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RESEARCH ARTICLE

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# PRELIMINARY STUDY AND CHARACTERIZATION OF A PRODIGIOSIN PRODUCING SOIL BACTERIA AND APPLICATIONS OF BIOPIGMENT IN NATURAL COSMETICS

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**A R T I C L E I N F O**

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**A B S T R A C T**

Demand for cosmetics and personal care products has increased world over. To enhance aesthetic potential, many cosmetics contain a botanical chromophore. Soil prokaryotes can be used as biofactories for harvesting prodigiosin, for commercial purposes. In this preliminary study, prodigiosin producer was isolated from garden soil from South Mumbai region. The isolate was identified based on general microbiological techniques and Bergey's manual. Cultural characterization studies were undertaken to establish enhanced biopigment production with longer stability. In this research, bacteria were chosen over botanical extracts due to general ease of handling, quick cultivation and rapid cost effective down streaming.

Prodigiosin extracted from *Serratia sp.* was used in designing cosmetic and personal care products. Nail paint and antibacterial beauty bars were made during this preliminary investigation. Future studies on Prodigiosin will help in establishing its promising role in other sectors like textile and leather industry.



## INTRODUCTION

### REVIEW OF LITERATURE

The current splurge in urbanization and privatization has led to increase in the number of MNC's. In most of these jobs there is always a constant need for working professionals to look their best.[1] Market today is flooded with diverse cosmetic and personal care products. Some of these products contain harmful and synthetic chemicals, which poses serious health hazards [6]. This has led to use of natural ingredients in cosmetics.

Many cosmetic products require to be coloured to enhance its aesthetic and cosmetic value. Colours added to these products can be extracted from natural sources like plants and microbial sources. Biopigments from microbes have been preferred over plants because of their rapid growth and their availability throughout the year because of cultivation technology[2,3].

Prodigiosin is a red colored biopigment belonging to the family prodiginines and has a tripyrrole in its structure (Grimont and Grimont, 1978, Venil *et al* , 2009 ,)[1,2,3,4,8,9].
Species of *Serratia*, *Pseudomonas and Streptomyces* are producers of extracellular red orange pigment prodigiosin [5]. This biopigment can be harvested from producer microbes by using simple extraction in organic solvents [9,10,13]. The microbial prodigiosin has found application in leather, tanning, textile , cosmetic, dye, Apiculture, food and FMCG industries[6,12, 13,14,].

The main aim of this project was to enhance the production of prodigiosin and extract the pigment from *Serratia sps* which is a normal soil flora. This chromogenic biotype owing to its antimicrobial and anticancerous properties (Carbonell *et al* 2000) can be used in natural cosmetics like nail enamels and beauty bars.

## MATERIAL AND METHODOLOGY

### *Isolation of Prodigiosin producer*

Soil sample was collected from South Mumbai region in a sterile container. Soil sample was used for isolation of prodigiosin producer after serial dilution and plating on sterile Nutrient Agar plates. Pigmented prokaryotic colonies were selected and further purified by serial subculturing method.

### *Characterization of Prodigiosin producer*

Identification studies were carried out using microscopy and staining techniques. Biochemical tests were carried out to further characterize and identify the selected prodigiosin producing bacteria.

### *Preliminary identification and Estimation of prodigiosin*

- Nutrient Broth with culture (O.D $_{500nm}$ - 0.85)was centrifuged at 10,000 rpm for 10 minutes.
- The supernatant was discarded and pellet was resuspended in 95% methanol to extract pigment from the cells.
- The suspended pellets were centrifuged at 10,000 rpm for 10 minutes. Debris were removed and supernatant was taken in two test tubes.
- The content of one of the tube was divided in to two parts. One part was acidified with a drop of conc. HCl and the other was alkalinized with conc. ammonia solution.

- A red or pink color in acidified tube and a pale yellow color in alkaline solution indicated a positive presumptive test for prodigiosin.

***Enhancement of pigment production***

Prodigiosin production was enhanced by growing isolated *Serratia sp*.on different microbiological nutrient media viz. Blood Agar, Luria Bertani LB Agar, Starch Agar and Peptone Glycerol Agar.

***Pigment Extraction Protocol***

**Step 1:** Inoculate 50ml of St.Nutrient Broth with pure culture of pigment producing organism, incubate at RT/ 24-48 hours.
**Step 2:** Add 200ml of ethanol to the acidified broth and transfer to shaker for 3 hours at room temperature.
**Step 3:** Decant the ethanol and rewash cell suspension with fresh ethanol.
**Step 4:** Filter using Whatmann filter paper 1.
**Step 5:** Now extract the pigment using petroleum ether in a separating funnel.
**Step 6:** Evaporate Petroleum ether in a crucible in incubator.

***Estimation of Prodigiosin***

Extracted prodigiosin was estimated using the following equation:

$$\textbf{Prodigiosin unit/cell} = \frac{[\ OD_{499} - (1.381 \times OD_{620})\ ] \times 1000}{OD_{620}}$$

$OD_{499}$ - pigment absorbance, $OD_{620}$ - bacterial cell absorbance, 1.381 - constant

***Product developmet using prodigiosin***

***Preparation of biopigment solution***

Nutrient broth with cultured cells was centrifuged. The cell free supernatant containing pigment was boiled in boiling water bath for 10 minute. The cooled pigment extract was plated on sterile nutrient agar plate to check for presence of any viable cells.

***Protocol for making antibacterial soap***

Step 1: Grind the pre weighed quantity of soap noodles in a mixer grinder.
Step 2: Heat the soap noodles in D/W till a liquid like consistency.
Step 3: Add required fragrance 6-7 drops and stir for 3-4 minutes.
Step 4: Add 5-10 ml solution of prodigiosin.
Step 5: Stir the mixture for 3-4 minutes.
Step 6: Pour the mixture in a mould and allow it to set at $4^0$C.

***Protocol for making nail enamel***

Step 1: Take 5.0 ml of nail top coat in a suspension tube.
Step 2: Add the required fragrance.
Step 3: Add about 0.5 ml of pigment. Vortex all ingredients well.
Step 4: Transfer aliquots to eppendorf tube.
Step 4: Apply the ready nail paint on artificial nails.

## RESULT AND DISCUSSION

Prodigiosin producer was isolated from South Mumbai soil. The organism was identified as *Serratia sp.* based on microscopic identification, biochemical testing and Bergey's Manual.

(Table1, Fig: 2.1, Fig 2.2, Fig 2.3, Fig 2.4)

The pigment was identified as Prodigiosin[4] [1-4methoxy-5-((5-methyl-4-pentyl-2H-pyrrol-2-ylidene) methyl)-2,2'-bipyrrole, molecular weight 323.43 Da. (Fig:1)

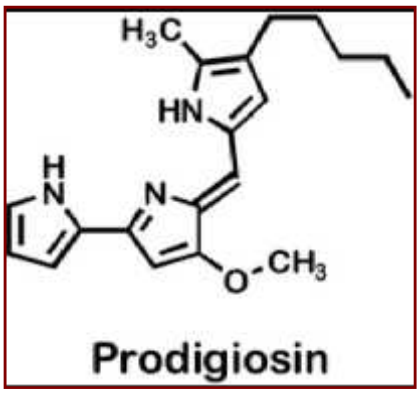


**Fig 1** Prodigiosin

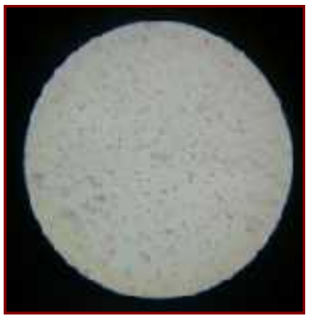

**Fig.2.1:**-Gram Staining

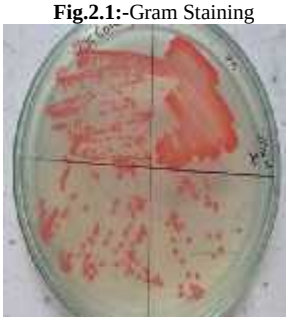

**Fig.2.2:**-Isolation on Nutrient Agar Plate

**Fig.2.3** Sugar fermentation tests

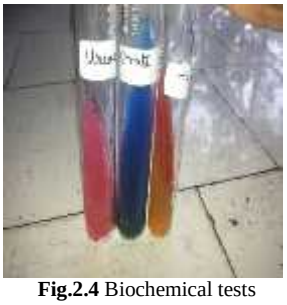

**Fig.2.4** Biochemical tests

**Table 1** Biochemical tests

| LAC | MALT | MANN | SUC | GLU | INDOLE | MR | VP | CITRATE | TSI |
|---|---|---|---|---|---|---|---|---|---|
| - | A | A | A | A | + | - | + | + | + $H_2S$ (48 HRS) |

Legend: Lac-Lactose
Malt-Maltose
Mann-Mannitol
Suc-Sucrose
Glu-Glucose
MR-Methyl Red Test
VP-Voges Proskaeur Test
Citrate-Simmon Citrate Test
TSI-Triple Sugar Iron Test

Pigment gave red and yellow color under acidic and alkaline conditions respectively which further proved that the extracted pigment was prodigiosin.

The color of colonies produced by the isolated *Serratia Sp*. on different media is listed in the following table. (Table 2) (FIG:3)

**Table 2** *Serratia* growing on different media

| Nutrient Agar | Blood Agar | Starch Agar | Luria Bertani Agar | Peptone Glycerol Agar |
|---|---|---|---|---|
| Orange colonies | Orange colonies | Orange colonies | Dark orange colonies | Red colonies |

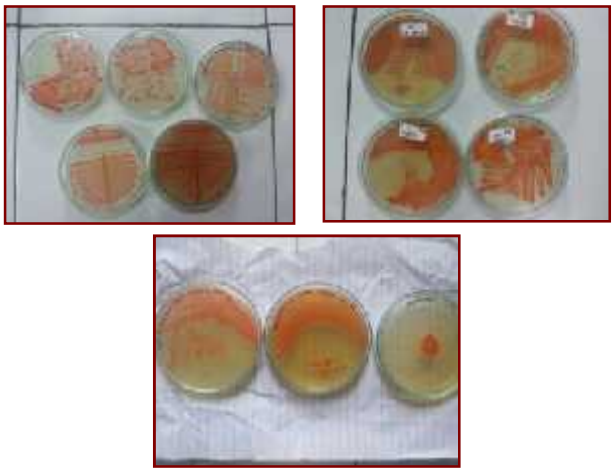

**Fig.3** *Serratia* growing on different media

Our studies showed that the intensity of pigment produced by *Serratia* remained fairly constant on prolonged incubation.
The Prodigiosin production was observed within 24 hrs at room temperature. The intensity of the color was fairly stable even upon prolonged incubation. The pigment was found to be stable when exposed to sunlight. (Table 3).

**Table 3**Absorbance of Prodigiosin measured at 500nm.

| Day | $O.D_{500}$ |
|---|---|
| 1 | 0.98 |
| 2 | 0.98 |
| 7 | 0.95 |
| 15 | 0.95 |
| 30 | 0.95 |

Prodigiosin was insoluble in water but soluble in organic solvents like chloroform, methanol, acetone etc. This property of prodigiosin enabled its extraction in ethanol (Fig:4).

**Fig.4** Prodigiosin extraction procedure

The extracted prodigiosin was used in production of nail paints and beauty bars

(Fig: 5, Fig.6).

**Fig.5** Lab scale Soap manufacturing

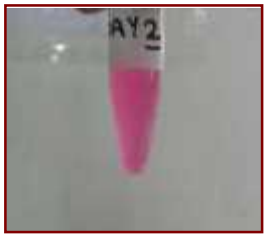


**Fig.6** Lab scale nail enamel

Water insoluble Prodigiosin was extracted using mixture of organic solvents like Methanol, Petroleum ether and ethanol. The pigment extracted was plated on sterile nutrient agar plate. The NA plate after incubation was free from any viable cells and hence was suitable candidate for its use in soaps and nail paints. Stable and attractive nail paints and skin friendly antibacterial beauty bars were made in the lab.

## CONCLUSION

Prodigiosin extracted from *Serratia sps* during this research work was found to be a suitable biopigment for development of soap and nail paint. It is easy to extract prodigiosin from bacterial cultures for commercial interest. Our research is in agreement of using bacteria as a potential producer of prodigiosin in comparison to plant sources for they are easy to handle, maintain and manipulate[1,13].

Our Prodigiosin is a suitable and promising candidate in various clinical, cosmetic and environmental applications. This research work proposes that prodigiosin is stable at room temperature and also when exposed to sunlight for a prolong period of time.

These properties of Prodigiosin can be utilized especially in cosmetics and personal care products.

A rapidly growing number of pyrrole alkaloids isolated from various sources deserves attention for their attractive biological properties[8] .Amongst them, the Prodigiosin family is particularly remarkable for their diverse range of biological effects, although much deeper insight into the mode of action of these compounds is required before a fully consistent and conclusive picture of Prodigiosin family is drawn. Future studies on this pigment will enable the establishment of Prodigiosin and its application in other fields like textiles, leather industry besides beauty products like hair colour, eye shadows, eye liner, lotions, creams etc.

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