



## Original Research Article

## Extraction of prodigiosin from *Serratia marcescens* and its application as an antibacterial spray

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## ABSTRACT

Prodigiosin is a natural pigment produced by *Serratia marcescens*. The pigment was grown in MacConkey agar and Luria Bertani agar. Growth of the pigment was higher in Luria Bertani agar when compared with MacConkey agar. Antibacterial activity was evaluated using test organisms such as *E.coli*, *Pseudomonas* sp. by well diffusion method. The pigment was impregnated on the surface of the test organism in different concentrations and incubated. The clear inhibition zones that formed around the well indicates antibacterial activity.

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### 1. Introduction

Plants and microorganisms are the two major sources to obtain natural pigments. The natural pigments obtained from plants have numerous drawbacks such as seasonal availability, instability against light, heat, pH and low water solubility.<sup>1</sup> Pigment production from microorganisms includes easy and fast growth in culture medium and it is independent from weather conditions.<sup>2</sup> Prodigiosin is a secondary metabolite produced by various microorganisms such as *Serratia*, *Streptomyces*, *Pseudomonas*, *Pseudoalteromonas* sp. having a common pyrrol dipyrromethane core structure.<sup>3</sup> *Serratia marcescens* is a single short rod bacteria belonging to the family Enterobacteriaceae, which is a gram negative, motile and facultative anaerobic in nature. Prodigiosin has a tripyrrole in its structure belongs to the family prodiginines. The production of prodigiosin is susceptible to temperature and is significantly inhibited at a temperature higher than 37° C in *Serratia marcescens* and utilized to produce antibacterial spray.

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#### 1.1. Prodigiosin

Prodigiosin is a natural red pigment produced by *Serratia marcescens*, the causative agent for Lyme borreliosis .

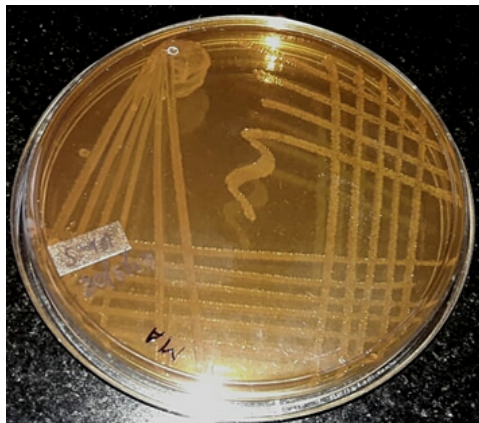
### 2. Aims and Objectives

1. To extract the prodigiosin pigment from *Serratia marcescens*.
2. To demonstrate the antibacterial activity on two bacterial isolates.

### 3. Materials and Methods

#### 3.1. Prodigiosin production grown on MacConkey agar

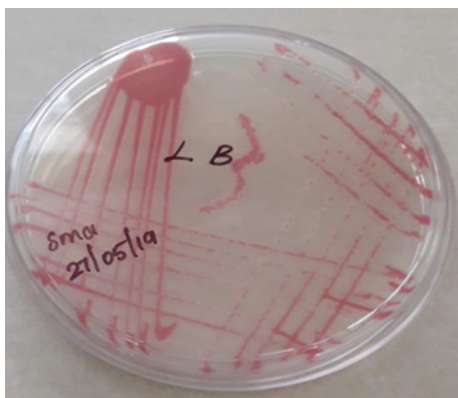
*Serratia* strain was extracted from the soil and streaked on MacConkey agar plate. The plate was placed in room temperature for 48 hrs. After 48 hrs, the plate was observed to have yellow coloured colonies. These colonies were inoculated into the LB broth and placed in CO<sub>2</sub> incubator under room temperature for 48 hrs. After 48 hrs, the prodigiosin pigment was observed.



**Fig. 1:** Colonies of *Serratia marcescens* on MacConkey agar

### 3.2. Prodigiosin production grown on Luria Bertani agar

*Serratia* strain was extracted from the soil and streaked on Luria Bertani agar plate. The plate was placed at room temperature for 48 hrs. After 48 hrs, the plate was observed to have pinkish red coloured colonies. LB broth was taken and pinkish red coloured colonies were inoculated into the LB broth and placed under room temperature for 48 hrs. After 48 hrs, the prodigiosin pigment was grown in the broth.



**Fig. 2:** Colonies of *Serratia marcescens* on Luria Bertani agar

### 3.3. Extraction of pigment grown on MacConkey agar

*Serratia marcescens* in MacConkey agar was centrifuged at 8000 g for 30 min. The Cell debris was formed in low concentration.

### 3.4. Extraction of pigment grown on Luria Bertani broth

*Serratia marcescens* in Luria Bertani broth was centrifuged at 8000 g for 30 min. The supernatant was discarded and

the cell pellets were collected. The acidified methanol was added with the extracted cell pellet and was centrifuged at 5000 g for 15 min. The supernatant was collected in a tube and placed in a dry bath at 60°C for 48 hrs to obtain a crude pigment.

## 4. Result and Discussion

### 4.1. Estimation of prodigiosin from LB agar

The cell absorbance of the bacterial culture was measured at 620 nm. For the pigment absorbance, the broth was mixed with methanol, subjected to centrifugation. The supernatant was used for the measurement of absorbance at 534 nm. The pigment prodigiosin was estimated by the formula,<sup>4</sup>

$$\text{Prodigiosin (unit per cell)} = \frac{[OD_{534} - (1.381 \times OD_{620})] \times 1000}{OD}$$



**Fig. 3:** Prodigiosin pigment

The pigment prodigiosin was estimated by the following formula and it was found to be 36.9 units/cell.

$$\text{Prodigiosin} = \frac{[OD_{534} - (1.381 \times OD_{620})]}{OD_{620}}$$

OD<sub>534</sub> = pigment absorbance; OD<sub>620</sub> = bacterial cell absorbance; 1.381 = constant

### 4.2. Antibacterial activity

### 4.3. Microbial strain

*Escherichia coli* and *Pseudomonas sp.* were used for the evaluation of antibacterial activity.

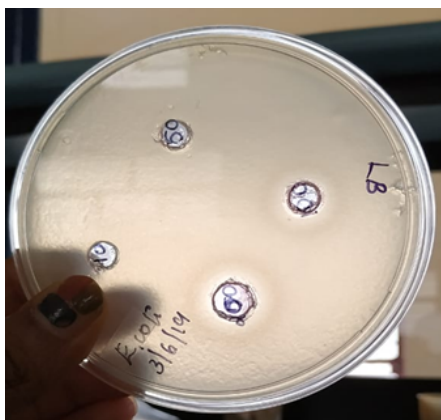
### 4.4. Nutrient agar

Nutrient agar was prepared (peptone -2.5 g, yeast -1.5 g, distilled water-500 mL, agar-10 g), stirred, boiled to dissolve and autoclaved at 121°C for 15 min. The hot medium was poured in a sterile petriplate. The medium was allowed to solidify for 15 min.

### 4.5. Agar well diffusion method

The antibacterial activity of the pigment was carried out using agar well diffusion method separately. The

test bacteria such as *E. coli* and *Pseudomonas sp.* were inoculated on the nutrient agar plate and incubated at 37°C. 20 mL of methanolic extract of this pigment was impregnated on the surface of test bacteria. Wells were made to inoculate the extracted pigment in different proportions on the surface of *E. coli* and *Pseudomonas sp.* The clear zones of inhibition were observed by incubating the plates at 37°C for 36 to 48 hrs.<sup>5</sup>



**Fig. 4:** *E. coli*



**Fig. 5:** *Pseudomonas sp.*

The results of extraction of prodigiosin from *Serratia marcescens* and demonstration of antibacterial properties on two bacterial isolates, obtained in this study, imply that the growth rate of the prodigiosin pigment is higher in LB agar when compared with MacConkey agar. Incubation of MacConkey agar plates under increased CO<sub>2</sub> has been observed to reduce growth of microorganism. The pigment from MacConkey agar was obtained in lower concentration. The antibacterial activity of the pigment from LB agar was estimated and checked by well diffusion method on *E. coli* and *Pseudomonas sp.* in different concentrations.

Demineralized water was mixed with the dry extract and then lavender essential oil was added in the mixture which has characteristics of antibacterial, antifungal activities and it has a pleasant smell. This indicates that, it can be useful to remove unwanted microbes in glassware which are used in laboratories and industries.

## 5. Conclusion

*Serratia marcescens* as an antibacterial activity revealed that the pigment prodigiosin can be considered as a possible alternative source of antibacterial spray in various laboratories and industries.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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