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Optimization of Pigment Production by *Micrococcus* and *Arthrobacter* species Isolated from Soil and Water

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ABSTRACT

A pigment is a substance that is substantially used in different industries that come in a wide range of coloration and few of which are soluble in water. Bacterial pigments have many applications in the modern day life. Some microorganisms have the characteristics feature to produce pigments which may additionally beneficial us in identification. Bacterial pigments have larger compatibility and greater biodegradability with the environment. Nontoxic nature of pigment produced with the aid of a huge range of microorganisms make them ecofriendly for use in foodstuff, dye, cosmetics, pharmacy and different industrial purposes. Pigments of bacteria provide various advantages compared to synthetic dyes. They are biodegradable in nature, easy to extract, easily available, cheaper substrates needed. The pigment produced by the bacteria can be used as anticancer agents, antibacterial agent and anti-proliferative agents. The object of the existing find out about was once to isolated pigment producing microorganism from water and soil samples and explores their properties. Based on their morphological and biochemical characteristics, they have been recognized as *Micrococcus* and *Arthrobacter*. The pigment isolated from bacterial spp. is used for the antimicrobial activity, anti-oxidant recreation and for optimization of pH, temperature and NaCl. It was once concluded that water and soil have various microorganisms and they have the capacity of producing pigments.

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INTRODUCTION

Pigments produced by microorganisms are the important characteristic feature of some microorganisms like bacteria which might also be beneficial in characterization and identification. Microbial pigments have a variety of functions due to their higher environment-friendly property. Bacterial pigments have numerous useful properties like immunosuppressive, anticancer, biodegradability, antiproliferative, antibiotic, and so forth (Raju and Radha, 2015). Different pigment producing microorganisms like fungi, bacteria, mould and yeast are used for the industrial manufacturing and have wide range of application generally in material industries, pharmaceutical industries and food industries (Kumar *et al.*, 2015). Some other advantages of microbial pigments: acts as antioxidant agent, anticancer, antimicrobial, protects from Ultra Violet rays, extreme cold and hot, acquisition of macro or micro nutrients such as carbon, iron and nitrogen (Pankaj and Kumar, 2016). Pigments can be classified based on their origin as

organic and inorganic. Synthetic and natural pigments are organic compounds; Inorganic pigments are observed in nature or produced through synthesis. Naturally occurring pigments are produced by using living organisms such as animal, plant and micro-organisms. The benefits of pigment manufacturing from microorganisms contain effortless and quick growth in the inexpensive culture medium, independence from climate conditions and colorings of one-of-a-kind shades (Sinha *et al.*, 2017).

Pigments are coloring compounds that exchange the colour of transmitted or reflected light as the end result of wavelength selective absorption. Colors have been used to add or enhance the aesthetic value of compound/products that we use (Samyuktha and Mahajan, 2016). Colors play an essential role in human lifestyles and in human culture. The color produced by microorganisms is known as bio colors (Bhatt *et al.*, 2013). Microbial communities are various and every place differs and has different microbial community which leads to various colors producing organisms. They have aesthetic traits like formation of different pigments, degradation of complex chemicals, by various microorganisms and can be isolated (Rokade and Pethe, 2016).

Bacterial pigment is an alternative source of natural pigments and have an extremely great potential for food utilization due to their natural coloration with safety to use, medicinal resources, nutrient like vitamins, manufacturing being independent of season and graphical climate with controllable and expected yield (Joshi *et al.*, 2003; Sharma, 2014).

MATERIAL AND METHODOLOGY

Water sample from Mula-mutha River in Pune and Soil samples from Bhosari dumping ground, Pune were collected. Sample were transported immediately to the laboratory and processed for further analysis.

Isolation of pigment producing bacteria

Soil & water samples were used serially diluted up to 10^6. 10^{-4} - 10^{-6} dilutions were spreaded on sterile Nutrient agar (NA) plates by spread plate technique and incubate for at 37°C for 24hrs to 48hrs.

The Nutrient agar plates had been found for growth after 48hrs of incubation time. The pigment producing microorganisms were used for in addition studies.

The selected pigmented colonies were selected and streaked on Nutrient agar slants and Nutrient agar plates to obtain a pure culture of the colony (Siddharthan *et al.*, 2020).

Characterization and Identification of isolated pigmented micro-organisms

The selected pigment producing bacterial isolates were identified on the basis of their morphological and biochemical characteristics. Colony characterization of pigment producing bacteria was done based on its size, shape, color, margin, opacity, consistency, elevation, Gram staining and motility. Later, the organism was identified using Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994; Goswami and Bhowal, 2014).

Enrichment of pigment producing bacteria

Identified bacterial isolates were inoculated in hundred ml sterile (NB) nutrient broth. Glycerol (2%) was added to the NB for enhancing the pigment production and one was inoculated without addition of glycerol to see the effect of glycerol in pigment production (Joshi *et al.*, 2019). This was kept on shaker incubator at 100 rpm for 3-7 days and was observed for pigment production till it was darkly pigmented and nearly opaque (Waghela and Khan, 2018).

Extraction of pigment

The yellow pigmented colony after incubation on rotatory shaker for 3 days was subjected to centrifugation at 6,000rpm for 15 minutes. The supernatant was discarded; the cells were washed with sterile distilled water and again centrifuged for 10 minutes at 4,000rpm. 4ml of ethyl acetate was added to the pellet and suspended it. The water bath was set at 60°C and the obtained suspension was incubated in it for 15 minutes till all the visible pigments were extracted (Joshi *et al.*, 2019). This was again subjected to centrifugation at 4,000rpm for 15 minutes. The extracts were analyzed by scanning the absorbance in the wavelength region of 200nm to 800nm using the spectrophotometer (Sinha *et al.*, 2017; Chandran *et al.*, 2014).

Optimization of pH, Temperature and NaCl concentration

pH

To determine the optimum pH for the selected pigmented colony, equal amount of the microbial isolate was inoculated in 50 ml sterile nutrient broth in 100ml flask with different pH viz, 5.0, 6.0, 7.0 and 8.0 and incubated at 37°C for 48 hrs, on rotatory shaker for 2 to 3 days. Then the O.D was taken at 540nm to assay for maximum production of pigment (Goswami *et al.*, 2010; Joshi *et al.*, 2011; Ratnakaran *et al.*, 2020).

Temperature

To determine the optimum temperature for the selected pigmented colony, isolates were streaked

on sterile nutrient agar (NA) plates and incubated at different temperature viz 4°C, 25°C, 30°C and 37°C for 48hrs after that it was observed for pigmented colony production (Ratnakaran *et al.*, 2020).

NaCl Concentration

To determine the optimum NaCl concentration for the selected pigmented colony, equal amount of the bacterial isolate was inoculated in 50 ml sterile nutrient broth in 100ml flask with different NaClviz, 0.5%, 1%, 2%, 4% and after that incubated at 37°C on rotatory shaker for 2 to 3 days. The O.D was taken at 540nm to assay for maximum pigment production (Ratnakaran *et al.*, 2020).

Antioxidant activity test

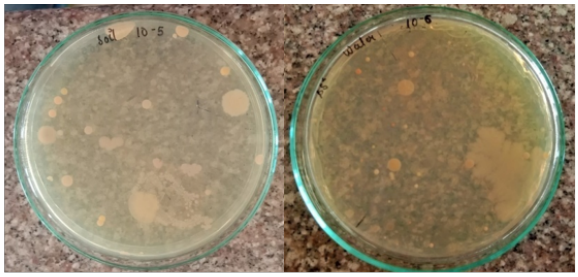
This approach is totally based on the principle of enlarge in the absorbance of the response mixtures. The absorbance will increase with the antioxidant activity will increases. The complete activity of antioxidant can be measured by way of the ferric reducing antioxidant power assay (FRAP). The phenolic and flavonoids acids both are presnt in the medicinal plant show off strong antioxidant activity and which is relying on their potential to form the complicated with metallic atoms, specially copper and iron (Rajurkar and Hande, 2011). The antioxidant compound existing in the samples varieties a colour complex with potassium ferricyanide, Trichloroacetic acid and ferric chloride, which is measured at seven hundred nm by way of Spectrophotometer (Fernandes *et al.*, 2016; Vijayalakshmi and Ruckmani, 2016).

Antibacterial assay

The antibacterial assay was performed by Kirby-Bauer Disk Diffusion method (Hudzicki, 2009). The sample micro-organisms were collected and pigment was tested for its antibacterial activity. The Nutrient agar plate was once seeded with 24hrs old microbial culture and wells had been bored in the NA plates. The wells have been filled by suitable quantity 20μlof pigment, it was once stored in fridge for half an hour after that it used to be incubated at 37^{0}C for 24hrs and the end result used to be found by using measuring zone of inhibition in diameter (Waghela and Khan, 2018; Singh *et al.*, 2012).

RESULTS AND DISCUSSION

The water & soil samples collected from different parts of Pune city have been used for isolation of pigment producing bacteria. Two pigment producing micro-organism have been identified and characterized which had been yellow and orange in coloration (Figure 1).



Yellow pigmented colony Orange pigmented colony

Figure 1: Colony of isolated pigment producing micro-organism from soil & water showing on Nutrient agar plate

Characterization of micro-organism was done by morphologically and various biochemical tests. This test gave us confirmatory results for identification of Microorganism. Thus given biochemical test followed by referring Bergey's manual (Holt *et al.*, 1994) gave confirmation of species as *Micrococcus* and *Arthrobacter* (Tables 1 and 2).

Various type methods had been used for the extraction of pigment producing micro-organism like filtration and centrifugation with ethanol addition so that cell gets lysed and intracellular pigment can be easily extracted. The extracted pigment had been yellow and orange in coloration (Figure 2). The optical-density of the pigment had been measured and in addition processed for antibacterial and antioxidant activity.

Yellow pigment Orange pigment

Figure 2: Culture of isolated pigment producing bacteria grown for pigment extraction

The impact of pH concentration on the production of pigment through each and every bacterial isolate was once determined through inoculating the pure cultures in sterile Nutrient broth. For impact of pH, sterile nutrient broth with pH 5, 6, 7, and eight was once used and incubated at 37^{0}C for about 24-48 hrs. Investigation on this study, both isolates show maximum production recorded on pH 7. Hence pH 7 was maintained optimization studies. This suggests significance of pH in the media considering that its altered value can both extend and limit the quantity of pigment (Graph 1). Isolates increase used to be most at impartial pH and pigmentation was once

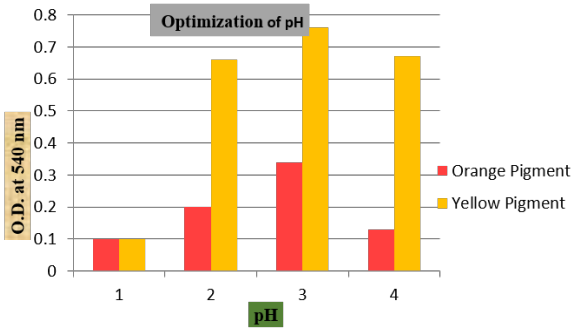
Table 1: Morphological and Biochemical characteristics of the Pigmented Bacterial isolates
(a)Morphological characterization

Shape	Size	Colour	Elevation	Margin	Opacity	Consistency	Gram-character
Circular	1mm	Yellow	Elevated	Irregular	Opaque	Smooth	Gram positive cocci
Circular	1mm	Dark orange	Elevated	Irregular	Opaque	Smooth	Gram positive rods

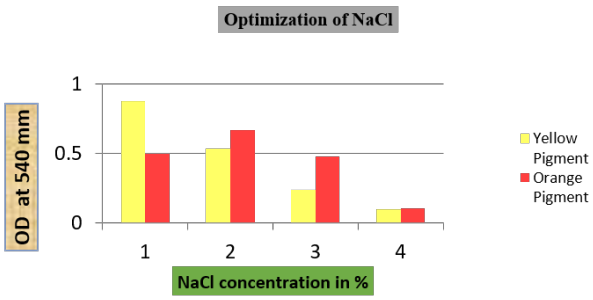
Table 2: Morphological and Biochemical characteristics of the Pigmented Bacterial isolates (b) Biochemical test

Test	Yellow Pigment	Orange Pigment
Catalase test	Positive	Positive
Oxidase test	Positive	Negative
Motility	Non- motile	Non- motile
Endospore staining	Non endospore forming	Non endospore forming
Sugar test		
Glucose	Positive	Negative
Galactose	Positive	Negative
Mannose	Positive	Negative
Maltose	Negative	Negative
Fructose	Negative	Negative
Mannitol	Positive	Negative
Raffinose	Negative	Negative
Sucrose	Negative	Negative
Lactose	Positive	Negative
Species	*Micrococcus*	*Arthrobacter*

found most when stored under refrigeration.



Graph 1: Optimization of pH



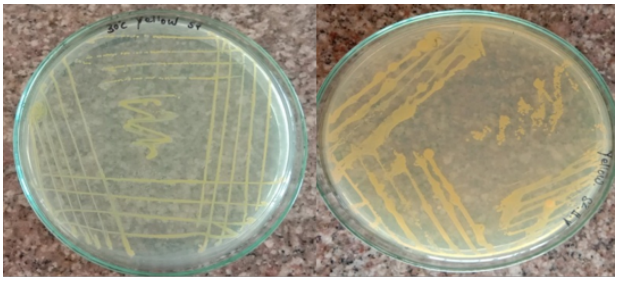
Graph 2: Optimization of NaCl in %

By observing the absorbance values obtained at different NaCl concentrations for yellow and orange pigmented colony. It can be concluded that at 0.5% NaCl concentration for yellow pigment and at 2% NaCl concentration for orange pigment production is the highest and can be considered as the optimum NaCl concentration for the production of pigment in large amount (Graph 2).

The yellow and orange pigment colony streaked on NA plates and incubated at different temperatures was observed for growth. It was observed that the yellow pigmented colony grows at 30°C and 37°C and orange pigmented colony grows at 25°C and 30°C. Therefore, it can be concluded that the yellow colony can grow between 30°C-37°Cand orange colony can grow between 25°C-30°C (Figure 3).

Total antioxidant was determined by using FRAP assay. The Absorbance used to be measured at seven-hundred nm used UV-VIS Spectrometer. The optical density at 700 nm for orange extract was 1.16, for yellow pigment it was 0.93 and Ascorbic acid as standard it was 0.203. The orange pigment has more antioxidant activity compared to yellow pigment.

Antibacterial activity was measured against 2 organ-

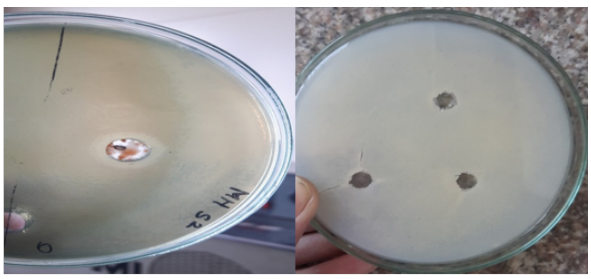


Yellow colony at 30°C Yellow colony at 37°C

Orange colony at 25°C Orange colony at 30°C

Figure 3: Optimization of different Temperature for yellow and orange pigment

isms like Escherichia coli and Pseudomonas. The antibacterial activity of orange pigment showed against the bacteria *Pseudomonas* and yellow pigment has no effect against *Pseudomonas.* Both yellow and orange pigment showed antibacterial activity against the *E.coli.* The Inhibition zone used to be measured to consider activity of antimicrobial (Figure 4).



Zone against *Pseudomonas* Zone against *E. Coli*

Figure 4: Antimicrobial activity of isolated pigmented organism as zone of Inhibition

From the results obtained, we observed that soil and water samples contain diverse microorganisms that have the ability to produce pigment as their secondary metabolite. Addition of glycerol tends to increase the pigment production. The pigment producing bacteria grows at neutral pH.

CONCLUSION

The results of present study confirm that extracted pigments can be used in various industries like pharmaceutical, paper printing industries etc. antibacterial and antioxidant activity of pigment can be of great benefit compared to synthetic dyes which are toxic and harmful to nature. They can be used as an achievablesource in textile, paper printing & food companies. From optimization results we can conclude that these organisms' best grow at 30°C temperature, neutral pH and 0.5% - 2% NaCl concentration. Soil and water are diverse in nature and can be screened for more pigment producing organism's and use their pigment as an alternative for synthetic dyes. More efforts are to be made for the use of cheaper substrates for the pigment producing organisms.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

REFERENCES

Bhatt, S. V., Khan, S. S., Amin, T. 2013. Isolation and characterization of pigment producing bacteria from various foods for their possible use as biocolors. *International Journal of Recent Scientific Research*, 4(10):1605–1609.

Chandran, M., Duraipandi, V., Yuvaraj, D., Vivek, P., Parthasarathy, N. 2014. Production and extraction of bacterial pigments from novel strains and their applications. *RJPBCS*, 5(6):584–593.

Fernandes, R. P. P., Trindade, M. A., Tonin, F. G., Lima, C. G., Pugine, S. M. P., Munekata, P. E. S., Lorenzo, J. M., de Melo, M. P. 2016. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. *Journal of Food Science and Technology*, 53(1):451–460.

Goswami, B., Bhowal, J. 2014. Identification and characterization of extracellular red pigment producing bacteria isolated from soil. *Int J Curr Microbiol App Sci*, 3(9):169–176.

Goswami, G., Chaudhuri, S., Dutta, D. 2010. Effect of pH and temperature on pigment production from an isolated bacterium. *Chemical Engineering Transactions*, 20:127–132.

Holt, J. G., Krieg, N. R., Sneath, H. A., Staley, J. T. 1994. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins U.S.A 9.

Hudzicki, J. 2009. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *American Society for Microbiology*, pages 1–8.

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.

Joshi, V. K., Attri, D., Bala, A., Bhushan, S. 2003. Microbial pigments. *Ind J Biotechnol*, 2:362–371.

Joshi, V. K., Attri, D., Rana, N. S. 2011. Optimization of apple pomace based medium and fermentation conditions for pigment production by Sarcina sp. *Indian J. Natural Product Res*, 2:421–427.

Kumar, A., Vishwakarma, H. S., Singh, J., Dwivedi, S., Kumar, M. 2015. Microbial Pigments: Production and their Applications in Various Industries. *IJPCBS*, 5:203–212.

Pankaj, V. P., Kumar, R. 2016. Microbial pigment as a potential natural colorant for contributing to mankind. *Res Trends Mol Biol*, pages 85–98.

Raju, V. N., Radha, T. 2015. Production of Extracellular Pigment from Microbes and its Application. *International Journal on Applied Bio-Engineering*, 9(2):23–29.

Rajurkar, N. S., Hande, S. M. 2011. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences*, 73(2):146–146.

Ratnakaran, P., Bhoir, M., Gupta, A. D. 2020. Isolation and characterization of pigment producing bacteria isolated from waste. *International Journal of Applied Research*, 6(4):252–260.

Rokade, M. T., Pethe, A. S. 2016. Isolation and identification of chromogenic bacteria from various sources. *European Journal of Pharmaceutical and Medical Research*, 3(5):295–299.

Samyuktha, S., Mahajan, S. N. 2016. Isolation and identification of pigment producing bacteria and characterization of extracted pigments. *International Journal of Applied Research*, 2(7):657–664.

Sharma, D. 2014. Understanding Biolocolor- A Review. *International Journal of Scientific and Technology*, 3(1):295–299.

Siddharthan, N., Sandhiya, R., Hemalatha, N. 2020. Extraction and Characterization of Antibacterial Pigment from Roseomonas Gilardii YP1 Strain in Yercaud Soil. *Asian Journal of Pharmaceutical and Clinical Research*, 13(3):116–120.

Singh, S., Kumar, P., Gopalan, N., Shrivastava, B., Kuhad, R. C., Chaudhary, H. S. 2012. Isolation and partial characterization of actinomycetes with antimicrobial activity against multidrug resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine*, 2(2):S1147–S1150.

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):119–124.

Vijayalakshmi, M., Ruckmani, K. 2016. Ferric reducing anti-oxidant power assay in plant extract. *Bangladesh Journal of Pharmacology*, 11(3):570.

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).