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Pyocyanin and its Bacteriostatic Effect toward Common Clinical Pathogens

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Abstract: *Pseudomonas aeruginosa,* a gram negative bacterium isolated from various clinical samples from which soluble pigment pyocyanin was produced. It was extracted using chloroform and the presence of secondary metabolite was confirmed by the addition of 0.2N HCl. Partial purification of the pigment was done by column chromatography and subjected to UV-vis spectrophotometer. A maximum absorption was observed at 277-278 nm. The partially purified pigment was subjected to antibacterial activity toward test pathogens such as *S. aureus, E. coli, P. vulgaris, Bacillus* sp. using disc diffusion method at various concentrations. The study revealed that pyocyanin from various strains of *P. aeruginosa* showed significant antimicrobial activity against test pathogens that is inhibiting the growth of the secondary pathogen in immune-compromised patients.
Key words: *Pyocyanin, Pseudomonas.*

Introduction

An alarming increase in gram negative infections now seems to be replacing a previous ground smell of gram positive infection which followed on the heels of the wide spread use of antibiotics. *P. aeruginosa*, a gram negative bacteria act as a secondary pathogen causing infection in post operative patients[1] which has drawn the attention of the microbiologist because the organism exhibited low sensitivity to various drugs and increase in incidence of infection with this organism in recent years. The characteristic feature of *P. aeruginosa* is the production of soluble pigment like pyocyanin which has the ability to show resistance to variety of drugs and inhibiting the growth of bacteria and fungi by producing pyrrolnitrin compound[2]. The present study deals with the production, extraction, purification, characterization of pigment and its determination of antibacterial activity against gram positive and gram negative bacteria. Secondary metabolite can be used as an bioactive compound against microbes infecting plants and human beings by structurally modifying it since, as such, it is little toxic to living population.

Materials And Methods

Identification of cultures

50 different clinical strains of *P. aeruginosa* were collected from Sharp laboratory, Perambur, Chennai. The isolate was maintained in Cetrimide agar slants and nutrient agar slants and stored at 4ºC.

Production and Extraction of Pigment

Liquid Media – *Pseudomonas spp.* isolates were grown in Potato glycerol broth and extracted with equal volume of chloroform formed a green to deep blue color and confirmed it to be highly positive for pyocyanin[3].

Solid Media – Pyocyanin production was demonstrated on solid media by inoculating it on nutrient agar and incubated over night at 37ºC.

Primary Screening Of Pyocyanin Compound

A total of 42 strains of *P. aeruginosa* were screened for antibacterial activity by cross streak method.

Cross Streak Method Of Pyocyanin Method

The following cultures were used for cross streak method *S. aureus, E. coli, Bacillus species, Proteus vulgaris* were procured from Department of Microbiology, Institute of Basic Medical Sciences, Chennai. *P. aeruginosa* was streaked diametrically across nutrient agar plates and were incubated at 37°C for 24 hours

The plates showed growth of the culture and the pigment was found diffused in the medium. The culture was then removed with a sterile glass slide. The plates were kept inverted and chloroform soaked filter paper was placed and left undisturbed for 30 minutes so that any traces of the bacteria was killed. The plates were then removed from the cabinet and traces of chloroform were eliminated on exposure to flowing air for a few minutes. A fresh inoculum of test organisms was streaked onto the chloroform treated plates at right angle to the original inoculum. Plates were then incubated for 24 hours at 37ºC for the demonstration of inhibition of bacterial growth[4].

Characterization Of Pyocyanin Compound

Among 42 strains, 10 strains of *Pseudomonas* pigment were efficient in cross streak method against test pathogens.

Purification Of Pyocyanin Pigment

Pigment was purified by column chromatography using silica gel G of column size 45 x 3.5 cm as stationary phase and chloroform and methanol (1:1) as mobile phase solvent[5].

Analysis of pigment by uv-visible spectrophotometer

Extracted pigment of the ten efficient strains of *P. aeruginosa* was subjected to UV-visible spectrophotometer and a maximum absorption was recorded by UV T-1800.

Antibacterial activity of partially purified pyocyanin pigment

The following bacterial cultures were used for the demonstration of antibacterial activity namely *S. aureus, E. coli, Proteus vulgaris* and *Bacillus* species.

The antibacterial activity of pyocyanin pigment was done by disc diffusion technique using sterile discs. The sterile discs were impregnated with various concentration of pyocyanin pigment *viz*. 5, 10, 15, 20 and 25ug/ml along with chloroform as control.

Results

Isolation of *Pseudomonas species*

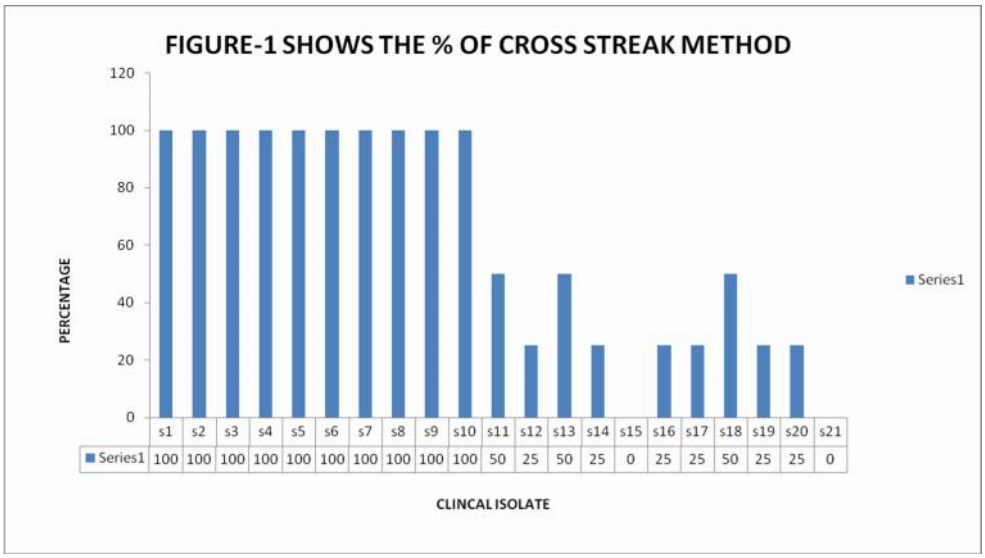
Out of fifty (50) clinical samples, forty two (42) strains were identified as *Pseudomonas spp.* based on Gram's staining, motility, cultural characteristic pigment production and by various biochemical reactions.

Pigment Production

Pigment production was observed after overnight incubation. Soluble pigment pyocyanin production was indicated by color change in the solid media. In case of liquid media, pyocyanin production was demonstrated in shades of bluish green color.

Primary Screening of Pyocyanin

Among 42 strains based upon the cross streak method, 10 strains were selected and used in this study as they showed maximum antibacterial activity (Fig 1,2).



Extraction Of Pyocyanin Pigment

After pigment was produced by using potato glycerol broth it was extracted by centrifugation there after pellet was discarded and to the supernatant 2-3ml of chloroform was added and bluish color compound was developed which was further confirmed by adding 0.2N HCl which confirmed presence of pyocyanin developing pinkish red color[6-8].

Partial Purification And Characterization Of Pigment

Extracted pigment was partially purified by column method and the fractions were collected by using an eluent chloroform and subjected to uv visible spectrophotometer for spectral analysis and a analyte peak was observed at a maximum range of 277-278 nm which was compared with the ATCC strain and further subjected to antimicrobial activity [4,9].

Antibacterial activity

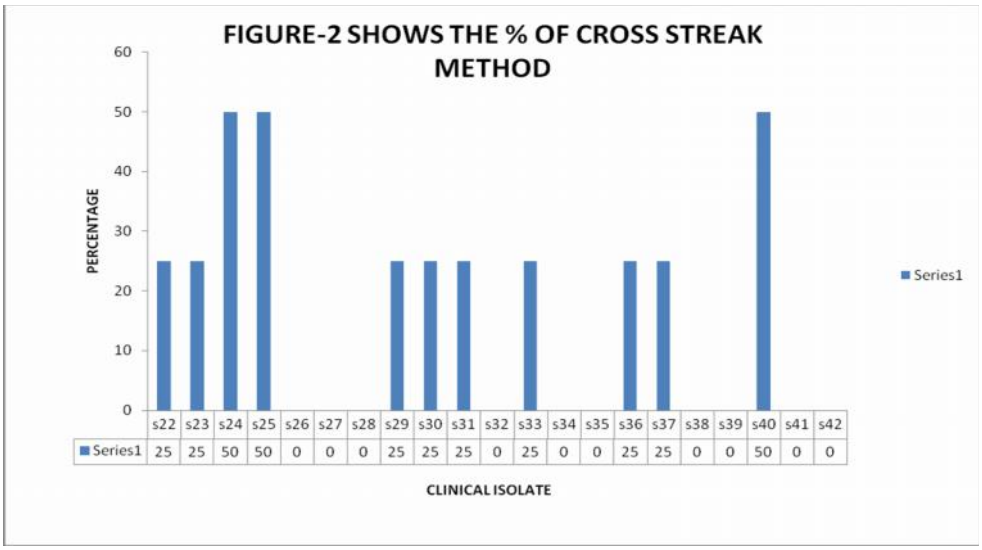
The antimicrobial activity of Pyocyanin pigment was found significant toward all the pathogens tested as in Table 1.1a & 1.1b.

Table 1.1a showing the susceptibility of bacteria towards pyacyanin pigment

S.No.	Bacterial Strain	Control	S1 (ug/ml)				S2 (ug/ml)				S3 (ug/ml)				S4 (ug/ml)				S5 (ug/ml)			
		mm	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
1.	*S. aureus*	-	0	8	11	14	0	9	10	12	0	7	9	12	0	13	13	15	0	7	9	12
2.	*E coli*	-	12	17	18	22	0	7	9	12	0	8	10	12	0	10	11	14	0	8	11	12
3.	*P. vulgaris*	-	7	12	14	15	8	12	12	17	0	12	15	17	7	9	11	15	7	12	12	14
4.	*Bacillus sps*	-	9	12	14	17	7	9	12	15	0	8	11	15	7	11	12	15	6	12	13	14

Table 1.1b showing the susceptibility of bacteria towards pyacyanin pigment

S.No.	Bacterial Strain	Control	S6 (ug/ml)				S7 (ug/ml)				S8 (ug/ml)				S9 (ug/ml)				S10 (ug/ml)			
		mm	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
1.	*S. aureus*	-	8	12	14	18	6	8	9	12	9	12	14	16	9	14	15	18	7	12	15	20
2.	*E coli*	-	9	14	15	16	9	13	14	18	10	12	14	17	7	13	14	17	10	12	14	19
3.	*P. vulgaris*	-	11	14	15	17	10	11	12	14	12	14	16	16	0	9	11	14	12	14	15	18
4.	*Bacillus sps*	-	11	13	14	15	11	12	12	14	0	0	10	12	0	0	10	13	12	14	17	20



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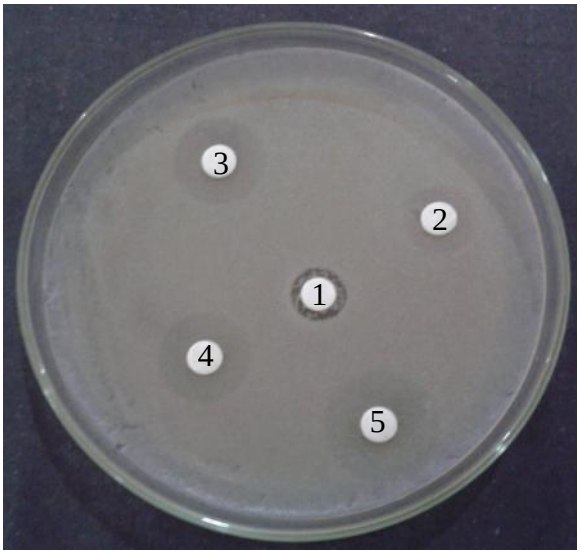

Fig. 3 Antimicrobial susceptibility of pyocyanin pigment toward E. coli by Disc diffusion method
1 – control; 2 – 5 ug/ml; 3 – 10 ug/ml; 4 – 15ug/ml; 5 – 20ug/ml

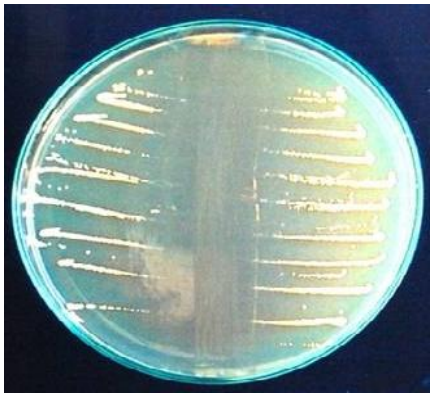

Fig. 4 Antimicrobial activity of pyocyanin pigment by Cross streak method toward *P. vulgaris*

Discussion

The characteristic feature of *P. aeruginosa* is the production of pyocyanin pigment which acts as secondary metabolite and inhibits the growth of bacteria and yeast. In present study the pyocyanin compound was produced using nutrient agar and potato glycerol broth, extracted using chloroform which impart blue colour and further confirmed by the addition of 0.2N HCl producing red colour which was in accordance with the work[8]. The extracted pigment was partially purified by column chromatography using silica gel as a column bed and blue colour fraction was eluted using methanol and chloroform which was correlating with the work[5]. The purified compound was dried, dissolved with chloroform which was further used for spectral analysis by UV spectrophotometer. The maximum absorption of all the selected strains was observed at 277 – 278 nm and compared with *P. aeruginosa* ATCC strain[9,10]. The pigment produced by selected strain was subjected to antibacterial activity using disc diffusion method at various concentrations of pigment *viz.* 5µg, 10µg, 15µg, 20µg/ml along with control. The secondary metabolite produced by the selected strain showed significant antibacterial activity against test pathogens such as *S. aureus, E coli, P. vulgaris, Bacillus sp.* A minimum of 5mm to a maximum of 20mm zone of inhibition was observed and tabulated by all the secondary metabolites. The results were slightly varied from previous study which may be due to strain variation and their source. The secondary metabolite present in the pigment arrested the electron transport chain of the tested organism[4]. In present study pyocyanin activity was seen for bacteria which was very effective and showed significant antibacterial activity.

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