**Introduction**

Biofilms are a hallmark feature of various pathogenic and opportunistic pathogenic bacteria that allow them to communicate with each other. *Pseudomonas aeruginosa* is an opportunistic bacterium that often results in serious infections in health-care settings, especially in immunocompromised patients. Our previous experimental results indicated that the polymicrobial biofilms formed by *P. aeruginosa* are not only more complex and stronger than the single species biofilms but also show much more resistance to the antibiotic treatment. There is an urgent need to work on new methods and ways to supplement our current antibiotics. Novel antimicrobial peptides and pigments isolated from various bacterial strains are gaining immense focus keeping in view of their relative ease of isolation and selective toxicity. Two such pigments that we have been studying in the lab are Prodigiosin - a red colored pigment from the laboratory bacterial strain *Serratia marcescens* and carotenoid - a yellow colored pigment from the laboratory bacterial strain *Micrococcus luteus*.

In the current study we optimized the production of the red pigment Prodigiosin from the bacterium *S. marcescens* and partially purified it using organic solvents. Maximum production of the pigment was observed after 48 hours and at 25°C. Prodigiosin showed significant antimicrobial activity against *pigment was observed after 48 hours and at 25 oC. Prodigiosin showed significant antimicrobial activity against Serratia marcescens strain.*

**Experimental Methods**

**Extraction & Quantification of the Prodigiosin Pigment**

Overnight actively growing *Serratia marcescens* was heavily inoculated onto a TSA plate and incubated at 25°C for 48 hours. The culture was then scraped and suspended in 20 mL distilled water. For the extraction process, 5 mL from above broth was taken in a test tube, and 4 mL of methanol was added. The mixture was vigorously vortexed for 2 min. The solution was then centrifuged for 10 min at 6000 rpm. To quantify the amount of Prodigiosin, 0.8 mL supernatant was further mixed with 0.2 mL of 0.05 N HCl: methanol mixture (4:1 v/v). Prodigiosin displays a characteristic absorption spectrum in acidified methanol, with a strong maximum at 534 nm. The absorbance of the resulting solution was read using a spectrophotometer at 534 nm. The absorbance at 595 nm. Experiments were performed in triplicates to ensure reproducibility.

**Results-1**

- **Figure 2:** Effect of temperature on Prodigiosin production
- **Figure 3:** Effect of bacterial incubation time on Prodigiosin production

**Biofilm Assay**

Overnight bacterial cultures were added to the sterile Tryptic Soy Broth (TSB) media in 12-well plates and were incubated at 37°C for 48-72 hours. The supernatant was discarded, and the adhered cells were rinsed with distilled water. 0.1% Crystal Violet (CV) solution was added to each well to stain the adhered biomass and organic solvent was used to release the bound CV dye from the biofilm, and the biofilm was quantified by reading the absorbance at 595 nm. Experiments were performed in triplicates to ensure reproducibility.

**Results-2**

- **Figure 4:** Anti-biofilm effects of Prodigiosin on *Pseudomonas aeruginosa*

**Conclusion**

Prodigiosin pigment isolated from the wild-type *Serratia marcescens* strain shows a broad antimicrobial activity against the gram-negative bacterial strains and also against *P. aeruginosa*. It is especially promising to note that the *P. aeruginosa* biofilm sensitivity to the antibiotic gentamicin has increased upon the pre-treatment of the bacterial cells with Prodigiosin. Most antibiotics are tested on the planktonic bacteria to calculate their MICs but in the complex microbial communities, biofilms and the group behaviors of the bacteria are very common. To combat the increasing problem of antibiotic resistance, an approach to identify anti-biofilm agents is very much needed and our work will be a contribution to those efforts. Prodigiosin pigment will need to be further purified using specialized chromatography techniques and dose-response experiments will be performed to further confirm the anti-biofilm activity against *Pseudomonas aeruginosa*.

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**References**