EXTRACTION OF PIGMENT PRODUCED BY *PSEUDOMONAS AERUGINOSA* AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Pseudomonas species are important opportunistic pathogens causing many types of hospital acquired infections. Apart from their negative role, they are important organisms used for bio-degradation of different types of xenobiotic compounds. An important characteristic feature of these organisms are production of secondary metabolites like pyocyanin and fluoroscein in both solid and liquid culture media. The study aims at production of the pigment and to assess the antimicrobial activity against clinical isolates. The production of the pigment was validated by using King's B liquid medium and extraction was done using chloroform. The pigment production was achieved after 24 hours of incubation. The antimicrobial activity of the pigment was found against E.coli, S.aureus and B.subtilis. Highest antimicrobial activity was achieved against S.aureus, whereas minimum inhibition of E.coli was observed against pigments.

KEY WORDS: Pseudomonas, pigment, antimicrobial activity.

INRODUCTION

Pseudomonas aeruginosa is an important gram negative opportunistic pathogen of human [5,8] including 10% of all hospital acquired infections.[1,3]. It is also the principal cause of morbidity and mortality in cystic fibrosis patients. Most of the *Pseudomonas* species, like *Pseudomonas aeruginosa* is also found associated with wound infections especially of burns.[2] Pseudomonas species are also involved in drug resistance towards various antibiotics.[3] *Pseudomoas aeruginosa* secretes a variety of pigments like pyocyanin, pyoverdine and pyorubin. These pigments have antibiotic activity against bacteria, fungi and protozoa. Besides these pigments, various other phenazine pigments like phenazine-1-

carboxylic acid, 1-hydroxy phenazine and phenazine-1-carboxamide are produced. [4] The study aims at production of the pigments and to assess the antimicrobial activity against clinical isolates.

Materials and Methods

Isolation and Identification of *Pseudomonas aeruginosa*. Collection of samples: The samples were collected from clinically suspected patients from Valsad district. They were inoculated into previously sterilized specialised microbiological media and incubated at 37 ^{0} C for 24 hours, isolated colonies were observed. Further, characterisation was done by using standard biochemical tests.

Production and Extraction of the pigment

Pseudomonas aeruginosa was inoculated in King's B liquid medium for maximum production of the pigment. They were incubated at 37 ^{0} C on a rotary shaker for 24 hours and observed for colour change.The broth culture was centrifuged at 10,000 rpm for 30 mins at 4 ^{0} C. The pigment was extracted using chloroform (1:2) and the aqueous phase was discarded The produced pigment was measured. Thin layer chromatography was also performed from the measured pigment. The measured pigment was tested for antibacterial assay.

Antibacterial Bioassay: Antibacterial activities were detected with a disk diffusion bioassay. Mixtures and pure compounds were suspended in the solvent and applied quantitatively to paper disks (0.55cm) [6,7]. The assay was performed by inoculating nutrient broth with test organisms like *E.coli*, *B.subtilis* and *S.aureus* incubating for 24 hours, and applying the bacterial suspension to an agar plate with a sterile swab. The dried prepared disks of different concentrations, including chloroform control were then placed on the swabbed plate using aseptic technique. The agar plate was incubated at 37^{o}C for 24 hours. Bacterial inhibition was measured in mm.

RESULTS:

ISOLATION OF *PSEUDOMONAS AERUGINOSA* ON KINGS B LIQUID MEDIUM

The strains were identified as *Pseudomonas aeruginosa* based on Gram's staining, motility, cultural characteristic, pigment production and by various biochemical reactions.

PIGMENT PRODUCTION

Pigment production was observed after overnight incubation. In liquid media, pigment production was demonstrated in shades of bluish green colour.

EXTRACTION AND ISOLATION OF CRUDE PIGMENT

After pigment was produced by using King's B liquid medium, it was extracted by centrifugation. The pellet was discarded. In a separating funnel chloroform was added and pigment was separated from aqueous phase after 4 hours. The pigment produced was measured as shown in TABLE-1.

THIN LAYER CHROMATOGRAPHY

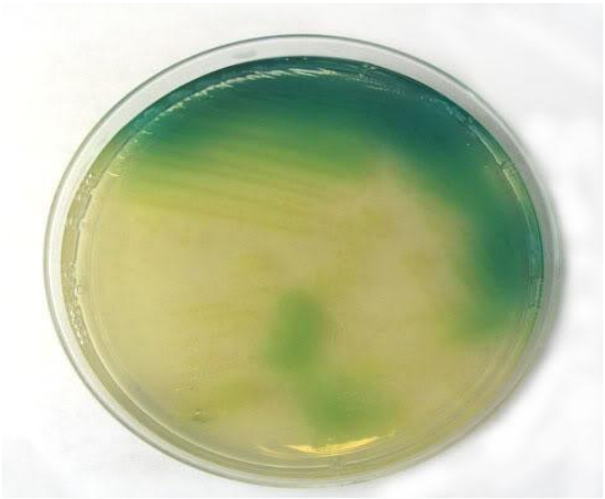
TLC was performed from the crude extract to monitor the progress of the reaction.

ANTIBACTERIAL ASSAY

The antimicrobial activity of the crude pigment was found significant towards *E.coli* and *S.aureus*. tested as in Table -2

ISOLATION OF *Pseudomonas aeruginosa* ON KING'S B MEDIUM

FIG -1



The above Figure-1 shows the presence of blue-green pigmentation on King'S B medium

FIG-2

PRODUCTION AND EXTRACTION OF PIGMENT

TABLE-1 EXTRACTION OF CRUDE PIGMENT BY CHLOROFORM SOLVENT

Solvent	Weight of empty petri plate (gm)	Weight of petriplate after drying (gm)	Weight of metabolite(gm)	Amt of distilled water (gm)	Con of metabolite(g/L)
Chloroform	**28.711**	**28.730**	**0.019**	**0.5**	**0.038**

FIG-3

THIN LAYER CHROMATOGRAPHY OF THE CRUDE PIGMENT

TABLE-2 SUSCEPTIBILITY OF BACTERIA TOWARDS CRUDE PIGMENT

Sr.No	Bacterial strain	Control Mm	P1 (µg/ml)			P2 (µg/ml)			P3 (µg/ml)		
		___	5	10	15	5	10	15	5	10	15
1	*E.coli*	___	0	8	10	8	8	12	8	10	12
2	*S.aureus*	___	10	12	20	12	12	22	10	12	22
3	*B.subtilis*	___	0	0	0	0	0	0	0	0	0

DISCUSSION

The bacterial strain was isolated from clinically suspected patients from Valsad district. The bacteria were further isolated on various medias like Nutrient .agar and King's B medium for identification of species. Gram's staining and motility were also performed. Various biochemical tests were done for primary characterisation of the isolated strain. The strain was found to produce blue-green pigment. The isolated bacteria were *Pseudomonas aeruginosa*. In the present study, the crude pigment was produced using King's B liquid medium and extracted using chloroform.[9] The crude pigment was further analysed using TLC. The pigment produced by the selected strain was subjected to antimicrobial activity using disc diffusion method at various concentrations of pigment viz 5µg, 10 µg, 15µg along with control. The pigment was subjected to antibacterial activity against the test pathogens like *S.aureus, E.coli,* and *B.subtilis.* In the present study, pigment activity was achieved against *S.aureus* with a zone of inhibition by 22mm, whereas minimum inhibition of *E.coli* was observed.

CONCLUSION

Based on the above study, the crude pigment has bioactive properties and is active against bacterial pathogens. The clinical strain *Pseudomonas aeruginosa* could be used to produce the pigment in large quantities and further purification method can be a promising one in the field of pharmaceutical application.

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