

Production of prodigiosin from *Serratia marcescens* isolated from soil

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Abstract: Prodigiosin is a promising drug owing to its reported characteristics of having antifungal, immunosuppressive and anti-proliferative activity. Prodigiosin was first characterized from *Serratia marcescens*. Investigations were made in the present study to screen new strains of *Serratia* that effectively produce prodigiosin. A new strain designated as *S. marcescens* NY1 was isolated. The parameters viz., temperature, pH, sugar substrate and oil substrate were optimized to increase the production of prodigiosin. It was observed that maximum amount of prodigiosin was produced at temperature 30°C and pH 7.0. Among the different sugar substrates tested maltose when amended in the medium yielded 425±40 mg/L of prodigiosin. Oil substrates play a vital role in prodigiosin production. Among the various oil substrates used the production of prodigiosin was maximum (535±45 mg/L) when the medium was amended with peanut oil.

Keywords: Prodigiosin, *Serratia marcescens*, biopigments

Introduction
Natural products represent one of the critical sources of chemical diversity and potential medicinal use. Pigments produced by organisms as reminiscence of its secondary metabolism are commonly referred as biopigments. These biopigments have wide synthetic and commercial application (Shirata *et al.*, 2000). Prodigiosin a red pigment produced by many strains of the bacterium like *Serratia marcescens* and some other unrelated microbial strains, such as *Vibrio psychroerythrus*, *Streptomyces griseoviridis* and *Hahella chejuensis* was found to exhibit antibacterial, antimycotic, immunomodulating, anti-tumor and anti-malarial properties (Frustner, 2003). Demain *et al.*, (1995) has reported that prodigiosin revealed a broad range of inhibitory activities against many bacterial, fungal and protozoan species while he tested these organism against the naturally produced anti microbial products.

Prodigiosin induced apoptosis in cancer cell lines by the characteristic DNA laddering pattern and condensed nuclei or apoptotic bodies were reported (Beatriz, 2000). Williamson *et al.*, (1992) had reported that sequencing and heterologous expression of the pig cluster responsible for prodigiosin biosynthesis in two *Serratia* sp. Srijith (2006) suggested that it is possible to genetically transform the enzymes responsible for prodigiosin production from *Serratia marcescens* to *Bacillus subtilis*.

The present study focuses on screening new strains of *Serratia* that produces prodigiosin and to optimize the culture medium for effective pigment production.

Materials and methods

Soil samples were collected from different parts of Yercaud hills and screened for *Serratia marcescens*. The collected soil samples were serially diluted and plated on nutrient agar plates. Red-pigmented colonies were isolated from the plates after incubation and preserved at 4°C. The isolated colonies were identified based on Bergey's classification of determinative bacteriology.

A loopful of culture was inoculated in pre-sterilized 100ml nutrient broth. The flask was kept in a shaker at 120 rpm for 16-18h at 30°C. The culture broth was centrifuged at 10,000 rpm for 20 min. Cell suspension was prepared using sterile distilled water and adjusted to 0.2 OD using UV Visible spectrophotometer. One percent (10⁵ CFU/ml) of the above suspension was used as inoculum for the production of Prodigiosin. The bacterial isolate was subcultured in 100 mL of nutrient broth and incubated in a rotary shaker for 48 h at 37°C.

Estimation of Prodigiosin (Slater *et al.*, 2003)

The organisms were harvested by centrifuging at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was resuspended in acidified ethanol (4% of 1M HCl in 96 mL ethanol). The mixture was vortexed and the suspension was centrifuged at 10,000 rpm for 10 minutes. The supernatant was transferred to a fresh vial and observed under UV-visible spectrophotometer at 534 nm. The prodigiosin produced was quantified using known concentrations of prodigiosin.

Effect of temperature on pigment production

Equal volume of the bacterial isolate was inoculated in nutrient broth and incubated at different temperature viz., 25, 30, 35 and 40 °C for 48 h. The prodigiosin production was estimated after incubation. The temperature at which maximum production of prodigiosin was observed was chosen and maintained in the following studies.

Effect of initial pH on pigment production

Equal volume of the bacterial isolate was inoculated in nutrient broth with various initial pH viz., 6.5, 7.0, 7.5 and 8.0. The flasks were incubated at 30°C for 48 h. The prodigiosin production was estimated after incubation. The initial pH at which maximum production of prodigiosin was observed was chosen and maintained in the following studies.

Effect of different sugar substrate on pigment production

To study the effect of different sugars, as substrate on pigment production the isolate was cultivated in the presence of different sugars viz., maltose, glucose, lactose and sucrose (2%) in nutrient broth. The pH of the

broth was adjusted to 7.0. Fresh-pigmented culture (5%) was added to each of the broth. The flasks were then incubated at 30°C for 48 hours. After incubation the amount of prodigiosin produced in each medium was estimated.

Effect of different oil substrate on pigment production

The effect of different oil substrates on pigment production were tested by adding 2 % peanut oil in 50 ml nutrient broth, 2% sesame oil in 50 ml nutrient broth, 2% coconut oil in 50 ml nutrient broth and 2% neem oil in 50 ml nutrient broth. The pH was adjusted to 7.0. Fresh-pigmented culture (5%) was added to each of the broth.

The cultures were incubated at 30° C for 48 hours. After incubation the amount of prodigiosin produced in each medium was estimated.

All the experiments were conducted in triplicates unless otherwise stated. Data points in tables and figures represent means, with all error bars shown (± 1 standard error of mean). Both mean and standard deviation performed were appropriate, using the statistical package on Microsoft® Excel version 2003.

Results

Twenty soil samples were collected from different parts of Yercaud hills and screened for prodigiosin producing organism. Among the 20 soil samples Red-pigmented colonies were isolated from 5 soil samples. The isolated colonies were identified based on their morphology and biochemical characteristics. The isolates were confirmed as *Serratia marcescens*.

The amount of prodigiosin produced by the 5 bacterial isolates was estimated. Among the 5 the yield was found to be more in 1, which was designated as *Serratia marcescens* NY1. This isolate was taken for further cultivation under submerged fermentation and the amount of prodigiosin was found to 335 ± 10 mg/L. The

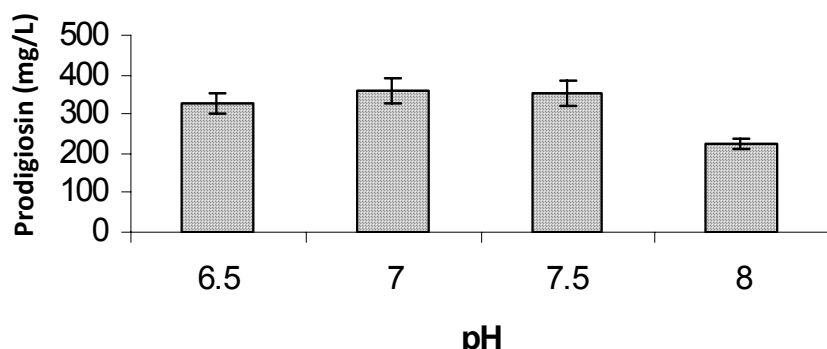


Fig. 2. Effect of pH on Prodigiosin production in *S.marcescens*

bacterial isolate was cultivated at different temperature and the amount of prodigiosin was maximum at 30°C (Fig. 1). The influence of initial pH of the growth medium on prodigiosin production was investigated. The prodigiosin production was maximum at pH 7.0 (Fig. 2).

The influence of sugar substrates (maltose/ glucose/lactose/ sucrose) in the growth medium on prodigiosin production was elucidated. The production was maximum in the presence of maltose. Moderate level of pigment production was observed in medium amended with lactose and sucrose. The presence of glucose in the medium did not increase the

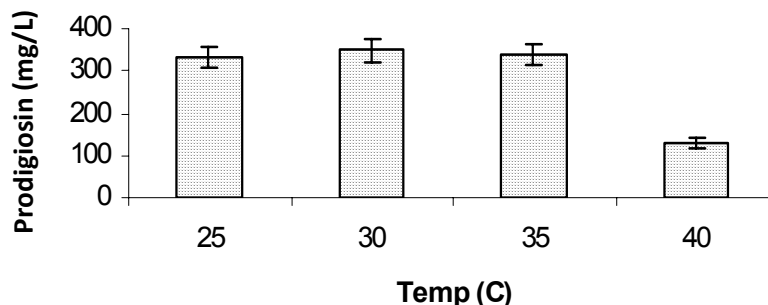


Fig. 1. Effect of temperature on Prodigiosin production in *S. marcescens*

production of prodigiosin (Fig 3).

Effect of different oil substrates on pigment production

The bacterial isolate was cultivated in medium amended with peanut, sesame, coconut and neem oil. It was observed that maximum amount of prodigiosin resulted in the medium supplemented with peanut oil followed by sesame oil, coconut oil and neem oil (Fig 4).

Discussion

Biopigments produced by bacteria possess enormous efficiency as medicinally important products. Prodigiosin, a red pigment, belongs to the family of tripyrrole was found to exhibit antibacterial, antimycotic, immunomodulating, anti-tumor and anti-malarial properties. These pigments were found associated with the cell wall vesicle of the bacteria. The present investigation centered on isolation of prodigiosin producing organisms from soil and formulating a production medium for effective production of prodigiosin.

The bacterial isolate elaborated the pigment at 30°C and the rate was reduced as the temperature increases. Williams & Hussain Quadri (1980) reported that no prodigiosin was produced when cultures were incubated at 38°C; however pigment production was observed when the temperature was shifted to 27°C. A complete block in prodigiosin was observed in most of the basically used media tested at 37° C was similar to the result observed by Pryce & Terry (2000).

While considering the basic role of carbon source in augmenting the pigment production, the addition of maltose or sucrose or lactose was shown to enhance the prodigiosin yield and addition of glucose was found to be ineffective. Anna R Oller (2005) reported that glucose and sorbitol had a repressive effect on prodigiosin synthesis. Chang *et al.*, (2000) has reported 3 mg/ml of prodigiosin when dextrose

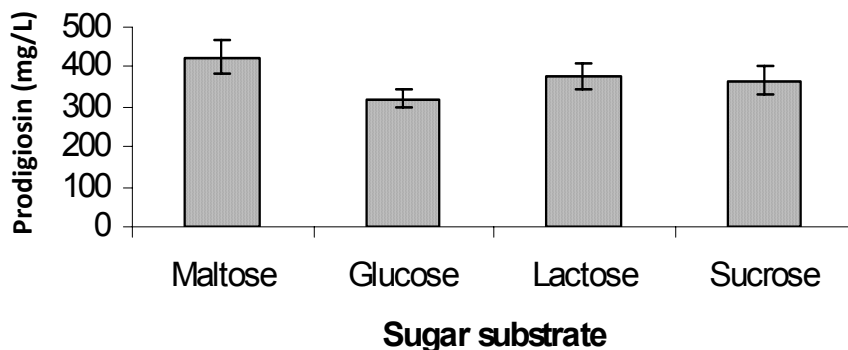


Fig 3. Effect of different sugar substrate on Prodigiosin production

was used in the medium. Jungdon *et al.*, (2001) reported that in a bioreactor study with an internal absorbent for prodigiosin, the final yield was 13 mg/ml in which dextrose was used in the culture broth.

The suitable fatty acid for pigment production was optimized by cultivating the bacterial isolate in the presence of peanut, sesame, coconut and neem oil. In the case of fatty acid alternatives, peanut oil broth gave a maximum yield (~560 mg/l) and this result was compared with the existing literatures that uses different carbon source. The observed enhancement in the yield of prodigiosin in peanut oil has been attributed to its higher saturated fatty acid content. On the other hand, sesame oil broth contains a higher level of unsaturated fatty acid (approx. 47%) than the rest. From these arguments it was speculated that the bonded fatty acids are of limited carbon source and less accessible by *S. marcescens*. Giri *et al.*, (2004) reported peanut seed powder increased the production of prodigiosin and they have concluded that the saturated form of fatty acid plays a role in enhanced cell growth and prodigiosin production.

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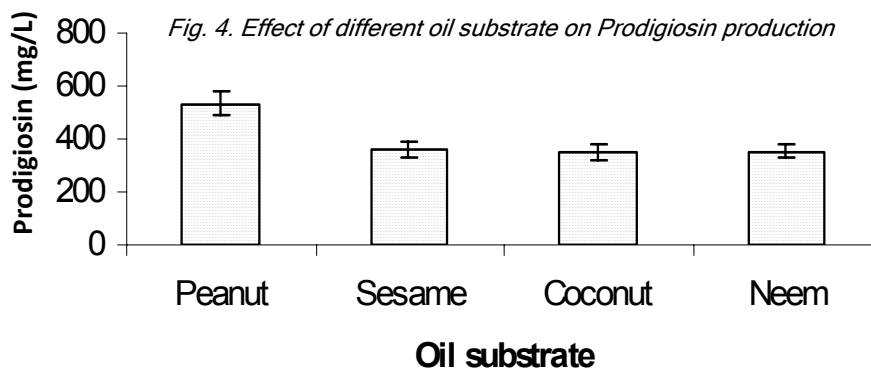


Fig. 4. Effect of different oil substrate on Prodigiosin production

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