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# ISOLATION AND IDENTIFICATION OF BIOACTIVE PIGMENT PRODIGIOSIN PRODUCED BY *SERRATIA MARCESCENS* AND ITS APPLICATION

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## ABSTRACT

*Serratia marcescens* is a Gram-negative member of the Enterobacteriaceae family, and it exhibits considerable variability in pigment production, particularly the red pigment known as prodigiosin. The production of prodigiosin is influenced by several factors, including the species type and the duration of incubation. Prodigiosin synthesis typically occurs in the later stages of bacterial growth, and while it doesn't serve any apparent physiological purpose in the organism, it has been reported to possess antimicrobial, antifungal, antiprotozoal, and antitumor properties. In terms of its antibacterial capabilities, prodigiosin *from Serratia marcescens* has demonstrated activity against a wide range of organisms. Furthermore, it's worth noting that when *Serratia marcescens* is cultured on tributyrin agar, it exhibits robust lipase production. This present study is carried out for the "Antibacterial activity of prodigiosin and lipase activity of Serratia marcescens".

**Keywords:** *Serratia Marcescens* , Prodigiosin ,Coconut , Antimicrobial Activity , Pigment Production

## I. INTRODUCTION

Natural products, whether synthesized or secreted by living organisms, represent a valuable source of potential medicinal applications. Among these, secondary metabolites stand out as low molecular weight compounds produced by organisms without any discernible function for the secreting cells. Secondary metabolites encompass a diverse range of substances, including pigments, steroids, enzymes, and antibiotics, which find extensive use in therapeutic treatments. Bioactive pigments are sourced from various origins, including plants, microorganisms, and more. Microbially produced bioactive pigments are often preferred over those from plants due to their stability and ready availability. In everyday life, both natural pigments and synthetic dyes play pivotal roles in multiple industries such as food, textiles, paper, printing inks, cosmetics, and pharmaceuticals. The importance of color in determining consumer acceptance is evident across these sectors. Given concerns about the toxicity of several artificial colorants, there is a growing trend toward utilizing natural additives. This has led to increased emphasis on the production of bio colors or natural colors extracted from fruits, vegetables, and microorganisms. The industrial production of natural colorants has already gained a strong foothold and continues to expand.

One noteworthy natural pigment is prodigiosin, which is synthesized by various bacteria, including Actinomycetes, Streptomyces, and *Serratia marcescens* . Prodigiosin has demonstrated significant therapeutic potential. as Dnase, lipase, and gelatinase. Additionally, this bacterium is characterized by its production of a cell-associated red pigment. Serratia species thrive on conventional media, similar to other Enterobacteriaceae, and can grow under both anaerobic and aerobic conditions using various carbon sources at pH 9 and temperatures ranging from 20-37°C. These bacteria are commonly found in soil, water, plants, insects, animals, and even in humans. The genus Serratia encompasses several species, including *Serratia pymuthica, Serratia odorifera, Serratia ficaria, Serratia liquefaciens, Serratia rubidaea, and Serratia fonticola*.

Secondary compounds produced by bacteria encompass a wide range of substances, such as enzymes, pigments, antibiotics, and more. These compounds have the potential to hold significant value for humanity in various applications. *Serratia marcescens* produce a pigment known as prodigiosin, is highly variable among species and is dependent on many factors such as species type and incubation time. Prodigiosin have been revealed to be associated in extracellular vesicles, or in intracellular granules. Prodigiosin, family of natural pigments, characterized by pyrrolopyrimidine common skeleton of low molecular weight, appearing only in later stages of bacterial growth and calledprodigiosinine, common to all the members of this family such as cycloprodigiosin, metacycloprodigiosin, dipyrrolildipirromethane, all which have a common pyrrolyldipyrrolyl-methane skeleton and it comprise three rings which form a pyrrolyl methane skeleton with the molecular formula C20H25N3O. Two of the rings are directly linked to each other and the third one is attached through a

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methane bridge. Macrocylicprodigiosins appear to be derived from undecycylprodiginine by oxidative cyclisation.

Chromogenic biotypes sourced from natural environments have rarely resulted in infections, and clinical isolates seldom display any pigmentation. The precise role of the red pigment known as prodigiosin remains enigmatic, with no clear physiological function identified within the producer strains. Prodigiosin has garnered significant interest across various domains, showcasing immense potential for clinical and environmental applications. Its versatility spans antibacterial, antifungal, anticancer, anti-neoplastic, antioxidant, anti-diabetic, anti-malarial, anti-tumor, anti-protozoal, cytotoxic, immune-suppressive, and anti-foulant properties.

Various prodigiosin differ primarily in the length of the alkyl side chain. cycloprodigiosin, meta cycloprodigiosin and nonyl prodigiosin have a cyclic side chain. A novel nalkylatedprodigiosin analogue 2, 2'-[3-methoxyl-1 amyl-5'methyl-4-(1'pyrryl)] di pyrrylmethane (MAMPDM) has been isolated from an organic solvent . Due to the frequent association of cell proliferation with both the immune system's functionality and the emergence of cancer cells, numerous anti-cancer agents tend to possess immunosuppressive properties. Prodigiosin's ability to inhibit the cell cycle and induce apoptosis renders it an appealing prospect for cancer treatment. The cytotoxic potential of the pigment was observed in the 60 cell lines panels of human tumour cells derived from lung, brain, ovarian, melanoma leukaemia, renal and colon. Prodigiosin pigment which are cytotoxic to human small cell lung carcinoma cells that are resistant to doxorubicin and over expressing multi drug resistance related protein. The presence of prodigiosin and metacycloprodigiosin in culture broth of *Serratia marcescens* observed selective and inhibition of polyclonal proliferation of T-cells as compared to that of B cells.

*Serratia marcescens* is known for its ability to produce a variety of extracellular enzymes, including chitinase. This bacterium is highly proficient in breaking down chitin, making it one of the most effective organisms in chitin degradation. *Serratia marcescens* produce three chitinases such as chi A, chi B, and chi C, chitobiose and chitin binding protein (CBP21). *Serratia marcescens* chino lytic machinery is immense, because it is one of the bacterium with synergisticinhibitory enzymes which was against spore germination of botrytis cinerea. *Serratia marcescens* is identified as they produce more concentration of prodigiosin. The cell lines were cultured in the Dulbecco's modified Eagle (DME) medium along with various concentrations of prodigiosin. After 24 hrs the percentage of cell viability was evaluated by 3- (4,5 dimethyl thiazol2yl) 2,5di phenyl tetrazolium bromide and neutral red. Prodigiosin showed dose dependent inhibition of cell proliferation . The findings from the research indicate that the prodigiosin pigment exhibits significant anticancer and apoptosis-inducing properties when tested against human cervical carcinoma cancer cells.

Fresh coconut meat is renowned for its exceptional nutritional value, boasting a wealth of saturated fats, natural sugars, and potassium, all of which contribute to a broad spectrum of health advantages. Unfortunately, the pristine quality of this coconut flesh is often compromised by the presence of a red pigment-producing bacterium known as Serratia. Microbial pigments are the natural pigments preferred the most among other natural sources of pigments. This is due to several advantages offered by them: Fast growth rate, Easy doubling time, cheap culture medium, Stability of microorganisms, Availability of cultural conditions throughout the year, Different shades of colour are obtained, High yield and Lower residues, etc

## II. AIM & OBJECTIVES OF THE STUDY

* Isolation and characterization of *Serratia marcescens* .
* Extraction and identification of bioactive pigment prodigiosin.
* To estimate the amount of prodigiosin per unit cell.
* To determine the antimicrobial activity of prodigiosin pigment.
* To determine lipase activity of *Serratia marcescens* .

## III. MATERIALS AND METHODS

**Collection of contaminated coconut**

Pinkish reddish discoloured spoiled coconut was collected and red pigment producing bacterium was isolated from spoiled coconut. The samples were brought to the laboratory and stored under refrigerator temperature.

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**Isolation of *Serratia marcescens* from contaminated coconut.**

In order to isolate *Serratia marcescens* from coconut samples, the pink coloured portion of spoiled coconut is directly inoculated to nutrient agar plates by using inoculation loop and incubated at 37°C for 24-48hrs. Since pigmented colonies of *Serratia marcescens* are visible in stationary phase of growth. The red pigment producing strain thus obtained was then subjected to subculture for obtaining pure culture. Then the isolate was subjected to morphological and biochemical analysis for identifying the organism.

**Morphological analysis by gram staining**

The bacterial smear was heat fixed. The slide was flooded with crystal violet staining reagent for one minute. The slide is again flooded with Gram's iodine solution for one minute. The slide was washed and decolorized with 95% ethanol for few seconds. The slide was again flooded with water and then the smear was immersed for two minutes in counter stain safranin. The slide was washed, dried and observed under microscope (10X/100X).

**Biochemical analysis of the isolate Indole test : -**

The isolates were subjected to biochemical studies. Standard Biochemical tests included Indole, Methyl red, Voges Proskauer and Citrate Test, TSI slant, Glucose, Lactose, sucrose and fructose test.

**Extraction of Pigment**

*Serratia marcescens* was cultured in 2% peanut seed medium (Giri et al., 2004). Peanut seed were finely powdered and 1g was added to 50 ml distilled water in a 250ml Erlenmeyer flask. The pH was adjusted to 7.0. This was sterilized at 121°C and 5% broth culture was inoculated. The medium was incubated for 3 days under static condition at 28°C for obtaining high amount of pigment.

**Presumptive test for prodigiosin:-**

The culture broth was centrifuged at 4500 rpm for 15 minutes 10 ml of 95% methanol was added to the cell pellet and centrifuged under the same condition. Debris was removed and the 2ml of the supernatant was taken into 2 test tubes. The content of one of the test tube was acidified with the drop of concentrated HCl and the other alkalinized with a drop of concentrated ammonia solution. The tubes were observed for colour change to red or pink colour in the acidified solution and yellow or tan colour in the alkaline solution. This gives a positive presumptive test for prodigiosin.

**Estimation of prodigiosin**

The bacterial cell absorbance of the culture broth was measured at 620nm. The relative concentration of prodigiosin produced by liquid grown cultures was quantified as follows: 1ml sample was harvested by centrifugation at 4500 rpm for 5 minutes. The supernatant was discarded and pellet resuspended in acidified ethanol (4% 1M HCl in ethanol) to extract prodigiosin from the cells. Cell debris was removed by a second centrifugation step and supernatant transferred to a cuvette for measurement of absorbance at 534nm.

Prodigiosin unit/cell

Prodigiosinunit/cell= ([OD534(1.381xOD620)])x1000/OD620

Where ,

OD-Optical density;

OD 534-Pigment absorbance;

OD 620-Bacterial cell absorbance

1. 381-Constant

**Applications Screening for antibacterial activity of prodigiosin:-**

The antibacterial activity of prodigiosin of *Serratia marcescens* was determined against various clinical isolates such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and species taken from Hindusthan College of Arts and Science. For the confirmation of these organism biochemical analysis also done. Their bacterial pathogens were swabbed into nutrient agar plates and a sterile cork borer was used to punch wells onto the plates. The prodigiosin pigment extracted was used to evaluate antimicrobial activity. To the wells prodigiosin was added and the plates were then incubated at 37°C for 24 hours. The zone of inhibition for each well was measured and recorded as antimicrobial effect.

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**Screening for lipase activity of *Serratia marcescens*:-**

The bacterium was identified as a strong lipase producer when streaked on ributyrin agar. For the enrichment of the culture, the bacterium was inoculated in enrichment medium containing beef extract (0.3%), peptone (1%), NaCl (1%), glucose (0.5%) and tributyrin (0.25%) at pH 7 for 24 hrs with incubation temperature 37°C. The enriched culture was then inoculated on the agar plates to check whether the strain was able to produce halo surrounding the colony, which indicates lipase production after incubating at 37°C for 18-24 hrs.

## IV. RESULT

**Isolation and identification of *Serratia marcescens* from contaminated coconut**

It was founded that Serratia marcescence is isolated from the contaminated coconut. The pink coloured portion of spoiled coconut producing bacteria, was picked up separately and purified by quadrant streaking in nutrient agar plates. After further incubation reddish colonies are observed on nutrient agar plates.

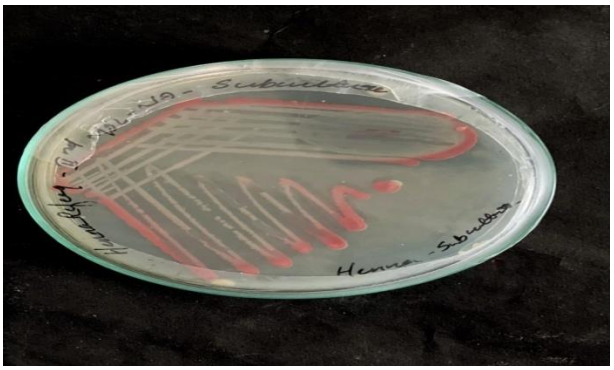

**Fig 1:** *Serratia marscesens* on nutrient agar plates

**Morphological analysis by gram staining**

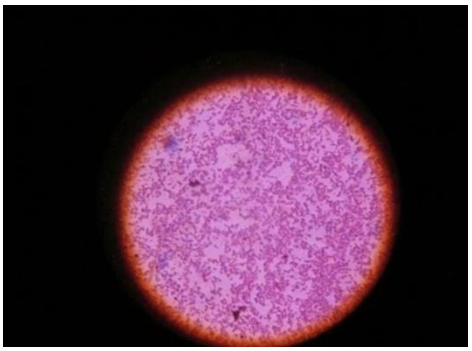

**Fig 2:** Gram negative rod colonies are observed by gram staining

24 hours old culture was gram stained and the slide was observed under microscope for gram reaction. Pink colored rod shaped cells were observed under microscope (100X) and it indicated, the organisms are gram negative rods.

**Biochemical analysis of *Serratia marcescens***

**Table(1):** characteristic of *Serratia marscescens*

| Biochemical tests | *Serratia marscescens* |
|---|---|
| Gram staining | Gram negative rod |
| Indole | -_ |
| Methyl red | +/- |
| Voges Proskauer | + |
| Citrate utilization test | + |
| Catalase | + |
| CHO Fermentation | - |
| Glucose | + |

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| | |
|---|---|
| Sucrose | + |
| Fructose | + |
| Lactose | + |

**Extraction of pigment**

Red coloured pigment production was enhanced by using peanut media incubated under the static condition. The red coloured pigment was extracted by repeated centrifugation followed by the repeated addition of 95% ethanol.

**Fig 3:** Before and after incubation

After incubation peanut seed medium becomes red colour due to the presence of prodigiosin pigment of *Serratia marcescens.*

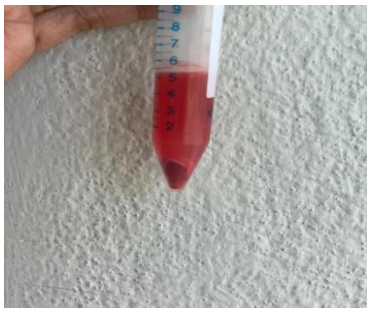

**Fig 4:** Extracted pigment Presumptive test for prodigiosin

On addition of 1 drop of concentrated HCl to the ethanol extract of pigment and to the cell free supernatant of the culture, the colour changed to red and pink respectively. When concentrated ammonia solution was added to ethanol extract and cell free supernatant, there was a colour change to yellow. This showed a positive presumptive test for prodigiosin.

Estimation of prodigiosin

The prodigiosin was estimated with the formula :

Prodigiosin unit/cell =([OD534-(1.381xOD620)])x1000/OD620

= ([0.37-(1.381x0.10])x1000/0.10

=414.3unit/cell

Screening for antibacterial activity of prodigiosin

Extracted pigment showed antibacterial activity against *Pseudomonas aerogenosa, Staphylococcus aureus* and *Escherichia coli* cultures taken from PG laboratory of Hindusthan College of Arts and Science.

Biochemical analysis of clinical samples.

**Table (2):** biochemical characteristics of *Staphylococcu saureus, Escherichia coli, Pseudomonas aeruginosa*

| CHARACTERISTICS | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* |
|---|---|---|---|
| INDOLE TEST | - | + | - |
| METHYL RED TEST | - | + | - |
| VOGES-PROSKAUER TEST | - | - | - |
| CITRATE TEST | + | - | + |

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| | | | |
|---|---|---|---|
| TRIPLE SUGAR IRON TEST | - | + | - |
| CATALASE TEST | + | + | + |
| OXIDASE TEST | - | - | + |
| UREASE TEST | - | - | - |
| NITRATE REDUCTION TEST | + | + | + |
| COAGULASE TEST | + | - | - |
| GELATIN HYDROLYSIS | _ | - | - |
| STARCH HYDROLYSIS | _ | - | - |
| MOTILITY TEST | non motile | Motile | Motile |

**Antibacterial activity:**

| Organism | Zone of inhibition(mm) |
|---|---|
| Pseudomonas aeruginosa | 5mm |
| Staphylococcus aureus | 8mm |
| Escherichia coli | 12mm |

Antibacterial activity of prodigiosin pigment is confirmed by measuring the zone of inhibition on nutrient agar plates swabbed by Pseudomonas aeruginosa Staphylococcus aureus, and Escherichia coli,

Screening for lipase activity of *Serratia marcescens*

Isolated organism shows lipolysis in Tributyrin agar upon incubation.

**Fig 5:** Plates showing lipase activity

The bacterium was identified as a strong lipase producer when streaked on tributyrin agar. On Tributyrin agar plated produce halo surrounding the colony, which indicates lipase production after incubating at 37°C for 18-24 hrs.

## V. DISCUSSION

In this present study, the prodigiosin pigment is produced by the bacterium *Serratia marcescens* and it has many applications such as dye, antimicrobial activity. Peptone glycerol broth containing 1.5 ml 87% glycerol *Serratia marcescens* was found to produce higher amount of prodigiosin, the production was also high in maltose containing medium. Frequently Prodigiosin production was done in nutrient broth and peptone glycerol broth. Protein was found to be highest in nutrient broth followed by powdered peanut broth and sesame seed broth. Nutrient broth and peptone glycerol broth consists of peptone, meat and yeast extract as the major components. Peptone is a commercially existing digest of plant or animal protein, made accessible to organisms as peptides and amino acids to aid the requirements for sulphur, nitrogen, carbon and energy. Peptone lacks certain essential minerals and vitamins in its composition. Yeast and meat extracts contain eukaryotic tissues which are extracted by boiling and then concerted to powdered form. Fastidious organisms often used these extracts as a source of amino acids, vitamins and coenzymes as growth factors. Glycerol was the carbon source in peptone glycerol broth. Seeds consist of vitamins, saturated and unsaturated fatty acids and these components differ from seed to seed. Nutrient broth is used for the pigment production as it is basically devoid of carbon sources; it is also associated with yeast extract and maltose. The peanut-based medium is also employed for the synthesis of prodigiosin. It serves as the best medium . The chief producer of prodigiosin pigment is *Serratia marcescens* and in this production carbon source may well play a critical role. There is a proof representing that the bacterium *Serratia marcescens* grow well on artificial media using different compounds as a sole carbon source . It was reported that the bacterium produced higher amount of prodigiosin at 30°C and at pH 7. It was found that the pH and temperature increases more than 30°C at pH 7 the amount of pigment production was decreased. In the prodigiosin pigment production there is diversity in

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*Serratia marcescens* strains and their optimal conditions. It was also found that most prodigiosin production occurred at 27°C. When the cultures were incubated at 38°C prodigiosin pigment production was not observed. In another study, prodigiosin pigment production was found to be stopped up at 37° C. Both organic and inorganic nitrogen sources were employed, with yeast extract proving to be the most favorable for promoting both biomass growth and prodigiosin pigment production. This led to the highest recorded biomass of 3.4g/L and prodigiosin pigment production at 31 mg/L. Consequently, yeast extract emerges as a promising option for optimizing both biomass yield and pigment production.

## VI. CONCLUSION

Based on the study, an attempt was carried out to isolate the pigment producing *Serratia marcescens* from soil samples. The red pigment producing bacteria was characterized using morphological and biochemical analysis. The red pigment was concluded as prodigiosin by presumptive test using HCl and ammonia solution. The highest yield of the pigment was obtained by using peanut media, and it was found to be the best and cheapest for the prodigiosin pigment production from Serratia marcescens. The prodigiosin pigment is a natural compound, it has antifungal, antibacterial, algicidal ,antiprotozoal, antimalarial , cytotoxic and anticancer properties. The prodigiosin estimated as 414.3 unit/cell. Also study the antibacterial activity of prodigiosin and lypolytic action of Serretia *marcescens* as applications with more economical importance.

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