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Isolation of Pigment Producing Bacteria and Testing of Antimicrobial Activity of Bacterial Pigments

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Abstract: ***The aim of the present study was to isolate the microorganisms from different locations, capable of producing pigments with anti-microbial activity. Soil and water samples were collected from different areas of college campus. A total of three pigmented colonies were isolated that produced different coloured intracellular pigments. Isolated organisms were characterized by the Bergey's Manual of Determinative Bacteriology. The pigments were extracted from the isolates in various organic solvents based upon their solubility and their antimicrobial activity was identified. The extracted pigments had inhibitory effect on both Gram positive and Gram negative bacteria as well on fungus. So it could be said that soil and water from the sample cite has diverse organisms which showed antibacterial and antifungal activity.***
Keywords: Bacterial Pigment, Antimicrobial activity.

I. INTRODUCTION

Pigments produced from natural sources are of worldwide interest and is gaining significance. Natural pigments are obtained from ores, insects, plants and microbes [10]. Natural pigments possess anticancer activity, contain pro-vitamin A and have some desirable properties like stability to light, heat and pH [13]. These pigments are synthesized by various types of microorganisms as secondary metabolites and not often found in all types of organisms. Pigments come in a wide variety of colors, some of which are water soluble [6]. Micro-organisms which have the ability to produce pigments in high yields include species of *Monascus, Paecilomyces, Serratia, Cordyceps, Streptomyces, Bacillus, Achromobacter, Yarrowia* and *Phaffia* also produce a large number of pigment [13]. Microorganisms produce various pigments like carotenoids, melanins, quinones, flavins, prodigiosins and more specifically monascins, violacein or indigo [8-12]. The pigments like carotenoids, melanin, flavin, violacein, prodigiosin showed distinct antimicrobial effect against many pathogenic bacteria [3, 7, 14, 15]. Pigments are compounds with uniqueness of importance to many industries viz. foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing [6]. In the food industry they are used as additives, antioxidants, color intensifiers, etc. Microbial colorants play a significant role as food coloring agent, because of its production and easy downstream processing [1-2]. Some pigments produced by the bacteria shows antimicrobial activity against pathogens. These antimicrobial agents or substance produced by the bacteria are successfully used for preventing and treating microbial diseases. Inspired by these facts, the aim of this research was to isolate the pigment producing microorganisms from natural habitat and evaluate the antimicrobial activity of pigments against human pathogenic bacteria. These antimicrobial agents or substance produced by the bacteria are successfully used for preventing and treating microbial diseases.

II. MATERIAL AND METHODS

Thirty six samples consisting of water and soil isolated from different places of Khyati Institute of Science, Ahmedabad were used for isolation of pigment producing bacteria. Out of total 36 isolates three of the pigment producing organisms which produced efficient pigment was further screened. These bacterial isolates were isolated and then screened for pigment production by using enrichment method. Pigment producing bacteria were isolated by serially dilution method by pour plate technique on Nutrient Agar (NA) medium. These isolates were purified and stored in a NA slants at 40°C for further studies. For extraction of pigment, the bacterial cells were first grown for 24 h in nutrient broth followed by centrifugation at 8000rpm for 15 min. Both the supernatant and bacterial cell pellets were extracted using either 95%(v/v) methanol or 99% (v/v) acetone in the ratio of 1:5 until the pellet was colorless, i.e., complete pigment extraction was achieved. The pigment extract was then analyzed by scanning the absorbance in the wavelength region of 400-600 nm using UV-Vis spectrophotometer. Antimicrobial activity of the pigments was tested by well diffusion method. Four human pathogens (*Escherichia coli, Pseudomonas, Staphylococcus aureus, Bacillus subtilis*) were used against extracted pigment to evaluate its antimicrobial activity. The plate was seeded with 24hrs grown pathogen culture and wells were bored in the plates. Then the wells were filled with appropriate amount of pigment (20µl) and it was kept in refrigerator for half an hour. After that it was incubated at 370 C for 24hrs and the result was observed by measuring zone of inhibition.

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III. RESULTS AND DISCUSSION

The soil and water samples collected from different parts of Khyati Institute of Science, Ahmedabad were used for isolation of pigment producing bacteria. three pigment producing bacteria were identified as pigment producing bacteira and characterized which were yellow (GF009), orange (GB006) and red (BF3) colored pigment (Figure 1). These bacteria were then identified and characterized with the help of morphological characteristics and biochemical tests (Table 1). Their identification at genus level was done with the help of Bergey's Manual of Determinative Bacteriology, looking to the biochemical profiling of the organisms with genus *Xanthomonas* (GF009) as yellow colored pigment producing bacteria, *Serratia* (GB006) as red colored pigment producing bacteria, and *Erythrobacter* (BF3) as orange colored pigment producing bacteria (Figure 2).

Table 1: Isolation of pigmented bacteria

Sample Code	Isolation of Pigmented Bacteria	No of colony and sources	
		Water	Soil
GF009	Yellow	1	3
	Red	5	0
	Orange	2	1
GB006	Yellow	1	0
	Red	5	2
	Orange	1	3
BF3	Black	0	1
	Green	3	1
	Red	2	1
	Yellow	4	0

Figure 1: Cultural characteristics

Figure 1 shows Nutrient agar showing pigment producing organism isolated from soil and water samples from the sample sites of Khyati Institute of Science, Ahmedabad. .

Table 2: Cultural Microscopic and Biochemical Characteristics

Characters	GF009	GB006	BF3
Cultural			
Size	Small	Small	Small
Shape	Regular	Regular	Regular
Margin	Entire	Entire	Entire
Elevation	Raised	Flat	Raised
Surface	Smooth	Smooth	Smooth
Consistency	Wet	Mucous	Dry
Opacity	Transparent	Opaque	Opaque
Pigmentation	Yellow	Orange	Red
Microscopic			
Gram's reaction	Gram positive	Gram negative	Gram negative
Shape of cell	Cocci	Short rod	Short rod
Spore	Absent	Absent	Absent
Flagella	Absent	Present	Absent
Capsule	Absent	Absent	Absent
Motility	Non motile	Motile	Non motile
Biochemical			
Diffusible pigment test	Negative	Positive	Negative
Indole test	Negative	Negative	Negative
Methyl red	Negative	Negative	Negative
Voges proskauer test	Negative	Negative	Negative
Citrate utilization	Positive	Positive	Negative
Nitrate reduction test	Positive	Positive	Positive
Urease test	Positive	Positive	Negative
Catalase test	Positive	Positive	Negative
Oxidase test	Negative	Positive	Negative
Starch hydrolysis	Negative	Negative	Negative
Casein hydrolysis	Positive	Negative	Positive
Lipid hydrolysis	Positive	Positive	Positive

For the extraction of pigment producing bacteria, various methods were used like centrifugation, filtration, and addition of ethanol so that cell gets lysed and intracellular pigment can be extracted. The pigments extracted were red, yellow, cream, light orange and dark orange in color (Figure.2). The optical density of the pigment were measured and further processed for antimicrobial activity (Figure 3).

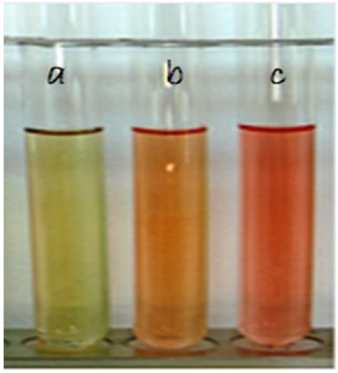

Figure 2: Pigment extracted in solvents a) Yellow pigment from Xanthomonas (GF009) b) red pigment from Serratia (GB006) c) orange pigment from Erythrobacter (BF3)

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The extracted pigments were dissolved with solvent DMF (dimethylformamide) to evaluate the antimicrobial activity against selected human pathogen by well diffusion method. Positive control was measured with streptomycin as a broad spectrum antibiotic to calculate the activity index of the antimicrobial property. The bacterial pathogens were *E.coli, Pseudomonas, Staphylococcus aureus* and *Bacillus subtilis.* The zone of inhibition was measured to evaluate antimicrobial activity with respect to positive standard. All the pigments isolated showed antibacterial activity against the test pathogens. Of all the different pigments tested, yellow pigment has shown the antimicrobial activity with maximum zone of inhibition 23 mm against *S. aureus* (Table 3). This study suggests that all the pigments showed better antibacterial activity against gram positive pathogens than gram negative pathogens. Mode of anti-bacterial action of most pigments was bacteriostatic. Antibacterial activity of the extracted pigments against the test pathogens are shown in Table 3.

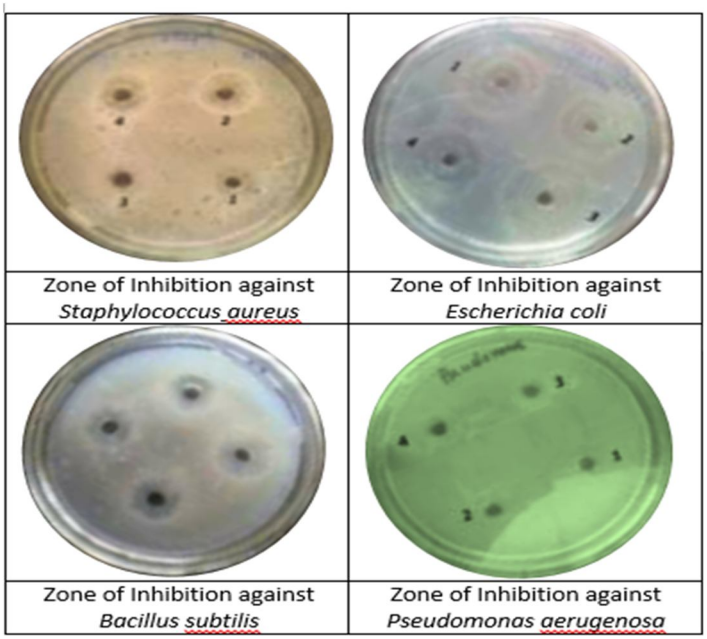

Figure 3: Antimicrobial Property of isolate on Gram positive and Gram negative bacteria

Table 3: Zone of inhibition showing antimicrobial activity

Sr. No.	Test Culture and Code	Zone of inhibition(mm)			Activity Index (A.I.)		
		GF009	GB006	BF3	GF009	GB006	BF3
1	*Escherichia coli* MTCC-425	22	19	16	0.91	0.791	0.666
2	*Pseudomonas aerogenosa* MTCC-11	21	22	19	0.875	0.916	0.791
3	*Staphylococcus aureus* MTCC-96	23	20	17	0.958	0.833	0.708
4	*Bacillus cereus* MTCC-430	23	16	21	0.958	0.666	0.875

*Note: Zone of Inhibition by Streptomycin as a standard drug= 24 mm (Mean Value)

All the pigments from the isolates can inhibit growth of both Gram-positive and Gram-negative bacteria and thus could be designated as broad spectrum. Zone of inhibition formed by each pigment was different in size even in case of isolates of same genus (*Staphylococcus*). Of the three pigments extracted from the different isolates, yellow pigment showed better anti-bacterial activity in terms of zone of inhibition. All other pigments showed moderate anti-bacterial activity against the test pathogens. This may be due to differences in composition of the different pigments. So it was concluded that soil and water has diverse microbial community which has shown antibacterial activity. The results obtained with the pigments against pathogenic organisms suggest that further studies might provide some illuminating data in the control of these diseases.

IV. CONCLUSION

Pigment producing bacteria are isolated, screened and studied in terms of biochemical, cultural, microscopic and antimicrobial properties from soil and water sample of Khyati Institute of Science campus. The isolated culture is known to possess antimicrobial property and activity against the selected test pathogens. Soil and water of Khyati Institute of Science, Ahmedabad is known to possess high diversity of microbial community that produces different pigments.

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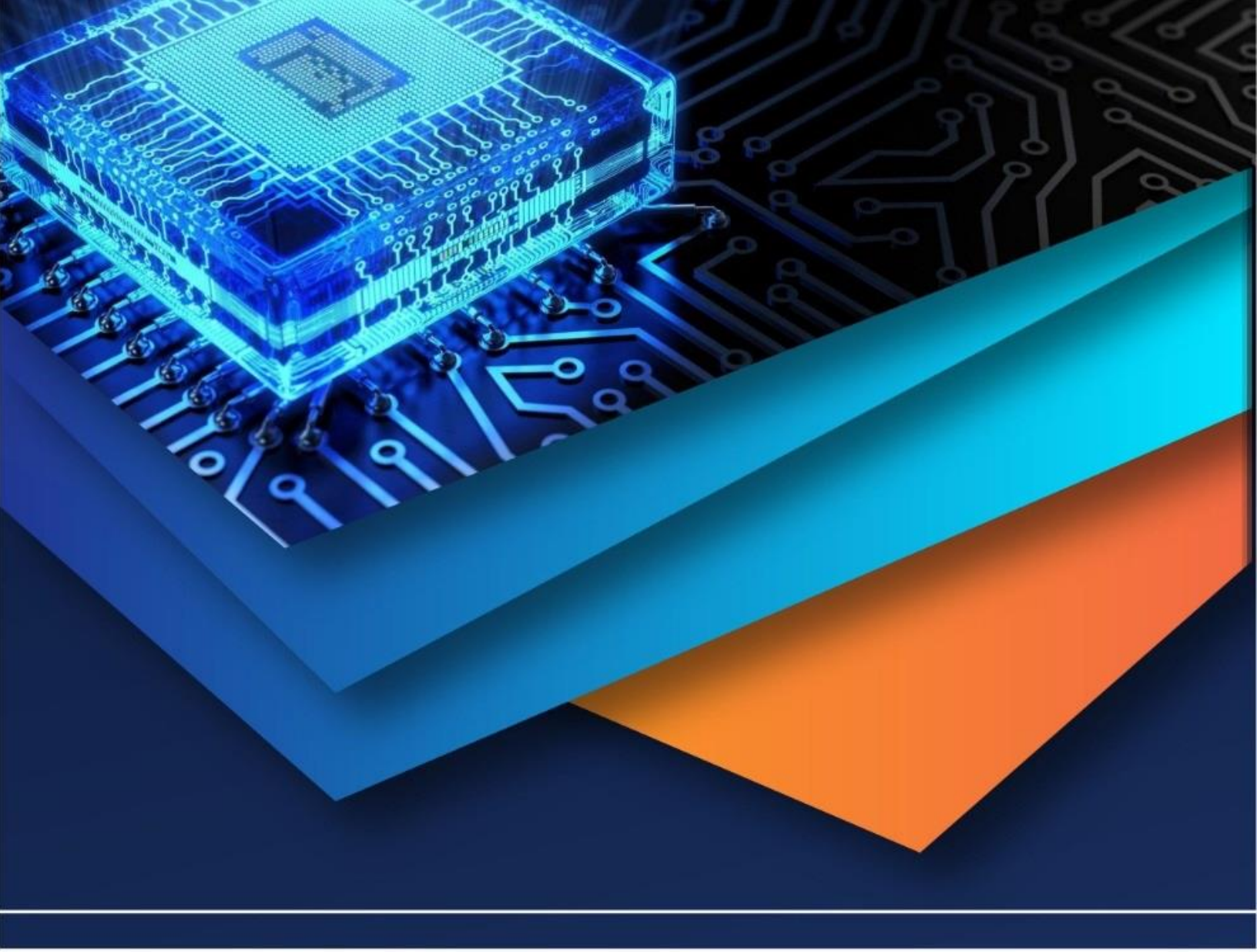

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