

Laboratory Scale Production, Optimization, and Characterization of Prodigiosin from *Serratia marcescens* isolated from soil sample of VSBT [18.1755° N, 74.6133° E]

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Abstract

Prodigiosin is a red tri-pyrrole secondary metabolite produced by several bacterial species, notably *Serratia marcescens*, and is known for its wide spectrum of biological activities including antibacterial, antifungal, anticancer, and immunosuppressive properties. The present study focuses on the isolation, laboratory-scale production, extraction, purification, characterization, and process optimization of prodigiosin produced by *Serratia marcescens* isolated from soil samples containing food waste. Screening of isolates was performed on nutrient agar, followed by morphological, staining, and biochemical characterization according to Bergey's Manual. Prodigiosin production was evaluated in different media, and peanut powder broth was found to support maximum pigment yield. Optimization studies revealed that pH 7, temperature 28 °C, NaCl concentration of 0.6%, and an incubation period of five days resulted in the highest prodigiosin production (5.3 g/L). The extracted pigment was confirmed using thin layer chromatography ($R_f = 0.62$), UV-Visible spectroscopy, and Fourier Transform Infrared (FTIR) analysis. Purified prodigiosin exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria and antifungal activity against selected fungal strains. This study highlights the potential of optimized laboratory-scale prodigiosin production for future pharmaceutical and industrial applications.

Keywords: Prodigiosin, *Serratia marcescens*, secondary metabolite, pigment production, optimization, antimicrobial activity

1. Introduction

Microbial pigments have attracted significant interest as sustainable alternatives to synthetic colorants due to their biodegradability, non-toxicity, and diverse biological activities [20–22]. Pigments are substances that impart color through selective absorption of visible light wavelengths,

unlike luminescent compounds that emit light [1]. Among these, prodigiosin is a prominent red pigment belonging to the prodiginine family, primarily produced by *Serratia marcescens* and several marine and terrestrial microorganisms [7,11].

Prodigiosin ($C_{20}H_{25}N_3O$; molecular weight $323.44 \text{ g mol}^{-1}$) is chemically described as 4-methoxy-5-[(Z)-(5-methyl-4-pentyl-2H-pyrrol-2-ylidene) methyl]-1H,1'H-2,2'-bipyrrole [2]. Its biosynthesis involves a complex pathway leading to the convergent assembly of three pyrrole rings derived from L-proline, L-serine, L-methionine, pyruvate, and 2-octenal intermediates [2,3,7,26]. The genes responsible for prodigiosin biosynthesis are clustered and tightly regulated by environmental and physiological factors [3,7,26].

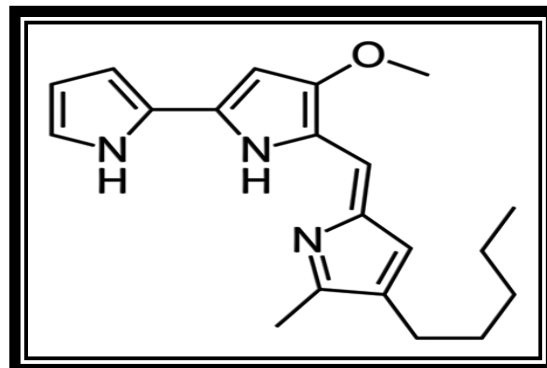


Fig 1: Structure of Prodigiosin

[<https://en.wikipedia.org/>]

Serratia marcescens is a Gram-negative, motile, facultative anaerobe belonging to the family *Enterobacteriaceae* and is widely distributed in soil, water, plants, insects, and animals [4]. Although the organism grows optimally at $37 \text{ }^\circ\text{C}$, prodigiosin production is suppressed at this temperature and is favored at temperatures below $30 \text{ }^\circ\text{C}$ [5,17]. Several studies have demonstrated that pigment production is influenced by nutrient composition, pH, temperature, phosphate availability, salinity, and dissolved oxygen levels [1,5,16–18].

Prodigiosin has been extensively studied for its antibacterial and antifungal activities [12,23], anticancer and pro-apoptotic effects [8,9,14], immunosuppressive properties [13], and insecticidal potential [15,24]. Despite its promising bioactivities, large-scale and cost-effective production remains a challenge. Therefore, the present study aims to isolate prodigiosin-producing bacteria from soil, optimize laboratory-scale production, and characterize the pigment to support future scale-up and application studies.

2. Materials and Methods

2.1 Sample Collection and Isolation

Soil samples were collected from garden soil and food-waste-containing soil, as nutrient-rich environments are known to harbor pigment-producing microorganisms [16]. Samples were serially diluted and spread on nutrient agar plates, followed by incubation at 28 °C for 24–48 h.

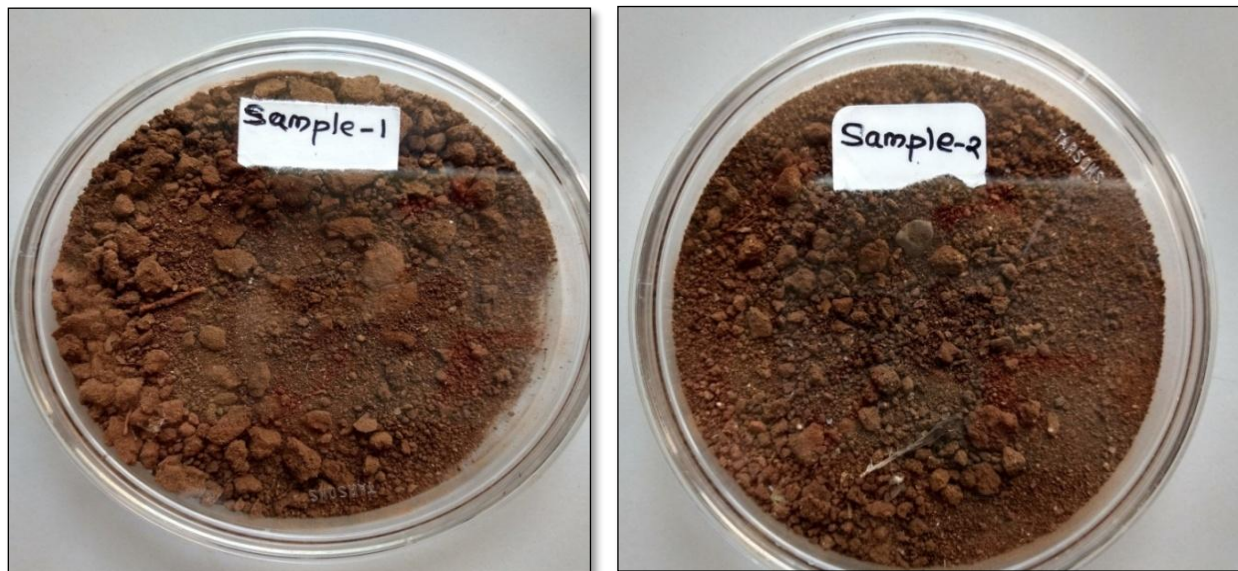


Fig 2: Soil Sample collected from VSBT garden [18.1755° N, 74.6133° E]

2.2 Screening and Identification of Prodigiosin-Producing Bacteria

Red-pigmented colonies were selected and purified by repeated streaking. Morphological examination, Gram staining, capsule staining, and motility tests were performed. Biochemical characterization was conducted according to Bergey's Manual, leading to identification of the isolate as *Serratia marcescens* [4].

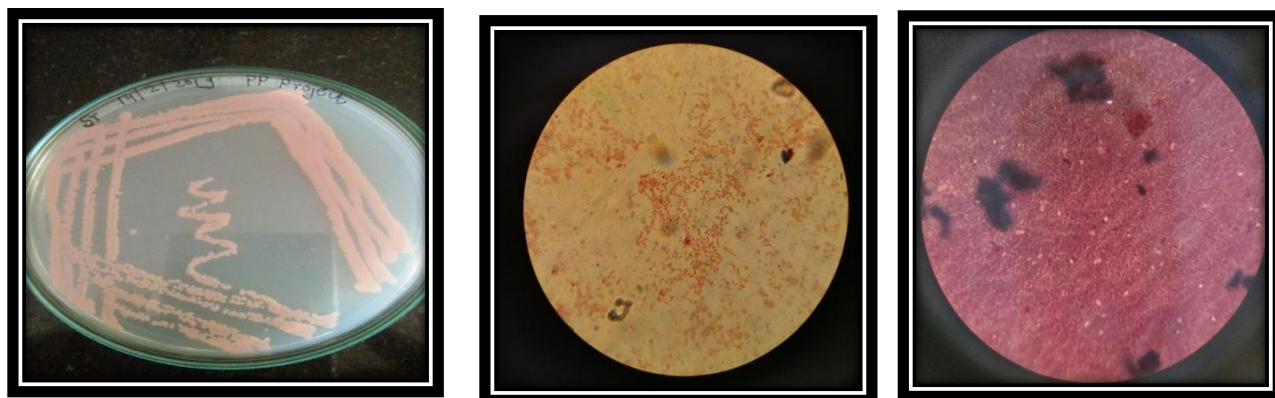


Fig 3: Streaked Colonies, Gram Staining, and Capsule stained

2.3 Production of Prodigiosin

The isolate was inoculated into different production media including nutrient broth, peptone–glycerol broth, and peanut powder broth, as different carbon and nitrogen sources significantly affect prodigiosin yield [5,16,17]. Cultures were incubated at 28 °C under shaking conditions.



Fig 4: Culture media

2.4 Extraction and Purification

Prodigiosin was extracted using acidified methanol, a commonly reported solvent for efficient pigment recovery [10,19]. Purification was performed using thin layer chromatography (TLC) on silica gel plates, and Rf values were compared with literature standards [19,25].

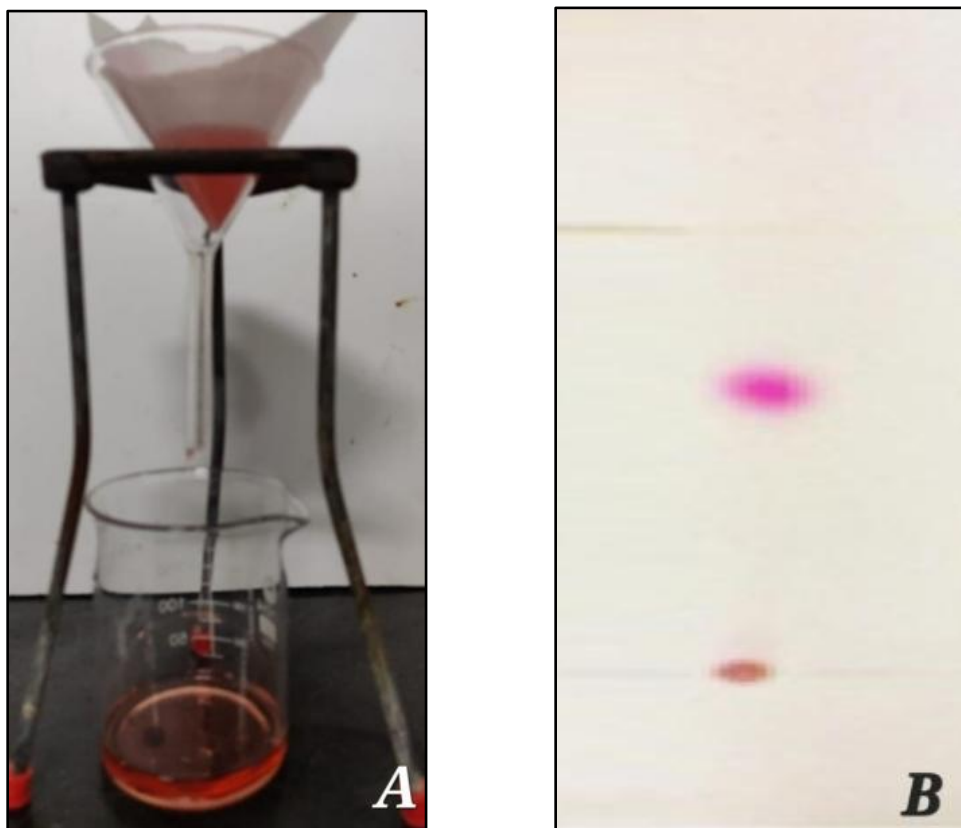


Fig 5: Extraction of pigment (A), TLC purification (B)

2.5 Characterization of Prodigiosin

UV–Visible spectroscopy was used to determine absorption maxima characteristic of prodigiosin [10,17]. FTIR analysis was performed to identify functional groups and confirm pigment structure [19,25].

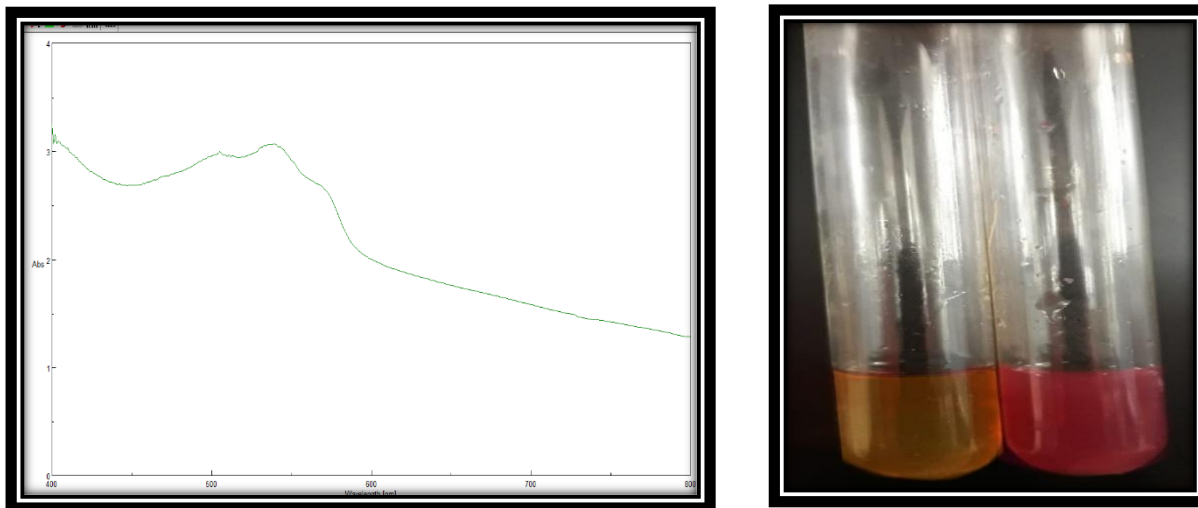


Fig 6: IR spectra and prodigiosin pigment with HCl, and Alkaline Ammonia solution

2.6 Optimization of Process Parameters

The effect of pH (5–9), temperature (20–37 °C), NaCl concentration (0–1%), and incubation period (1–7 days) on prodigiosin production was studied, as these parameters are known to regulate prodiginine biosynthesis [1,5,17,18]. The laboratory-scale production of prodigiosin from *Serratia marcescens* was systematically optimized by evaluating multiple process parameters to achieve maximum pigment yield. Among the four culture media tested, peanut broth supplemented with peanut powder (1g), yeast extract (0.1g), NaCl (0.5g), and maltose (0.05g) at pH 7.0 demonstrated the highest prodigiosin concentration of 2.7 g/liter with a dry cell weight of 2.13 g/100ml, significantly outperforming nutrient broth (1.6 g/l), LB broth (1.7 g/l), and peptone-glycerol broth (2.1 g/l). Temperature optimization revealed that an incubation temperature of 28°C was optimal, yielding 4.5 g/liter of prodigiosin, which was 2.1-fold higher than at 22°C (1.4 g/l) and substantially greater than at 25°C and 31°C (both 1.6 g/l). pH optimization studies showed that neutral pH 7 was most conducive to prodigiosin biosynthesis, producing 2.2 g/liter, while acidic (pH 6: 1.6 g/l) and

alkaline conditions (pH 8-9: 1.7-1.8 g/l) resulted in reduced yields. NaCl concentration significantly influenced pigment production, with 0.6% NaCl yielding the maximum concentration of 2.7 g/liter, compared to lower concentrations (0.2-0.4%: 1.6-2.6 g/l) and the absence of NaCl (1.7 g/l), indicating the salt's role as an osmotic regulator and energy source. Finally, incubation period optimization demonstrated that prodigiosin production was time-dependent, with accumulation beginning on day 2 (2.7 g/l) and progressively increasing to day 5 (5.3 g/l), representing an 84% increase over the initial two-day yield. These collective findings establish the optimal production parameters as peanut broth medium at pH 7, incubated at 28°C with 0.6% NaCl for five days, which collectively achieve maximum prodigiosin yield of 5.3 g/liter for scaled-up industrial production.

| Sr. no. | Parameters | Variables | Pellet dry weight mg/100ml | Prodigiosin conc. in g/10ml. | Prodigiosin concentration in g/lit |
|---------|-----------------|------------------------|----------------------------|------------------------------|------------------------------------|
| 1. | Different media | Nutrient broth | 1.24 | 0.016 | 1.6 |
| | | L.B. Broth | 0.92 | 0.017 | 1.7 |
| | | Peptone-Glycerol Broth | 0.5 | 0.021 | 2.1 |
| | | Peanut Broth | 2.13 | 0.027 | 2.7 |
| 2 | Temperature | 22°C | 0.9 | 0.014 | 1.6 |
| | | 25°C | 0.10 | 0.016 | 1.6 |
| | | 28°C | 0.24 | 0.045 | 4.5 |
| | | 31°C | 0.08 | 0.016 | 1.6 |
| 3. | pH | 6 | 0.09 | 0.016 | 1.6 |
| | | 7 | 0.18 | 0.022 | 2.2 |
| | | 8 | 0.16 | 0.018 | 1.8 |
| | | 9 | 0.12 | 0.017 | 1.7 |
| 4 | NaCl | 0.2% | 0.8 | 0.016 | 1.6 |
| | | 0.4% | 0.12 | 0.026 | 2.6 |

| | | | | | |
|----|---------------|-------------|-------------|--------------|------------|
| | Concentration | 0.5% | 0.28 | 0.021 | 2.1 |
| | | 0.6% | 0.28 | 0.027 | 2.7 |
| | | 0.8% | 0.14 | 0.017 | 1.7 |
| 5. | Days | 1 | 0.00 | 0.000 | 0 |
| | | 2 | 0.10 | 0.027 | 2.7 |
| | | 3 | 0.16 | 0.036 | 3.6 |
| | | 4 | 0.23 | 0.045 | 4.5 |
| | | 5 | 0.28 | 0.053 | 5.3 |

Table 1: Optimization of process parameters

2.7 Antimicrobial Activity

Antibacterial activity against Gram-positive and Gram-negative bacteria was evaluated using the agar well diffusion method [12,23]. Antifungal activity was tested against selected fungal strains following standard protocols [10,23]. The extracted prodigiosin pigment from *Serratia marcescens* demonstrated significant antibacterial activity against both Gram-negative and Gram-positive bacteria, as assessed through disc diffusion assays with varying concentrations (10, 20, and 30 µg/ml). Against *E. coli*, the highest zone of inhibition was observed at 24 mm with 30 µg/ml prodigiosin, outperforming other tested strains, while *Salmonella typhi* showed 16 mm at the same concentration. *Staphylococcus aureus* exhibited 20 mm inhibition at 30 µg/ml, and *Bacillus subtilis* displayed the lowest activity with just 4 mm at 10 µg/ml, confirming prodigiosin's dose-dependent efficacy and selective potency, particularly against *E. coli*.

Antifungal activity was similarly evaluated, revealing prodigiosin's inhibitory effects on common fungal pathogens. *Aspergillus niger* produced the largest zone of 16 mm at 30 µg/ml, followed by *Rhizopus* at 14 mm and *Aspergillus flavus* at 13 mm under identical conditions, with no inhibition in controls across all tests. These results underscore prodigiosin's broad-spectrum antimicrobial potential, supporting its applications in combating bacterial and fungal infections as a natural bio pigment.

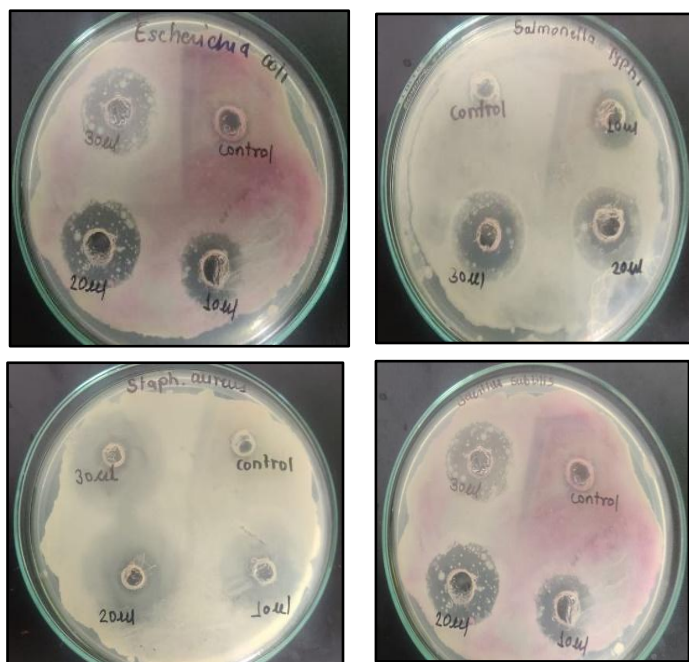


Fig 7: Antibacterial activity of pigment against *E. coli*, *S. typhi*, *S. aureus*, *B. subtilis*.

| Sr. no | Test Organism | Concentration of Prodigiosin in ug/ml | Zone of inhibition in mm |
|--------|--------------------------|---------------------------------------|--------------------------|
| 1 | <i>E.Coli</i> | Control | 0 |
| | | 10 | 8 |
| | | 20 | 16 |
| | | 30 | 24 |
| 2 | <i>Salmonella typhi</i> | Control | 0 |
| | | 10 | 6 |
| | | 20 | 12 |
| | | 30 | 16 |
| 3 | <i>Staph. Aureus</i> | Control | 0 |
| | | 10 | 12 |
| | | 20 | 16 |
| | | 30 | 20 |
| 4 | <i>Bacillus subtilis</i> | Control | 0 |
| | | 10 | 4 |
| | | 20 | 9 |
| | | 30 | 14 |

Table 2. Antibacterial activity, test organism Vs Zone of inhibition

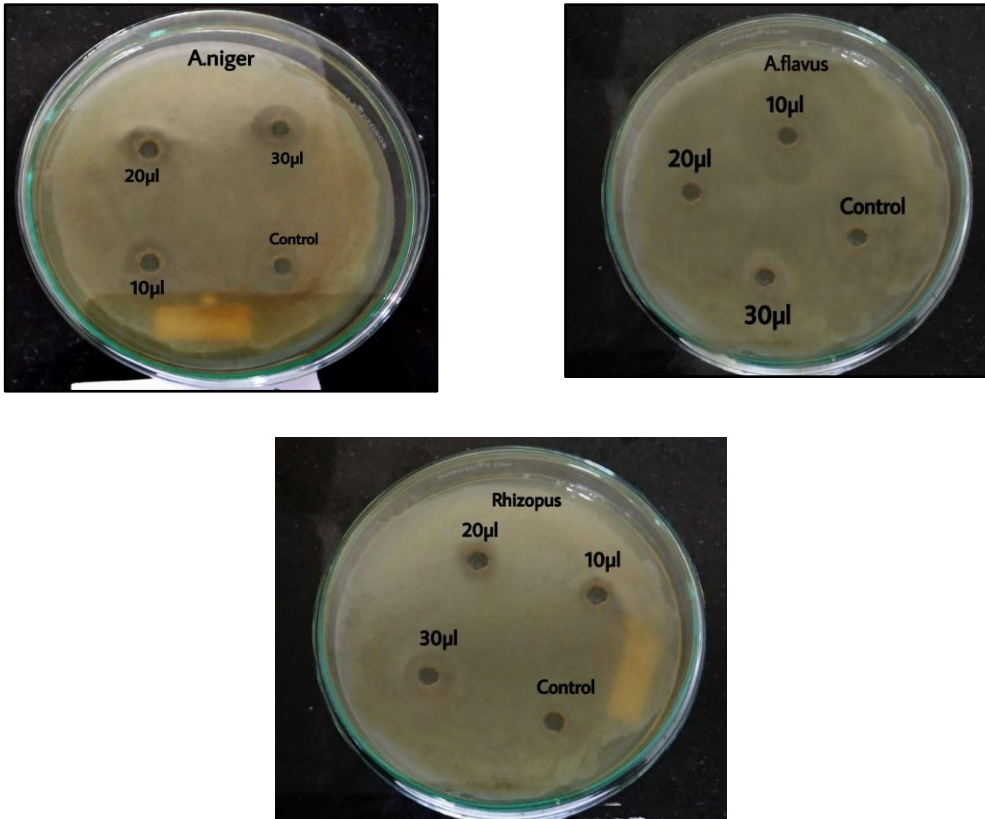


Fig 8: Antifungal activity of pigment against *A. niger*, *A. flavus*, *Rhizopus*.

| Sr. no | Test Organism | Concentration of Prodigiosin in ug/ml | Zone of inhibition in mm |
|--------|------------------|---------------------------------------|--------------------------|
| 1 | <i>A.niger</i> | control | 0 |
| | | 10 | 9 |
| | | 20 | 14 |
| | | 30 | 16 |
| 2 | <i>A.flavous</i> | Control | 0 |
| | | 10 | 8 |
| | | 20 | 11 |
| | | 30 | 13 |
| 3 | <i>Rhizopus</i> | Control | 0 |
| | | 10 | 10 |
| | | 20 | 12 |
| | | 30 | 14 |

Table 3. Antifungal activity, test organism Vs Zone of inhibition

3. Results and Discussion

Isolation from food-waste-containing soil yielded a potent prodigiosin-producing *Serratia marcescens* strain, supporting earlier reports that nutrient-rich substrates enhance pigment production [16,17]. Among the tested media, peanut powder broth resulted in the highest prodigiosin yield, likely due to its lipid and carbon-rich composition, which favors secondary metabolite synthesis [16].

Optimization studies revealed that prodigiosin production was maximal at pH 7 and 28 °C, consistent with previous findings that acidic or alkaline stress and higher temperatures suppress pigment biosynthesis [5,17,18]. The presence of NaCl was essential, with optimal production at 0.6%, supporting reports on osmotic regulation of prodigiosin biosynthetic genes [5,18]. Maximum pigment yield (5.3 g/L) was observed after five days of incubation, corresponding to the stationary phase of growth, as reported earlier [2,7].

TLC analysis showed an R_f value of 0.62, matching reported values for purified prodigiosin [19,25]. UV–Visible and FTIR analyses further confirmed the identity and purity of the pigment. The extracted prodigiosin exhibited strong antibacterial and antifungal activities, in agreement with earlier studies demonstrating its broad-spectrum antimicrobial potential [12,23].

4. Conclusion

The study successfully demonstrates the isolation, laboratory-scale production, optimization, and characterization of prodigiosin from *Serratia marcescens*. Optimization of nutritional and environmental parameters significantly enhanced pigment yield. The purified prodigiosin exhibited potent antimicrobial activity, reaffirming its promise as a bioactive compound for pharmaceutical, agricultural, and industrial applications. These findings provide a strong foundation for future scale-up, genetic regulation studies, and formulation development.

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