilardii yp1 strain in yercaud soil. In this study, a soil sample was collected from a coffee plantation in Yercaud, Salem district, and a pigment-producing bacterial strain was isolated. The pigment was extracted using acidified ethanol and identified as *Roseomonas gilardii.* The pigment was characterized using ultraviolet spectroscopy, thin-layer chromatography, and Fourier transform infrared (FT-IR) analysis. It also demonstrated antibacterial activity against the tested pathogens. Based on these findings, the pigment extracted from *R. Gilardii* has potential applications in the food, textile, and cosmetic industries.

e-ISSN 2347 – 517X
Review Article

S. Priyadharshini, M. Sri Aravind Lal, A. Zibia Kasturi Gratia and M. Davamani Christober,2021 studied on Production and Characterization of Yellow Pigment from Pomegranate and its application. In this study ACPFM04 isolated from the Pomegranate produces yellow colour pigment. After gram staining it show gram negative cocci. The isolated bacteria were incubated to produce a yellow pigment. Methanol and ethanol were employed as solvents for pigment extraction. The pigment was identified using various techniques, including NMR, GC-MS, TLC, UV-Vis, FTIR, HPLC, and gel permeation chromatography. These pigments are utilized as food colorants in the food industry.

Dr. Jenitha K, 2022 performed on Isolation and identification of bacteria producing pigments from garbage dumped ground soil. In this study, pigment-producing bacteria were isolated from a garbage dumping site. The identified bacterial strains were *Serratia sp.*, *Pseudomonas sp.*, and *Staphylococcus sp.*, which produced green, yellow, and orange pigments, respectively. The pigments were extracted using a solvent extraction method. These extracted pigments show antimicrobial activity against human pathogen. These pigments also have other applications such as antibacterial, antifungal, viricidal, anti-oxidant, anticancer activities etc. Therefore, these extracted pigments were applied on the textile industry.

Shashi Bhushan Chaturwedi, Swostika Mainali and Richa Chaudhary, 2024, studied on Antibacterial activity of pigment extracted from bacteria isolated from soil samples. In this study, soil samples were collected from various locations, including forests, agricultural fields, riverbanks, and dumping sites in the Kathmandu and Lalitpur districts. Colored pigments were isolated and identified as S4O (*Staphylococcus aureus*), S11Y (*Micrococcus luteus*), S14P (*Micrococcus roseus*), and S17G (*Pseudomonas aeruginosa*). Characterization using UV-Vis spectrophotometry and TLC analysis revealed that the pigments from isolates S4O, S11Y, and S14P were carotenoids, while the pigment from isolate S17G was identified as pyocyanin. The pigments extracted from these pigmented bacteria exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria, highlighting their potential to control the growth of harmful pathogens.

RESULT :

Pigmented bacteria were isolated from soil, water and food samples. These bacteria were identified and characterized by morphological characteristics and biochemical tests (Sinha S et al., 2017). From identification isolates were identified to be *Serratia, Micrococcus, Erythrobacter* and *Azorhizobium* (Sinha S et al., 2017), *Xanthomonas sp., Sarcina sp., Rhodotorula sp.* (P. Senthamil Selvi at.al.), *Micrococcus luteus* (Minal. R. Dave at.al.), *Serratia marcescens* (Sapna Chaudhari et al., 2019), *Roseomonas gilardii* by 16S rRNA sequencing (Siddharthan N et al.,2020), *M. luteus, K. turfanensis, Cryseobacterium spp., and C. pallidum* (Rupali Koshti et al., 2019), *Staphylococcus aureus, Micrococcus luteus, Micrococcus roseus* and *Pseudomonas aeruginosa* (Shashi Bhushan Chaturwedi et al., 2024), *Micrococcus* (Pratik P Joshi et al.,2019). There were many factors like pH which affect the production of pigment at pH 2 (S. Samyuktha et al., 2016), pH7 (Minal. R. Dave et al., 2016), and pH8 (S. Priyadharshini et al., 2021) maximum pigment production was observed. At 4°C (S. Samyuktha et al., 2016), 27°C (Minal. R. Dave et al.,2017), and 30°C (S. Priyadharshini et al., 2021) maximum production were observed. The maximum pigmentation was observed at 4% (S. Samyuktha et al., 2016), and 2% (S. Priyadharshini et al., 2021) salt concentration. The pigments were extracted using solvent such as chloroform (S. Samyuktha et al., 2016), ethanol (Sinha S et al.,2017), acidified ethanol (Megha Waghela et al., 2018), and methanol (Rupali Koshti et al., 2019) (Chaturwedi, S. B. et al., 2024).

The colorimetric analysis showed that the λmax for each pigment was 440nm (Samyuktha, S. et al., 2016), UV-visible Spectrophotometer analysis of pigment was observed between the wavelength of 200-700nm. The peak optical density was at 450nm (Sinha S et al., 2017), (Siddharthan, N. et al.,2020) (Priyadharshini, S. et al., 2021), Specific pigments like pyocyanin exhibited maximum absorbance at 276 nm, while carotenoids displayed peaks at 492 nm, 470 nm, and 386 nm (Chaturwedi, S. B. et al., 2024). Thin layer chromatography analysis shows the Rf value obtained from pigments were found to be 0.99 (Selvi, P. S. et al.,2018), 0.70 (Joshi, P. P. et al.,2019), 0.82 (Siddharthan, N. et al.,2020), 0.79, 0.51, 0.75, and 0.622 (Koshti, R. et al., 2022), 0.78, 0.88 and 0.65, 0.74 (Chaturwedi, S. B. et al., 2024) which show the presence of carotenoids and pyocyanin. The antimicrobial activity of the pigments was evaluated against human pathogens. All pigments showed inhibition zones against gram-positive and gram-negative bacteria. Notable inhibition zones included 23 mm for a yellow pigment against *E. coli* (Sinha, S. et al., 2017), 18mm of yellow pigment against E. coli (Waghela, M. et al.,2018),30mm against E.coli (Siddharthan, N. et al., 2020), 26mm green pigment against Proteus (Chaturwedi, S. B. et al., 2024) was observed. . Additionally, *M. luteus* (milky yellow pigment) and *C. pallidum* (yellow pigment) exhibited significant UV-protective activity in UV-cling film assays, making them suitable for sunscreen formulations (Koshti, R. et al.,2022). Yellow orange and pink red pigment were used to dye cotton cloth (Selvi, P. S. et al.,2018).

DISCUSSION:

The result of this study shows that different pigmented bacteria were isolated from different sample. All bacteria produces their own unique pigment. Bacteria produce pigment at temperature 37°C but maximum production was observed at 4°C, 27°C and 30°C in some bacteria. *M. luteus* bacteria show high production at 27°C. pH also affects the production of pigment as at pH at 2,7 and 8 maximum production of pigment was observed. For solvent extraction, solvents like chloroform, ethanol and methanol were used. By UV-visible Spectrophotometer analysis maximum absorbance was observed in between 200-700nm. Most of the pigment was found to be carotenoids by observation. All the pigments show antimicrobial activity against human pathogen. From observation maximum zone of observation was observed against E. coli bacteria rather than other bacteria. Additionally, pigments from *M. luteus* (milky yellow) and *C. pallidum* (yellow) demonstrated UV-protective properties, making them suitable for sunscreen formulations. This microbial pigment used to dye cotton cloths and so used in textile industry.

CONCLUSION :

From this literature study it concludes that the microbial pigment can be used as an antimicrobial agent as it shows antimicrobial activity against human pathogen. These pigments are also used in sunscreen as it also gives protection from UV rays and also used as a dye to colour the different cloths. In summary, microbial pigments offer an eco-friendly and versatile alternative to synthetic colors, benefiting various industries and promoting environmental sustainability.

REFERENCES:

Chaturwedi, S. B., Mainali, S., & Chaudhary, R. (2023). Antibacterial Activity of Pigment Extracted from Pigment Producing Bacteria.

e-ISSN 2347 – 517X
Review Article

Chaturwedi, S. B., Mainali, S., & Chaudhary, R. (2024). Antibacterial activity of pigment extracted from bacteria isolated from soil samples. *BMC Research Notes*, *17*(1), 169.

Joshi, P. P., Yewatkar, S. K., Ghumare, R. R., & Trupti, D. (2019). Isolation, Characterization of Yellow Pigments Producing Bacteria. *EC Clinical and Experimental Anatomy*, *2*, 316-322.

Koshti, R., Jagtap, A., Noronha, D., Patkar, S., Nazareth, J., Paulose, R., ... & Chakraborty, P. (2022). Evaluation of antioxidant potential and UV protective properties of four bacterial pigments.

Priyadharshini, S., Lal, M. S. A., Gratia, A. Z. K., & Christober, M. D. (2021). Production and Characterization of Yellow Pigment from Pomegranate and its application.

Samyuktha, S., & Mahajan, S. N. (2016). Isolation and identification of pigment producing bacteria and characterization of extracted pigments. *Int J Appl Res*, *2*(7), 657-664.

Selvi, P. S., & Iyer, P. (2018). Isolation and characterization of pigments from marine soil microorganisms. *IJLSSR*, *4*, 2003-2011.

Shetty, M. R. D. R. (2018). Screening and extraction of microbial pigment from organism isolated from marine water. *Int. J. Sci. Res.(IJSR)*, *7*, 60-66.

Siddharthan, N., Sandhiya, R., & Hemalatha, N. (2020). Extraction and Characterization of Antibacterial Pigment from Roseomonas Gilardii YP1 Strain in Yercaud Soil. *EXTRACTION*, *13*(3).

Sinha, S., Choubey, S., Kumar, A. A., & Bhosale, P. (2017). Identification, characterization of pigment producing bacteria from soil and water and testing of antimicrobial activity of bacterial pigments. *Int J Pharm Sci Rev Res*, *42*(2), 119-24.

Waghela, M., & Khan, S. (2018). Isolation, Characterization of Pigment Producing Bacteria from various food samples and testing of antimicrobial activity of bacterial Pigments. *DAV International Journal of Science*, *7*(1).