Isolation, Characterization of Pigment Producing Bacteria from various food samples and testing of antimicrobial activity of bacterial Pigments

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ABSTRACT

Microbial pigments are the characteristic feature of some bacteria to produce pigments which may be useful in identification. Bacterial pigments offer promising avenues for various applications due to their better biodegradability and higher compatibility with the environment. The aim of the present study was to isolate the microorganisms from different food samples capable of producing pigments with antimicrobial activity. A total of three pigmented colonies were isolated and an antibacterial activity of the pigments was studied against Gram positive and Gram negative bacteria. The extracted pigments had inhibitory effect on both Gram positive and Gram negative bacteria indicating antibacterial nature of the isolated pigments.

Keywords: Biocolours, pigments, bacteria, antibacterial activity

INTRODUCTION

Microbial pigments are the characteristic feature of some bacteria which may be useful in identification. Bacterial pigments offer promising avenues for various applications due to their better biodegradability and higher compatibility with the environment. Microbial pigments have numerous beneficial properties like anticancer, antiproliferative, immunosuppressive, antibiotic, biodegradability etc. Many microorganisms, including bacteria, fungi, yeast and mould etc. are employed for the industrial production of various pigments by using fermentation technology. These microbial pigments have broad area of application, mainly in food industries, pharmaceutical industries and textile industries (Abhishek Kumar *et al*., 2015).Some functions of microbial pigments: protection from UV rays, acts as antioxidant, protects from extreme heat and cold, functions as antimicrobial and anticancer, acquisition of nutrients like iron, nitrogen and carbon. (Vivek Prakash Pankaj *et al*., 2016). Pigments can be classified based on their origin as natural, synthetic or inorganic. Natural pigments are produced by living organisms such as plants, animals and microorganisms. Natural and synthetic pigments are organic compounds. Inorganic pigments are found in nature or produced by synthesis. The advantages of pigment production from microorganisms comprise easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades (Sinha *et al*., 2017). Natural pigments that are mostly extracted

from plants and animals such as Anthocyanins (fruits), Betataines (beetroot), Flavone (vegetables), Curcumin(turmeric), Quinones (roots, barks), Polyphenols (tea leaves). Microbial pigments- from Bacteria**:** -*Achromobacter* (creamy), *Bacillus sp*. (brown), *Brevibacterium sp*.(orange, yellow), *Pseudomonas sp.* (yellow), *Rhodococcus maris* (bluish red), *Streptomyces* (yellow, red, blue). Molds include *Aspergillus sp*. (orange, red), *Monascus purpureus* (yellow, orange, red), *H.avenae* (bronze colour). Yeasts include *Cryptococcus sp.* (red), *Phaffia rhodozyma* (red). Synthetic pigments include annatto, Caramel, Iron oxides, Manganese violet, White pigments such as zinc oxide, titanium dioxide.

Presently research is going on these pigment producing bacteria because the antimicrobial substance produced by these bacteria have been successfully used for clinical therapy. The aim of this research study was to isolate the pigment producing microorganisms from various food samples and evaluate the antimicrobial activity of pigments against human pathogenic bacteria.

MATERIALS AND METHODS

Sample collection: Different food samples were collected from the local market of Mumbai, India. Various food samples collected were mango, bread, spinach, apple, curd, okra etc. From these samples, pigment producing bacteria were isolated and used for the present study. The antimicrobial activity of the pigments was studied against *Escherichia coli, Pseudomonas, Staphylococcus aureus* and *Streptococcus spp*.

Media used: The media used for enrichment and isolation of pigmented bacteria were Nutrient Agar, Nutrient Broth, Nutrient Agar (containing glycerol for enhancement of pigmented bacteria) which were obtained from Hi media, India.

Isolation of pigment producing organisms: Food samples were collected from the local market to Mumbai, India. Loopful of food samples suspension was streaked on sterile Nutrient agar plates and kept for incubation at 37°C for 48 hr. The plates were observed for growth after incubation. Only the pigmented bacterial colonies were selected and used for further studies. The isolated pigment producing bacteria were further streaked on sterile NA plate to obtain pure culture.

Characterization and Identification of pigment producing bacterial isolates:

Colony characterization of pigment producing bacteria from NA plate was done based on colony size, shape, color, margin, opacity, consistency, elevation, Gram staining and motility. The biochemical tests performed were Indole test, Methyl Red (MR), Voges Proskauer (VP), Simmon's Citrate test, Catalase test and sugar fermentation tests. Identification of isolates obtained in pure cultures were characterized by morphology, growth characteristics on Nutrient agar media , microscopically by Gram staining, and various biochemical tests recommended in the Bergey's Manual of Determinative Bacteriology (Sinha *et al*., 2017).

Screening of pigment producing bacteria

Isolated colonies of identified cultures were suspended in Nutrient broth containing 2% glycerol in a flask and were incubated on rotary shaker for 48hr. Extensive growth of pigment producing bacteria was seen in the flasks after 48hr.

Extraction of pigments from pigment producing bacteria

The pigment producing bacteria was harvested by centrifugation at 2000rpm for 20 mins. The supernatants were discarded and the pellets were resuspended in acidified ethanol. The mixture was vortexed and the suspension was centrifuged at 2000rpm for 10 mins and supernatant was collected. The absorbance of the filtrates was measured on UV- visible spectrophotometer in the range of 300- 400nm. The solvent containing pigment was then used to evaluate its antimicrobial activity against human pathogens along with its control.

Antimicrobial activity

Antimicrobial activity of the pigments was tested against four human pathogens (*Escherichia coli, Pseudomonas, Staphylococcus aureus, Streptococcus spp* by well diffusion method by using nutrient agar plates. The plates were seeded with 24hrs grown pathogen culture and wells were bored in the plates. The wells were filled with appropriate amount of pigment (20µl) and it was kept in refrigerator for 15-20 mins. After that the plates were incubated at 37°C for 24 hrs and after incubation the anti-microbial activity of pigments was observed by measuring zone of inhibition.

RESULTS AND DISCUSSION

Identification and characterization of the pigment producing bacteria

The food samples collected from local market of Mumbai were used for isolation of pigment producing bacteria. Three pigment producing bacteria were identified and characterized which were yellow, light orange and dark orange in colour. These bacteria were then identified and characterised with the help of morphological characteristics and biochemical tests.

Table 1:- Morphological, Biochemical and Physiological characteristics of the Pigment producing bacterial isolates

Colour	Size	Margin	Shape	Opacity	Consistency	Elevation	Gram characters	Motility
Yellow	1mm	Entire	Circular	Opaque	Butyrous	Raised	Gram positive cocci	Non motile
Dark orange	1mm	Entire	Circular	Opaque	Butyrous	Flat	Gram positive cocci	Non-motile
Orange	1mm	Entire	Circular	Opaque	Butyrous	Flat	Gram positive cocci	Non-motile

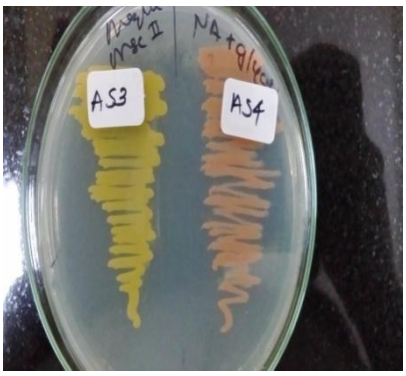
Biochemical tests

Pigments	Maltose	Mannitol	Dextrose
Yellow	-	-	-
Dark orange	-	+	-
Orange	-	-	-

IMVIC test, nitrate, catalase test

Pigment	Indole	Methyl red	Voges Proskauer	Nitrate test	Catalase	TSI
Yellow	-	-	-	-	+	No growth
Dark orange	-	-	-	+	+	No growth
Orange	-	-	-	-	+	No growth

+ = positive test : – = negative test



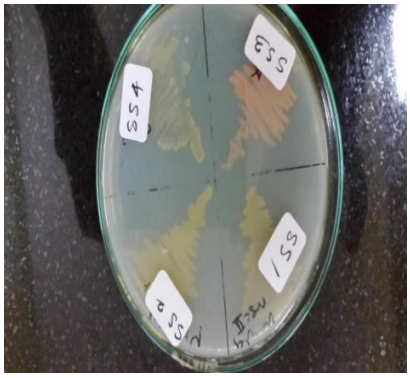


Fig 1: Nutrient agar showing pigment producing organism isolated from food samples.

Extraction of pigments from pigment producing bacteria

For the extraction of pigment producing bacteria, various methods were used such as centrifugation, filtration and addition of acidified ethanol so that cells get lysed and intracellular pigment can be extracted. The pigments that were extracted were yellow, dark orange and orange. The optical density of the pigments were measured and further processed for antimicrobial activity.

Antimicrobial activity against bacterial pathogens

The extracted pigments were dissolved with solvent acidified ethanol in order to evaluate the antimicrobial activity against human pathogen by well diffusion method. The bacterial pathogens were *E.coli, Pseudomonas, Staphylococcus aureus and Streptococcus.* The zone of inhibition was measured to evaluate antimicrobial activity.

Yellow pigment Orange pigment Dark orange pigment

Fig 2: Visual observation of culture of isolated pigment producing organism

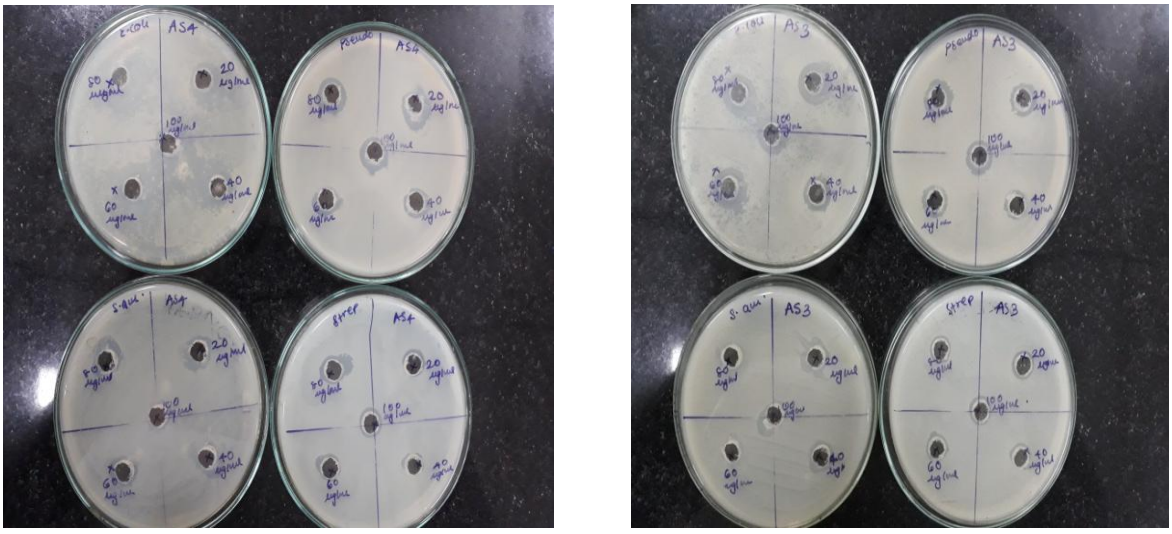


Fig 3: antimicrobial activity of isolated pigmented organism as zone of inhibition.

All the pigments isolated showed antibacterial activity against the test pathogens. Of all the different pigments tested, yellow pigment showed maximum zone of inhibition (23 mm) antimicrobial activity against *S. aureus* (Table 3). Antibacterial activity of the extracted pigments against the test pathogens are

shown in Table3. All the pigments from the isolates were found to inhibit the growth of both Gram-positive as well as Gram-negative bacteria and thus could be designated as broad spectrum antimicrobial agents. Zone of inhibition formed by each pigment vary in size even in case of isolates of same genus (*Staphylococcus*). Of the three pigments that were extracted from the different isolates, yellow pigment and dark orange pigment showed better antibacterial activity in terms of zone of inhibition whereas the orange pigment showed moderate anti-bacterial activity against the test pathogens. So it was concluded that the isolated bacteria could synthesise pigments that possessed antimicrobial activity against certain human pathogenic bacteria.

Table 2: Zone of inhibition showing antimicrobial activity of pigments

Pigment	Test organism	Diameter of zone of inhibition in mm				
		20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Yellow	*E.coli*	18.00	13.00	16.00	16.00	14.00
	Pseudomonas spp	14.00	12.00	13.00	15.00	13.00
	Staphylococcus aureus	15.00	12.00	10.00	12.00	12.00
	Streptococcus spp	12.00	11.00	12.00	11.00	12.00
Dark orange	*E.coli*	13.00	13.00	16.00	13.00	10.00
	Pseudomonas spp	15.00	13.00	15.00	15.00	15.00
	Staphylococcus aureus	14.00	12.00	12.00	13.00	11.00
	Streptococcus spp	11.00	9.00	9.00	12.00	12.00
Orange	*E.coli*	13.00	14.00	11.00	9.00	16.00
	Pseudomonas spp	11.00	10.00	11.00	9.00	9.00
	Staphylococcus aureus	9.00	-	7.00	9.00	11.00
	Streptococcus	10.00	-	-	10.00	11.00

CONCLUSION

The present study suggested that the local microbial isolates were able to produce pigment with anti-bacterial activity against human pathogens like *S. aureus*, *Pseudomonas*, *E.coli*, and *Streptococcus*. Hence it was concluded that food samples has diverse organisms producing different pigments which showed antibacterial activity.

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