ECRONICON OPEN ACCESS

EC CLINICAL AND EXPERIMENTAL ANATOMY

Research Article

Isolation, Characterization of Yellow Pigments Producing Bacteria

Pratik P Joshi*, Saikiran K Yewatkar, Ruchita R Ghumare and Trupti D Aglave

Student, Department of Microbiology, New Arts, Commerce and Science College, Savitribai Phule Pune University, Pune, Maharashtra, India

***Corresponding Author:** Pratik P Joshi, Student, Department of Microbiology, New Arts, Commerce and Science College, Savitribai Phule Pune University, Pune, Maharashtra, India.

Received: August 19, 2019; **Published:** August 28, 2019

Abstract

The pigment which has synthetic origin eventually creates a toxicity problem that's why it creates mounting interest toward natural pigment. Among this the pigments which are originated from microbial resources are potentially good alternative ones to synthetic pigment. Basically, natural pigments can be obtained from two major sources, plants and microorganisms. The pigments obtained from plants have numerous drawbacks such as instability against light, heat or adverse pH, low water solubility and are often non-availability throughout the year. The latter are of great interest owing to the stability of the pigments produced and the availability of cultivation technology. The advantages of pigment production from microorganisms is due to its easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades. Carotenoid is the yellowish pigment produced by organisms of the genus *Micrococcus*, is among the more conspicuous pigments extant in the microbial world. Hence, pigment production from microbial resources is now one of the emerging fields of research to demonstrate its potential ability for various industrial applications.

Keywords: *Natural Pigments; Carotenoid; Genus Micrococcus; Industrial Application*

Introduction

Now a day there is rapid emergence of antibiotic resistant pathogens causing life threatening infections so it creates demand for new antibiotic in spite of considerable progress in the fields of chemical synthesis and engineered biosynthesis of antimicrobial compounds [1].

Pigment is the important group of organic constituents of bacterial protoplasm. Some of these, like prodigiosin, pyocyanin, violacein, phenazine, pulcherrimin, iodinin, indigoidine and melanin are metabolic by-products formed under special circumstances [2]. Microorganisms produce various pigments like carotenoids, melanins, flavins, quinones, prodigiosins and more specifically monascins, violacein or indigo [3].

There is growing interest in the food industry in the use of natural ingredients. Ingredients, such as colors, are considered natural when derived from biological sources like plants or microorganisms [4]. The industry is now able to produce some microbial pigments for applications in food, cosmetics or textiles [5]. In nature, color rich and pigment producing microorganisms (fungi, yeasts, and bacteria) are quite common [6].

Currently emerging and reemerging infectious diseases caused by different drug resistant strains are a major problem in public health and global economies. Such pathogens are increasing significantly over time because they are becoming progressively more resistant to conventional antibiotic compounds. Most wild-type strains of *S. marcescens* produce a characteristic secondary. This pigment was isolated from other species such as: *Serratia plymuthica*, *Serratia rubidaea*, *Hahella chejuensis*, *Vibrio gazogenes* [1]. The pigment has no defined

role in the physiology of producing strains, but have been reported to have antifungal, antibacterial, algicidal, antiprotozoal/antimalarial activities, immunosuppressive and anticancer activities [8].

Pigments like carotenoids, melanin's, flavins, monascins, violacein, indigo, prodigiosin are the type of pigments obtained from microorganism [3]. Carotenoids is a group of bioactive compounds and are responsible for bright yellow, orange, red pigments consisting various plants, microorganisms and animals and are widely distributed in the nature. They also used as colorants in the food industry which colours salmon, trout and poultry flesh (or) to identify the colour of egg yolk.

The search for new antimicrobial agents is a field of utmost importance. Antimicrobial substances, such as antibiotics, have been successfully used for preventing and treating microbial diseases for many decades. To date, thousands of antimicrobial substances have been identified and applied in clinical therapy. Pigments make the nature colourful. Many pigment producing microorganisms such as fungi, yeast, bacteria are abundant in nature.

Materials and Methods

Collection of sample

Soil sample were collected from different areas of Botanical garden of new arts college Ahmednagar, Maharashtra India Soil sample were collected from the roots of the plants by scraping the soil surface with spatula. All the soil samples were collected in clean and dry container and immediately transfer to the laboratory.

Isolation of pigment producing bacteria

Nutrient agar with pH 7.2 was used as isolating media. Isolation of pigment producing bacteria was done by spread plate count. The plates were then incubated at 37°C for 24 to 72 hours. After incubation, different colored colonies were observed from which yellow colored colonies was selected for further study.

Identification

Identification of isolated colony was performed by using gram staining as well as Morphological characteristics and biochemical tests.

Screening of pigment production

Yellow colored colony which is grown on nutrient agar (pH 7.2) was used for pigment production. A 2 - 3 loopful cultured was added into sterile 100 ml broth and incubated for 24 hours on a rotary shaker at room temperature.

Extraction of pigment

The different solvents like Chloroform, Ethanol, and Methanol was used for extraction of pigment. The 100 ml of incubated nutrient broth was mixed with 50 ml of chloroform. After mixing, the mixture was kept on rotary shaker at 100 rpm for 30 minutes. After 30 minutes this mixture was taken into extraction flask and this flask was kept for 5 - 10 minutes to settle down the mixture. After 10 minutes two separate layers of mixture were formed. The upper layer was collected in Petri plate was kept in hot air oven at 45°C up to evaporation. After evaporation dried pigment was collected and dissolved in 2% DMSO solution. The purified pigment was obtained and this pigment was subjected for further characterization.

Characterization of extracted pigments

Thin layer chromatography (TLC)

Extracted pigment was characterized by thin layer chromatography (TLC). A pre-coated silica gel sheet of 0.25 mm thickness was used. For the development of Chloroform: Methanol: Ethyl acetate (1:6:3) was used as a mobile phase. The spot of extracted pigment was applied on the silica gel TLC sheet. The TLC sheet was then dipped in the solvent system until the solvent reached up to $3/4^{th}$ of the TLC sheet. After that, the sheet was kept in oven for 5 minutes for drying. No developing reagent was used for observation of spot, but instead the sheet was observed under UV light for spot observation. R_f value of the spot was calculated by using formula:

$$R_f = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by solvent}}$$

FT-IR Spectroscopy

The sample was dried at 60°C and the dried powder was studied by using FT-IR spectrum. The prepared sample was recorded in a Shimadzu FT-IR in the range of 4000 - 400 cm^{-1} at a resolution of 4 cm^{-1} using KBr pellet methods.

Antibiotic susceptibility testing

Susceptibility testing of isolated strain assessed by antibiotic susceptibility testing. A sterile MHA plate (large petri-plate) was inoculated with 0.3 ml culture of isolated strain. The inoculum was spread evenly on medium using sterile glass spreader. Plate was kept in refrigerator for 10 min for pre-diffusion of culture. An antibiotic disc of HiMedia, Dodeka G-1 plus of known potency containing 12 standard antibiotics in specified concentration was placed on the medium. After that plate were incubated at 28°C for 24 Hrs. After incubation plates were observed for zone of inhibition and the diameter of zones were measured.

Result and Discussion

Isolation of bacteria

After incubation, colored colonies were observed on plate. However only the yellow coloured colony was selected as potential isolate. The isolate was labeled as yellow.

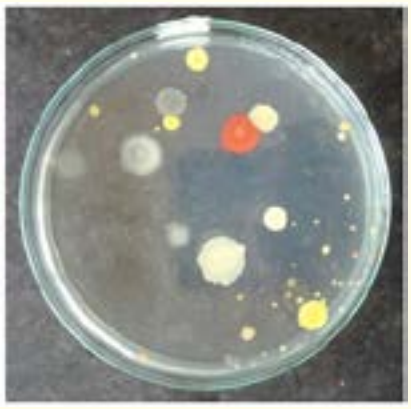

Figure 1: *Plate containing colored colony.*

Morphological and Biochemical characteristics of isolate:

After growth of isolate on Nutrient agar the morphological characters were noted. The biochemical characters were performed. The result of morphological and biochemical method are mentioned in table 1-3.

Characters	Observation
Color	Yellow
Size	0.1 mm
Shape	Circular
Margin	Entire
Elevation	Convex
Opacity	Opaque
Consistency	Sticky
Gram Staining	Gram Positive, Cocci
Motility	Motile

Table 1: *Morphological characterization of isolate.*

Test	Observation
Indole	Positive
Methyl Red	Negative
Voges Proskauer's	Positive
Citrate utilization	Negative
Gelatinase	Positive
Catalase	Positive
Oxidase	Negative

Table 2: *Biochemical Characterization of isolate.*

Sugars	Observation	
	Acid	Gas
Glucose	Positive	Negative
Sucrose	Positive	Negative
Mannitol	Positive	Negative
Fructose	Positive	Negative

Table 3: *Sugar utilization test of isolate.*

Thin layer chromatography

The extracted pigment was characterized by TLC. For this Silica plates (Aluminium) were used. The solvent system was composed of Chloroform: Methanol: Ethyl acetate (1:6:3). The Rf value of the spot was determined using formula. The average Rf value of pigment obtained was found to be 0.70.

FT-IR (Fourier transform-infrared spectroscopy)

The FT-IR spectra of the yellow pigment showed that it has several degrees of similarity to the spectra of Carotenoid.

Sr. No.	Wave Number (cm^{-1})	Functional Group
1	3500.80	N-H
2	2926.01	Aromatic C-H
3	2854.65	Stretching of Methylene group
4	1643.35	N-H
5	1535.34	Aromatic C=C
6	1371.39	C-O

Table 4

Antibiotic susceptibility testing

Disc containing antibiotics are from the various families like Aminoglycoside, Cephalosporin, fluoroquinolone etc. The isolate shows susceptibility against all the antibiotics present on disc which are Cefoperazone, Gentamycin, Clarithromycin, Sparfloxacin, Ciprofloxacin, Cefadroxil, Cefuroxime, Azithromycin, Roxithromycin, Ampicillin, Amikacin, Cefotaxime etc. Zone of inhibition was observed which conclude that isolate is susceptible to used antibiotics and if any causes happens due to this species it will be treat with the help of this antibiotics.

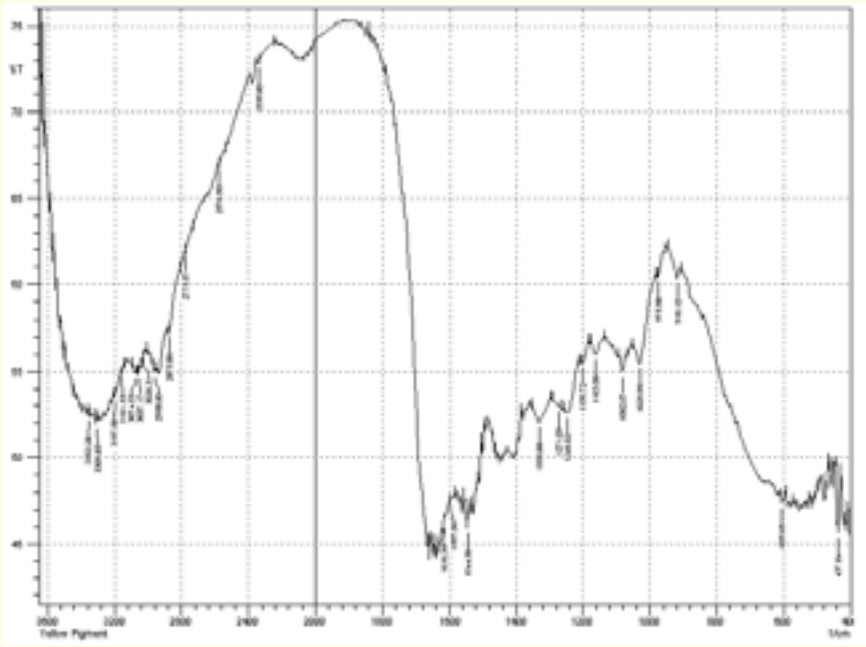

Figure 2: *FTIR spectra of yellow pigment producing bacteria.*

Figure 3: *Antibiotic susceptibility testing of the isolate.*

Sr. No.	Name of Antibiotic	Potency (mcg/units)	Zone of Inhibition (mm)	Susceptible/Resistance
1	Cefoperazone (CPZ)	75	28	Susceptible
2	Gentamycin (GEN)	10	16	Susceptible
3	Clarithromycin (CLR)	15	30	Susceptible
4	Sparfloxacin (SPX)	5	32	Susceptible
5	Ciprofloxacin (CIP)	5	34	Susceptible
6	Cefadroxil (CFR)	30	18	Susceptible
7	Cefuroxime (CXM)	30	18	Susceptible
8	Azithromycin (AZM)	30	16	Susceptible
9	Roxithromycin (RO)	15	18	Susceptible
10	Ampicillin (AX)	30	18	Susceptible
11	Amikacin (AK)	30	18	Susceptible
12	Cefotaxime (CTX)	30	20	Susceptible

Table 5

Conclusion

Carotenoid is the yellowish pigment produced by organisms of the genus *Micrococcus*, is among the more conspicuous pigments extant in the microbial world. Hence, pigment production from microbial resources is now one of the emerging fields of research to demonstrate its potential ability for various industrial applications.

Acknowledgements

The authors would like to express sincere thanks to the Principal Dr. B.H. Zaware and all staff members of Department of Microbiology, New Arts, Commerce and Science College, Ahmednagar.

Bibliography

1. Carbonell GV., *et al*. "Clinical relevance and virulence factors of pigmented Serratia marcescens". *FEMS Immunology and Medical Microbiology* 28.2 (2000): 143-149.
2. Giri AV., *et al*. "A novel medium for the enhanced cell growth and production of prodigiosin from Serratia marcescens isolated from soil". *BMC Microbiology* 4 (2004): 11.
3. Dufosse L. "Pigments, Microbial". Encyclopedia Microbiology 4 (2009): 457-471.
4. Pattnaik P., *et al*. "Biocolours: New Generation Additives for Food". *Indian Food Industry* 16.5 (1997): 21-32.
5. Bello E Kizito and Nwankwo EO. "Antibiotic Activity of Streptomyces Isolates Collected From Soil of Kogi Central, Nigeria". *IOSR Journal of Pharmacy and Biological Sciences* 8.4 (2013): 78-84.
6. Babu S and Shenolikar IS. "Health and nutritional implications of food colors". *Indian Journal of Medical Research* 102 (1995): 245-249.
7. Ramina Mekhael and Samira Y Yousif. "The Role of Red Pigment Produced by Sarritia marcescens as Antibacterial and Plasmid curing". *Agent* 12.1 (2009) 268-274.
8. Chidambaram Kulandaisamy Venil and Perumalsamy Lakshmanaierumalsamy. "An Insightful Overview on Microbial Pigment, Prodigiosin". *Electronic Journal of Biology* 5.3 (2009): 49-61.
9. Indra Arulselvi P., *et al*. "Screening of Yellow Pigment Producing Bacterial Isolates from Various Eco-climatic Areas and Analysis of the Carotenoid Produced by the Isolate". *Journal of Food Processing and Technology* 5 (2014): 1.
10. Kirti Kushwaha., *et al*. "Antibacterial Activity by Pigmented Psychrotrophic Bacterial Isolates". *Indian Journal of Applied Research* 4.10 (2014): 70-73.

11. L Selvameenal., *et al*. "Antibiotic Pigment from desert soil actinomycetes biological activity, purification and chemical screening". *Indian Journal of Pharmaceutical Sciences* 71.5 (2009): 499-504.

12. Md Mamunar Rashid., *et al*. "Anti-Bacterial Activity of Pigments Isolates From Pigment-Forming Soil Bacteria". *British Journal of Pharmaceutical Research* 4.8 (2014): 880-894.

Volume 2 Issue 7 September 2019