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Isolation and Characterization of Pigment Producing Bacteria from Solid Food Waste Samples

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ABSTRACT

Due to increasing advertence of hazards of chemical colours, biocolours produced by pigment producing microorganisms can be helpful option to be used commercially. In the current study it was attempted to isolate pigment producing bacteria from solid food waste samples which are rich in microflora. Three isolates designated as IS-1, IS-2 and IS-3 were obtained which were later tentatively identified as *Staphylococcus roseus* (red pigment), *Staphylococcus saprophyticus* (pink pigment) and *Micrococcus luteus* (lemon yellow pigment), respectively on the basis of morphological and biochemical characteristics. Each pigment was further extracted and its antimicrobial activity was checked against *Staphylococcus aureus, Candida albicans, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The pigment produced by *Staphylococcus roseus* showed antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. Hence, this red pigment has potential to be used in food industry and also in skin creams. Further work on this pigment for its feasibility in above use is in process.

Key words: *Microbial pigments, Antimicrobial, Solid food waste.*

Introduction

Any compound to sustain in the commercial market needs to look attractive. The attractive look comes from nice packaging and color. The color to the compounds is given using colorant which are commonly known as pigments. A pigment is a coloured material that is completely or nearly insoluble in water.

Animals, plants (Joshi *et al.,* 2013) and microorganisms serve as major sources of biopigments. Any coloured material obtained from biological system is known as biopigment. It was found that many microbes have the ability to produce pigments like carotenoids, flavines, melanins, violacin, indigo (Dufosse, 2006). These pigments are synthesized as secondary metabolites and not often found in all bacteria. Pigments are beneficial to bacteria in providing protection against UV radiation and antibiotics and also found helpful in photosynthesis and pathogenesis. These pigments can be applied as additives, antioxidants, color intensifiers and functional food ingredients. Microbial pigments have number of beneficial properties like anticancer, anti proliferative, immunosuppressive, antibiotic etc. Microbial pigments can be produced in industries commercially because they are more stable and soluble than plant or animal pigments (Poorniammal *et al.,* 2018). Many microbes have the potential to synthesize pigments but amongst them bacteria are most promising. Pigments have a broad set of applications in food industry, pharmaceutical and textile industry. Therefore, this study was undertaken to isolate potential pigments producing bacteria.

Materials and Methods

Collection of sample

Solid food waste samples were collected from local food cafeteria of malkapur, Karad.

Isolation of Pigment producing bacteria (Bhatt *et al.*, 2013)

1 g of solid food waste was serially diluted using sterile saline and spread inoculated on 2% glycerol nutrient agar (peptone-1g, meat extract-0.3g, Nacl-0.5g, glycerol- 2 ml, agar-agar-2.5g, D/W-100 ml) of pH-7. The plates were incubated for 24-48 h at 28 °C. After incubation isolated pigmented colonies were picked up, purified and maintained at 4 °C on slants for further studies.

Characterization of pigment producing isolates (Bhatt *et al.*, 2013)

Isolates were further characterized on the basis of colony, morphological and biochemical characteristics.

Colony characteristics

Isolated and purified organisms were studied for their colony characters such as size, shape, colour, margin, opacity, elevation and consistency.

Morphological characteristics

Gam nature, motility and spore staining were studied by Hucker's modified Gram staining method, hanging drop preparation and Dorner's spore staining method, respectively.

Biochemical characteristics

Biochemical characteristics like catalase, oxidase, urease, caseinase production, citrate utilization, gelatin liquification, starch hydrolysis, nitrate reduction, Methyl red test, Vogus Proskauer and sugar fermentation tests were studied.

On the basis of these characteristics isolates were tentatively identified using Bergey's Manual of Systematic Bacteriology 9th edition (2000).

Extraction of pigments

To obtain sufficient growth of bacteria 0.1 ml of suspension of each isolate was spread inoculated on 2% Glycerol nutrient agar medium and incubated at 28 °C for 72 h. Growth obtained was collected in 2 ml methanol in test tube and vortexed for 3 h. The mixture was centrifuged at 10,000 rpm for 10 min. Supernatant was collected and evaporated completely. The pigment obtained was suspended in sterile D/W and was used to assess the antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Klebsiella pneumoniae.*

Assessment of antimicrobial activity of pigments (Srilekha *et al.*, 2017)

For assessing antimicrobial activity, extracted pigment was suspended in 1mL sterile D/W. Test organisms viz.. *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Klebsiella Pneumoniae* were spread inoculated on Mueller- Hinton agar medium. Then the wells were prepared by cork borer and 0.1 ml of pigment suspension was added into wells. Sterile D/W was taken as a control. Plates were incubated at 37 °C for 24-48 h and zones of inhibition were observed and recorded.

Results and Discussion

After the isolation of pigment producing bacteria on 2% glycerol agar medium total 3 promising isolates were obtained (Table 1, Photoplate 1, 2 and 3).

After the isolation process the 3 isolates were designated as IS-1, IS-2, IS-3.

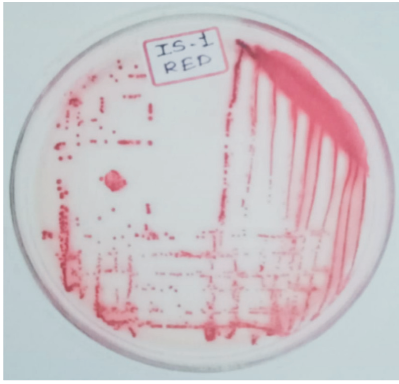
Table 1. Source and designation of isolates

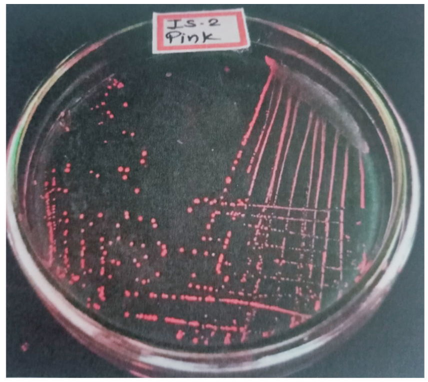
Source	Colour of pigment produced by isolates	Designation of isolates
Solid food waste samples from food cafeteria	Red	IS-1
	Pink	IS-2
	Lemon yellow	IS-3

Colony characteristics of the isolates was studied. It was observed that the colour of all three isolates were different (Table 2). The characters like margin, opacity, elevation and consistency were similar.

The morphological characteristics like Gram nature, motility and spore staining was studied and it was found that all 3 isolates was Gram positive in nature and they were non-motile and non-spore forming (Table 3). All isolates were non-motile.

Biochemical characteristics of the isolates were studied and the positive results were designated as '+' and negative results as '-'(Table 3). All produced catalase, urease, and caseinase enzymes. Isolate 1,2 and 3 showed positive results for glucose fermenta-





(Photoplate 1) (Photoplate 2) (Photoplate 3)

Photoplate 1. Petriplate showing red coloured colonial growth of isolate-1.
Photoplate 2. Petriplate showing pink coloured colonial growth of isolate-2.
Photoplate 3. Petriplate showing lemon yellow coloured colonial growth of isolate-3.

Table 2. Colony characteristics of pigment producing isolates on Glycerol nutrient agar at 28 °Cfor 48 h:

Colony → characteristics Isolates ↓	Size	Shape	Colour	Margin	Opacity	Elevation	Consistency
IS-1	2mm	Circular	Red	Entire	Opaque	Convex	Moist
IS-2	1mm	Circular	Pink	Entire	Opaque	Convex	Moist
IS-3	2mm	Circular	Yellow	Entire	Opaque	Comvex	Moist

tion and maltose fermentation. Isolate 1 and 2 showed positive results for sucrose, maltose xylose and fructose fermentation. While isolate 3 showed positive results for lactose fermentation.

Table 3. Biochemical characteristics

Sr. No.	Test	Isolates		
		IS-1	IS-2	IS-3
1	Vogas Proskauer	+	+	-
2	Citrate utilization	+	-	-
3	Catalase production	+	+	+
4	Oxidase production	-	-	-
5	Gelatine liquefication	-	-	-
6	Urease production	+	+	+
7	Casein hydrolysis	+	+	+
8	Starch hydrolysis	-	-	-
9	Glucose fermentation	+	+	-
10	Sucrose fermentation	+	+	-
11	Maltosefermention	+	+	+
12	Mannitol fermentation	+	+	-
13	Lactose fermentation	-	-	+
14	Xylose fermentation	+	+	-
15	Fructose fermentation	+	+	-
16	Methyl Red	-	+	-
17	Nitrate reduction	+	+	+
18	**Amylase production**	**-**	**-**	**-**

After the study of morphological, cultural and biochemical characteristics the isolates were tentatively identified. It was found that IS-1, IS-2, IS-3 were *Stapylococcus roseus*, *Staphylococcus saprophyticus* and *Micrococcus luteus* respectively (Table 4).

The antibacterial activity of the extracted pigments was studied. It was found that Isolate-1(IS-1) showed antibacterial activity against *Staphylococcus aureus* and *Candida albicans* while other isolate pigments did not show anti microbial activity (Photoplate 4 and Table 5)

Table 4. Tentative identity of isolates.

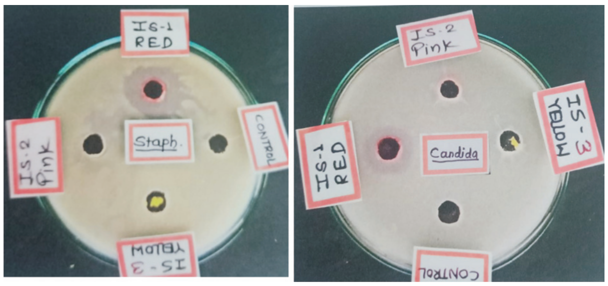
Sr. No.	Isolate	Tentative identification
1.	IS-1	*Staphylococcus roseus*
2.	IS-2	*Staphylococcus saprophyticus*
3.	IS-3	*Micrococcus luteus*

Conclusion

Promising pigment producing bacteria were isolated successfully. The isolates were tentatively

Table 5. Antimicrobial activity of pigments of isolates

Isolates	Zone of inhibition in cm against following species			
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumoniae*
IS-1	-	2.7 cm	0.8 cm	-
IS-2	-	-	-	-
IS-3	-	-	-	-



Photoplate 4: Antimicrobial activity of pigments of isolates against indicator test organisms:

identified as *Staphylococcus roseus*, *Staphylococcus saprophyticus* and *Micrococcus luteus* on the basis of cultural, morphological and biochemical characteristics. The isolates produced red, pink and yellow coloured pigments. It was found that pigment of IS-1 showed antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. The pigment IS-1 is promising in control of Staphylococcal and candida infections.

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Conflict of interest

No conflict of interest is there amongst the authors.

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