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**Research Article**

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# Biosynthesis, antibacterial activity of pyocyanin pigment produced by *Pseudomonas aeruginosa* SU1

**T. Sudhakar[1], S. Karpagam[2] and J. Premkumar[1]**

[1]*Department of Biomedical Engg., Faculty of Bio & Chemical Engineering, Sathyabama University, Chennai*
[2]*Department of Botany, QMC, Chennai*

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**ABSTRACT**

*Pseudomonads are well known for their degradative abilities and play an important role in the environmental cleanup and they are well known for their opportunistic pathogenic properties. The characteristic feature of Pseudomonas aeruginosa is the production of soluble pigments like Pyocyanin, which is an secondary metabolite that is produced in both solid and liquid culture media. Pyocyanin isolated from Pseudomonas aeruginosa SU1 showed antibacterial activity. The study also includes optimization of the pigment production on various solid and liquid media. Further, the pigment pyocyanin was extracted using the solvent chloroform extraction method. The spectrophotometric analysis of the pigment showed maximum absorbance at 521nm. The antimicrobial property of the pigment was found similar to siderophore. They include specific depression under conditions of $Fe^{3+}$ deficiency, and a very high affinity for $Fe^{3+}$, together with a lack of affinity for $Fe^{2+}$. The properties of the pigment make it an important bioactive compound which has the ability to arrest the electron transport chain of fungi and exhibit antibacterial activity towards E.coli, Proteus spp. S aureus and klebsiella spp..*

**Keywords:** *Pseudomonas aeruginosa*, Pyocyanin, Bioactive compound, Antibacterial activity, siderophore

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## INTRODUCTION

*P. aeruginosa* is an optimistic pathogen of humans, belonging to the bacterial family Pseudomonadaceae which is wide spread in the environment. *P. aeruginosa* is widely distributed in nature and is commonly present in moist environment in hospitals. It causes disease in humans with impaired immune response [1,2]. The characteristic feature of *Pseudomonas aeruginosa* is the production of soluble pyocyanin pigment. Pyocyanin is a water soluble blue green phenazine pigment produced in large quantities by active cultures of *P. aeruginosa*. Pyocyanin has antibiotic activity against bacteria fungi and protozoa. The present study deals with biosynthesis, and characterization of pyocyanin pigments produced by *P. aeruginosa.* The purified pigment was used as bioactive compound to study the *in vitro* antagonistic activity of pyocyanin pigment against various pathogenic bacteria.

## EXPERIMENTAL SECTION

### ISOLATION OF BACTERIAL STRAINS

*P. aeruginosa* SU1 was isolated from soil sample collected from Sathyabama University using spread plate method. The isolated colonies were identified using gram staining and standard biochemical test. The identified culture was preserved in nutrient agar slants and stored at 4ºC in refrigerator.

**PRODUCTION AND OPTIMIZATION OF PIGMENT**

To study the production of soluble pigments pyocyanin and fluorescein, *P. aeruginosa SUI* was inoculated in nine different solid and nine different liquid medium. They were incubated at 37ºC for 24 hours and were observed for color change. The pigment was extracted using Chloroform solvent system.

**EXTRACTION OF PIGMENT**

*Pseudomonas* broth (PB) medium was used for the extraction of pigment where the organism was inoculated and incubated for 2-3 days at 37C. The change in color of the pigment to bluish green indicated the pigment production. After the color change, 5 ml of sample was centrifuged and 3ml of chloroform was added to the supernatant and mixed well until blue color is obtained. It was further confirmed by adding 0.2 N HCl to the blue color compound which turned red [3,4].

**PURIFICATION OF THE PIGMENT**

The pigment pyocyanin was separated by column chromatography using silica gel as a column bed. The fraction was eluted by using chloroform which act as a solvent system [5].

**UV- SPECTROPHOTOMETRIC ANALYSIS**

Red color pigment obtained by adding 0.2N HCl was separated and further subjected to UV-spectrophotometer and the maximum absorbance of pigment was read by using a distilled water as blank [3].

**DEMONSTRATION OF ANTIBACTERIAL ACTIVITY**

The broth was inoculated with organism such as *E. coli, Staphylococcus aureus, Proteus sp., Klebsiella sp.* and *Pseudomonas sp.* for 3 hrs at 37°C and the turbidity was checked using 0.5 Mcfarland standard. Then 100µl of bacterial suspension was poured on the surface of MHA spread by L- shape glass rod and left for 10 minutes to settle down the bacteria and 20µl of purified pyocyanin was added to the prepared wells in the same plate and therefore incubated at 37°C for 24 hrs and the diameter of zone was measured and the results were recorded [3].

## RESULTS AND DISCUSSION

**ISOLATION OF *PSEUDOMONAS* SP. SU1**

The colony morphology and cultural characteristics of the isolated organism was identified as *Pseudomonas aeruginosa SU1*. Gram staining and motility showed gram negative rods with actively motile organism. (**Figure 1**).

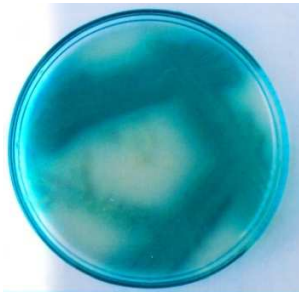

**Figure 1 – *Pseudomonas aeruginosa* in Cetrimide Agar**

Pigment production was accomplished after overnight incubation. Soluble pigments namely pyocyanin production were indicated by color change in the solid media. In case of liquid media, pyocyanin production was demonstrated in shades of green color.

The change in color of the pigment to deep pink observed upon addition of chloroform and 0.2N HCl that confirmed the presence of pyocyanin. The absorbance of this solution was maximum at 512 nm. This peak indicates the presence of pyocyanin compound [3]. (**Figure 2**).

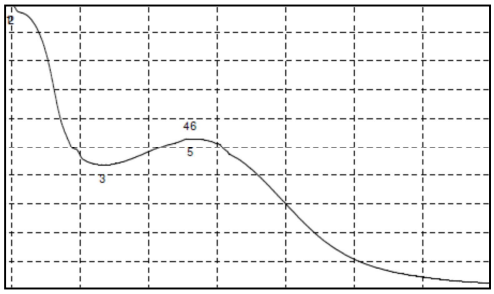


**Figure 2 – Analysis of pyocyanin by UV-Vis spectophotometer**

**Purification of The Pigment**

The pigment on column chromatography yielded one single fraction of Light blue color. It was then eluted with Chloroform and methanol.

**Antimicrobial Activity**

The pigment produced by p aeruginosa suI was subjected to antibacterial activity using *E coli*, *S aureus*, *Proteus* and *klebsiella* spp (Table1)

**Table 1 showing the antibacterial activity of Pyocyanin**

| S.No. | Bacterial isolates | Zone of Inhibition (in mm) |
|---|---|---|
| 1. | *E. coli* | 20mm |
| 2. | *S. aureus* | 17mm |
| 3. | *Proteus sp.* | 18mm |
| 4. | *Klebsiella sp.* | 16mm |

## DISCUSSION

Pyocyanin was synthesized, purified, and the nature of its antibacterial action was determined for several bacteria and yeast. The pigment was shown to be bactericidal for all organisms tested. The bactericidal effect was dependent upon concentration of pyocyanin pigment where increased concentration reduces the viability of bacterial strains. The gram-positive were more susceptible than the gram-negative bacteria. All apyocyanogenic *Pseudomonas* tested were totally resistant to the pigment, suggesting that resistance may be a characteristic of the genus. *P. aeruginosa*, the producer organism, was essentially unaffected by high concentration of pyocyanin. Facultative anaerobes were twofold or manifold resistant to the action of the pigment under fermentative conditions. However, the inhibitory effect does not require oxygen since denitrifying bacteria were susceptible during anaerobic respiration than during aerobic respiration.

The most widely used criteria for distinguishing *Pseudomonas aeruginosa* from closely related organisms is by the production of pyocyanin pigment in various solid and liquid media. In the present study *Pseudomonas aeruginosa* SU1 obtained from environment produced pigment in different media. The results are in accordance with the work of Young[11] who reported that *Pseudomonas aeruginosa* SU1 isolated from various samples produced pigments in various media [7] reported that production of pyocyanin pigment on various media indicated a wide variation in yields depending on the composition of the media, but satisfactory yield of this pigment was obtained. This report was co-relating the present study.

In the present study, pyocyanin pigment was produced by *Pseudomonas aeruginosa* SU1 on various liquid and solid media. The pigment was extracted using chloroform which produced blue color and turned to red on addition of 0.2N HCl. The extracted pigment showed a maximum absorbance at 512nm confirmed the presence of pyocyanin which was co-relating with the work of Wa Ad Mahmood Ra'oof *et al*.[10]. Then the confirmed pigment was purified by column chromatography, where only one light blue color fraction was obtained and its was found in accordance with the work [5].

*Pseudomonas spp.* produces a variety of metabolites [8] which some exhibit antimicrobial activity of which antimicrobial substance pyrrolnitrin has been known to possess antifungal activity.

Antibacterial activity was tested against *E. coli, S. aureus, Proteus sp., Klebsiella sp*. and *Pseudomonas sp.* using pyocyanin pigment from *P. aeruginosa* SU1 which showed antimicrobial activity. The maximum activity was seen in *E. coli, S. aureus, Proteus sp.,* and *Klebsiella sp.,* whereas *Pseudomonas* showed resistance which was quite varied with the work [3].

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