

## Isolation, Identification, Optimization of Prodigiosin Pigment Produced by *Serratia Marcescens* and its Applications

Srimathi R, Priya R, Nirmala M, Malarvizhi A\*

Department of Microbiology

Vivekanandha College of Arts and Sciences for Women (Autonomous)  
Elayampalayam-637 205, Tiruchengode, Namakkal Dt, Tamilnadu, India.

---

**Abstract:** Natural products either synthesized or secreted by organisms represent one of the critical sources of potential medicinal use. Both natural pigments and synthetic dyes have been widely used in different fields in on a daily basis life such as foods or feeds, textiles, cosmetics, pharmaceuticals, paper printing inks, etc. Secondary metabolites of bacterial origin include various enzymes, pigments, antibiotics etc, which could be of importance to mankind in many ways. Prodigiosin pigment was produced by the bacterium *Serratia marcescens*, the pigment production is highly uneven among numerous species and is dependent on several factors such as species type and incubation time. Pigment producing microorganisms was isolated from soil samples. It was biochemically characterized and identified as *Serratia marcescens*. Production of prodigiosin was optimized with respect to different environmental parameters such as pH, temperature, incubation, different media, nitrogen source, sugar substrates. Crude extract of pigment was further purified by thin layer chromatography. Prodigiosin was tested for range of applications such as dyeing, antibacterial and antifungal activity. The dyed in cotton cloth showed good colour tone therefore, the prodigiosin pigment can be suggested for dyeing the textiles in the large scale production of the pigment will make it an alternate for the chemical dyes and future study can be done on anti cancer activity of red pigment from *Serratia marcescens* in human cervix carcinoma cells.

**Keywords:** Dyes, Prodigiosin, *Serratia marcescens*, Thin layer chromatography.

---

### I. Introduction

Natural products either synthesized or secreted by organisms represent one of the critical sources of potential medicinal use. One of these less significant molecular weight natural products secreted by organisms and are having no demonstrable function on the secreting cells are known as secondary metabolites that includes pigments, steroids, enzymes and antibiotics. These products are widely used for therapeutic treatment. Bioactive pigments are obtained from plants, microorganisms and many other sources [1]. Bioactive pigments produced by microorganisms are mostly preferred when compared with plants because of their stability and availability [2] [3] [4]. In daily life both natural pigments and synthetic dyes have been widely used in various fields such as foods, feeds, textiles, paper, printing inks, cosmetics, pharmaceuticals, etc.[5]. Since colour is an important aspect that determines the consumer acceptance in textiles, food and in many industries. Due to the toxicity of several artificial colorants, the use of natural additives is increasing that put string importance on the production of bio colours or natural colours extracted from fruits, vegetables and microorganisms [6]. The industrial production of natural colorants is already well established and expending. Prodigiosin pigment is synthesized from different bacteria that includes *Actinomycetes*, *Streptomyces* and *Serratia marcescens* and it also has more therapeutic values.

*Serratia marcescens* is a Gram negative bacterium, classified in the large family of *Enterobacteriaceae*. *Serratia* can be well-known from other genera by its production of three particular enzymes such as DNAase, lipase and gelatinase. Another characteristic feature of this bacterium *Serratia marcescens* is the production of cell associated red colour pigment. *Serratia*, grow well on ordinary media like other *Enterobacteriaceae*. They also grow well on synthetic media under anaerobic and aerobic conditions using various compounds as a single carbon source at pH 9 and at temperatures from 20-37 °C. It mainly occurs in soil, water, plants, insects, animals and also in man [7]. There are several species of the Genus *Serratia* such as *Serratia pymuthica*, *Serratia odorifera*, *Serratia ficaria*, *Serratia liquifaciens*, *Serratia rubidaea*, and *Serratia fonticola* [8].

#### 1.1 Prodigiosin structure

Secondary metabolites of bacterial origin include various enzymes, pigments, antibiotics etc which could be of importance to mankind in many ways. *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. Prodigiosins, family of natural pigments, characterized by pyrrolyl pyrromethane common skeleton of low molecular weight, appearing only in later stages of bacterial growth and called as prodigiosininde, which is

common to all the members of this family such as cycloprodigiosin, metacycloprodigiosin, dipyrrolil dipirromethane, all which have a common pyrrolyl dipyrrolylmethane skeleton and it comprise three rings which form a pyrrolylmethane skeleton with the molecular formula  $C_{20}H_{25}N_3O$  [9] Two of the rings are directly linked to each other and the third one is attached through a methane bridge [10] [11]. Macrocylic prodigiosins appear to be derived from undecycyl prodiginine by oxidative cyclisation [12]. Chromogenic biotypes from the natural environment have rarely resulted in infections and the clinical isolates are rarely pigmented. The function of the red pigment prodigiosin remains uncertain and no function has been clear for it in the physiology of the producer strains [13].

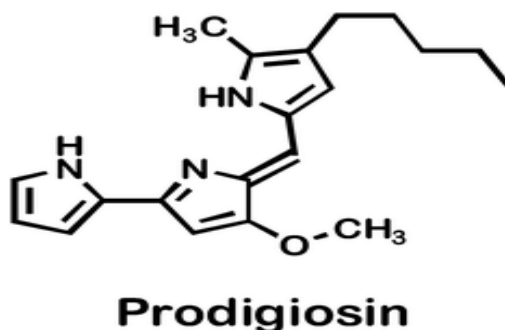


Fig 1.1. Prodigiosin structure [14]

Though, the interest of this prodigiosin pigment has been reported in different areas and it has great potential for clinical and environmental applications like antibacterial, antifungal, anticancer, anti-neoplastic, antioxidant, anti-diabetic, anti malarial, anti-tumour [15] [16], anti-protozoal [17], cytotoxic activities [18], immune suppressive and as anti foulant [19]. Various prodigiosin differ primarily in the length of the alkyl side chain. cycloprodigiosin, meta cycloprodigiosin and nonylprodigiosin have a cyclic side chain. A novel n-alkylated prodigiosin analogue 2, 2'-[3-methoxy-1-amy-5'-methyl-4-(1'pyrryl)] di pyrrolylmethane (MAMPDM) has been isolated from an organic solvent. Tolerant marine bacteria *Serratia marcescens* [20] contains two pyrrole rings attached through the nitrogen atom and alkyl side chain attached to the nitrogen atom of the third ring [21]. Since cell proliferation is a common occurrence between function of immune system and the development of cancer cells, many anticancer agents are immune suppressive. The cell cycle inhibitory and apoptosis inducing activity of prodigiosin makes an attractive candidate for anticancer therapy. The cytotoxic potential of the pigment was observed in the 60 cell lines panels of human tumour cells derived from lung, brain, ovarian, melanoma leukemia, renal and colon, [22]. The anti-proliferative activity and the cytotoxic effects of prodigiosin were not only observed in cultured tumour cell lines but also in human primary cancer cells taken from B-cell chronic lymphocytic leukaemia patients [23]. 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 [24]. The presence of prodigiosin and metacyclo prodigiosin in culture broth of *Serratia marcescens* observed selective and inhibition of polyclonal proliferation of T-cells as compared to that of B cells (Han *et al.*, 2001). The immuno suppressive activity has been observed for other prodigiosin analogue such as cycloprodigiosin, CprG, MAMPDM, nonylprodigiosin. Prodigiosin suppressed the proliferation of lymphocytes stimulated with concanavalin A, phorbol myristate acetate and ionomycin. These prodigiosin inhibit both T cell receptor dependent and independent proliferation of T cells [25].

*Serratia marcescens* secretes range of extracellular enzymes including chitinase [26]. The bacterium is one of the most efficient in the degradation of chitin [27]. *Serratia marcescens* produce three chitinases such as chi A, chi B, and chi C, chitobiase and chitin binding protein (CBP21) [28]. *Serratia marcescens* chitinolytic machinery is immense, because it is one of the bacterium with synergistic inhibitory enzymes which was against spore germination of *botrytis cinerea*. *Serratia marcescens* is identified as they produce more concentration of prodigiosin [29]. 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) 2,5-di phenyl tetrazolium bromide and neutral red. Prodigiosin showed dose dependent inhibition of cell proliferation. These results from the study described that the prodigiosin pigment has high anticancer and apoptosis activity against human cervical carcinoma cancer [30].

Microbial pigments is an alternative basis for the natural food grade pigments and has great potential in food application owing to their natural colour, medicinal properties, production in independent of time, expected yield and environmentally safe [31]. Production of microbial pigments is one of the emerging field and microorganisms such as yeast, bacteria and fungi were rich in pigment [32]. Nowadays scope of commercial

production of bio pigments like chlorophyll, melanin, monascins, flavins and prodigiosins are increasing [33]. The present study focuses on the importance of prodigiosin pigment in their antimicrobial activity and application in textile dyeing.

## **II. Materials and Methods**

### **2.1 Collection of Soil Samples**

Rhizosphere soil samples were collected from the depth of 10 -15cms at different sites in Salem and Namakkal districts using sterile glass containers.

### **2.2 Isolation and Identification of Bacteria**

The soil samples were serially diluted and 0.1ml of diluted samples from each dilution was spread over the Nutrient agar surface. Then the plates were incubated at 37 °C. After 24 hrs, morphologically distinct colonies were taken and identified by biochemical characterization using Bergey's manual of systematic bacteriology. The identified bacterial isolates were used for further studies.

### **2.3 Presumptive test for prodigiosin pigment**

The isolated organism was inoculated in the nutrient broth and incubated for the observation of pigment production. The culture broth was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was suspended in 95% methanol to extract the pigment from the cells. The suspended pellet was centrifuged at 10,000 rpm for 10 minutes. Debris was removed and the supernatant was taken in two tubes. The content of the first tube was acidified with a drop of concentrated hydrochloric acid and the second tube was alkalinized with a drop of concentrated ammonia solution. Then the colour change was observed and the results were interpreted.

### **2.4 Optimization for enhancing prodigiosin production**

#### **2.4.1. Different media**

Powdered sesame, peanut and groundnut seed were mixed in distilled water and sterilized. The pH of all the media was maintained at 7.0. Then the fresh culture was inoculated separately in each sterilized media. The prodigiosin production was estimated at time intervals of 24, 48, 72 and 96 hours.

#### **2.4.2. Effect of incubation period**

From the overnight culture of the isolates, 1ml was inoculated in nutrient broth and incubated in a shaker incubator at room temperature. The pigment production was estimated at time intervals of 24, 48, 72 and 96 hours.

#### **2.4.3. Effect of sugar substrates**

The carbon sources such as dextrose, fructose, mannitol, lactose, sucrose were supplemented at 1% w/v concentration in nutrient broth at room temperature. Overnight culture of the isolates was inoculated into the media supplemented with different carbon source. The pigment production was estimated at time intervals of 24, 48, 72, and 96 hours.

### **2.5 Effect of physico – chemical factors on prodigiosin production**

#### **2.5.1. Effect of pH**

1ml of overnight culture of the isolates was inoculated in to nutrient broth maintained at pH 6, 7, 8, 9 and 10. The flasks were incubated at room temperature. Prodigiosin production was estimated after 10, 24, 48, 72 and 96 hours of incubation period from each flask.

#### **2.5.2. Effect of temperature**

The effect of temperature on prodigiosin production was observed by inoculating the bacteria in isolates in nutrient broth and incubated at different temperatures such as 28 °C, 30 °C, 37 °C, 39 °C, 40 °C.

#### **2.5.3. Effect of nitrogen source**

1ml of the overnight culture was inoculated in nutrient broth. The broth was supplemented with 0.5% w/v of nitrogen sources like urea, tryptone, peptone, beef extract powder, yeast extract powder and incubated at room temperature. After 10, 24, 48, 72 and 96 hours of incubation prodigiosin production was estimated.

### **2.6 Extraction of the prodigiosin pigment**

Bacterial cultures from the liquid broth were centrifuged at 10,000 rpm for 15 minutes. The supernatant and cell pellet were extracted with acetone and ethyl acetate. Then the cell pellet was repeatedly centrifuged to obtain white pellet. The pigment extracts of ethyl acetate fraction and acetone fraction were evaporated separately in evaporating dishes at room temperature till the powder appears.

### **2.7 Estimation of prodigiosin pigment**

The absorption pattern was chiefly checked with different wavelengths and found that the absorption was maximum at 499nm as well as prodigiosin pigment was also absorbs at the maximum wavelength. At this wavelength the absorptions were recorded using the formula

$$\text{Prodigiosin unit/cell} = \frac{[\text{OD} - (1.381 \times \text{OD})] \times 1000}{\text{OD}}$$

OD= optical density; OD=Pigment absorbance; OD =Bacterial cell absorbance; 1.381 =constant [34].

## 2.8 Purification of prodigiosin pigment

### 2.8.1. Thin Layer Chromatography

Thin layer chromatography is a technique used to separate non volatile mixtures. The prodigiosin pigment was separated using TLC plate coated with silica gel. In the chromatographic tank the developing solvent such as chloroform, methanol and acetone (4:2:4v/v) was standardized and poured, then it was saturated with a filter paper soaking in the mobile phase. Then the Rf value of the chromatogram was observed in the TLC plates.

## 2.9 Applications of prodigiosin pigment

### 2.9.1. Antibacterial activity

Antibacterial activity of prodigiosin pigment was studied against different species of Gram positive bacteria such as *Staphylococcus aureus*, *Bacillus spp.* and Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia* and *Proteus spp.* The test cultures were swabbed on Muller Hinton agar plates and activity of prodigiosin was studied using well diffusion method.

### 2.9.2. Antifungal activity

The antifungal activity of prodigiosin was checked against different species of fungal pathogens including *Aspergillus niger*, *Candia albicans*. The cultures were swabbed on potato dextrose agar plates and the activity of prodigiosin was checked by well diffusion method.

### 2.9.3. Potential effect of prodigiosin on dyeing cloth

For dyeing process, the cotton fabric was pre-treated by scouring (To increase the absorbency of the fabric and also to leave the fabric in hydrophilic condition). The fabric was washed with commercial detergent at 50°C for 25 minutes followed by water and dried [35]. Then 1g of dried fabric was immersed in 20 ml of bacterial culture and heated up to 80-90°C for 1 h. After an hour, the fabric was allowed to cool and washed with cold water [36]. The dyed fabric were immersed with 0.5 g/L mordant at 60°C for 20 min keeping the material to liquid ratio (MLR) at 1:20 [35] [36] [37]. The mordants used are ferrous sulphate (FeSO<sub>4</sub>) and copper sulphate (CuSO<sub>4</sub>) as metal mordants, Sodium bicarbonate (NaHCO<sub>3</sub>) as an alkali mordant, also lemon as acidic natural mordant. After mordating the dyed fabric was washed with tap water to remove excess mordants and dried.

## III. Results

### 3.1 Characteristics of the Soil Sample

This study revealed that ten Rhizosphere soil samples were analyzed with respect to bacteria, where as *Serratia marcescens* was the most prevalent species. The physiochemical properties of soil play an important role in the growth of microorganism. The Rhizosphere soil was slightly acidic. The humidity, moisture content, and temperature were 35%, 45% and 25°C, respectively.

### 3.2 Isolation and Identification of Bacteria from soil samples

The collected soil samples consists large number of different groups of pigment producing bacteria, out of this red colour pigment producing bacteria with different morphology and individual colonies were picked up separately and purified by quadrant streaking in nutrient agar plates (Fig.3.2.1) for the isolation of bacterium *Serratia marcescens*. The preliminary identification of the bacterial isolates revealed the presence of *Serratia marcescens* and was further confirmed with the biochemical characterization (Fig.1.2.2) using Bergey's manual of systematic bacteriology (Table 3.2). *Serratia marcescens* was found to be red colour pigmented, yellow coloured and bright red pigmented colonies on nutrient agar, macconkey agar and peanut medium respectively. Non- lactose fermenting is the reason where *Serratia marcescens* appeared in yellow colour on the macconkey agar.

Table 3.2. Identification of *Serratia marcescens*

Preliminary and Biochemical Tests	<i>Serratia marcescens</i>
Gram Staining	Gram negative rod
Motility	Motile
Catalase	Positive
Oxidase	Negative
Indole	-
Methyl red	+/-

Voges-Proskauer	+
Citrate utilization test	+
Nitrate reduction test	+
Triple sugar iron test	AK/A,H <sub>2</sub> S-
Glucose	AG <sup>+</sup>
Sucrose	AG <sup>+</sup>
Lactose	AG <sup>-</sup>
Maltose	AG <sup>+</sup>
Mannitol	AG <sup>+</sup>



Fig 3.2.1. *Serratia marcescens* on nutrient agar



Fig 3.2.2. Biochemical analysis of *Serratia marcescens*

### 3.3 Production of pigment in nutrient broth

In the present study nutrient broth was used for the production of the pigment, the yield of prodigiosin pigment on nutrient agar is 1336.14 mg/L. It was observed only after 96 hours of incubation.

### 3.4 Presumptive test for prodigiosin pigment

The presumptive test results in a pink colour in the acidified solution of hydrochloric acid and orange colour in the alkaline ammonia solution was observed. These colour changes indicate positive presumptive result for prodigiosin pigment.

### 3.5 Optimization for enhancing prodigiosin production

#### 3.5.1. Different media

Peanut medium was found to be suitable medium for prodigiosin production (2131.19µg/ml after 96 hours of incubation) followed by ground nut medium and sesame seed medium the rate of pigment production increased after every 24 hours of incubation (Fig 3.5.1).

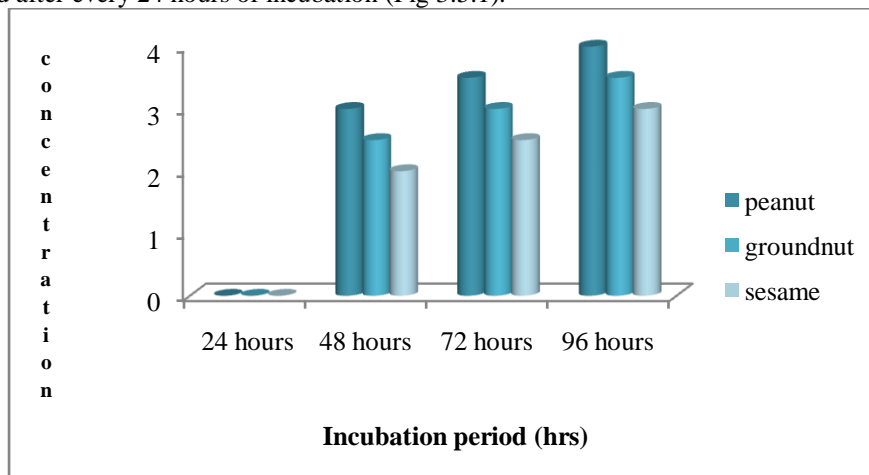


Fig 3.5.1. Different media on prodigiosin pigment production

#### 3.5.2. Effect of incubation period

The OD value of the cultures was observed using spectrophotometer at different incubation period like 24, 48, 72 and 96 hours. The results showed maximum pigment production at the 96<sup>th</sup> hour of incubation. Prodigiosin production was found to commence after 24 hours of incubation and its production increased with the increase in the incubation period (Fig 3.5.2).

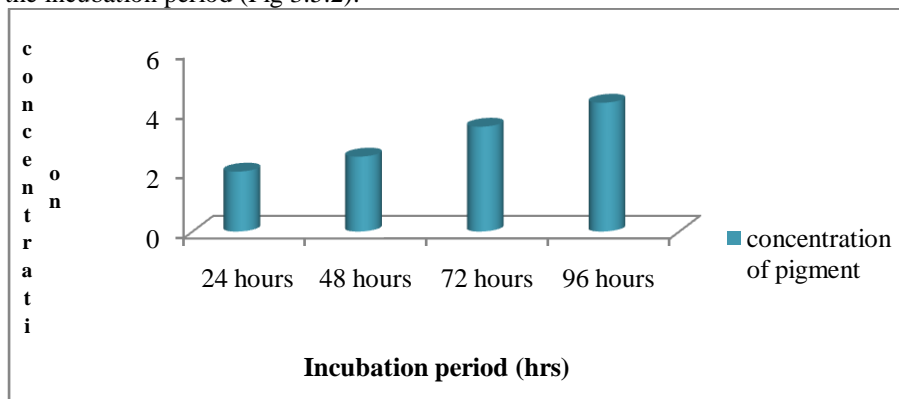


Fig 3.5.2. Effect of incubation period of prodigiosin production

### 3.5.3. Effect of sugar substrates

Nutrient broth amended with mannitol favoured in the highest production of prodigiosin pigment that increased every 24 hours of incubation. After 96 hours of incubation the concentration of prodigiosin was found to be 1167.21 mg/ml spectrophotometrically followed by fructose lactose, dextrose and finally sucrose (Fig 3.5.3).

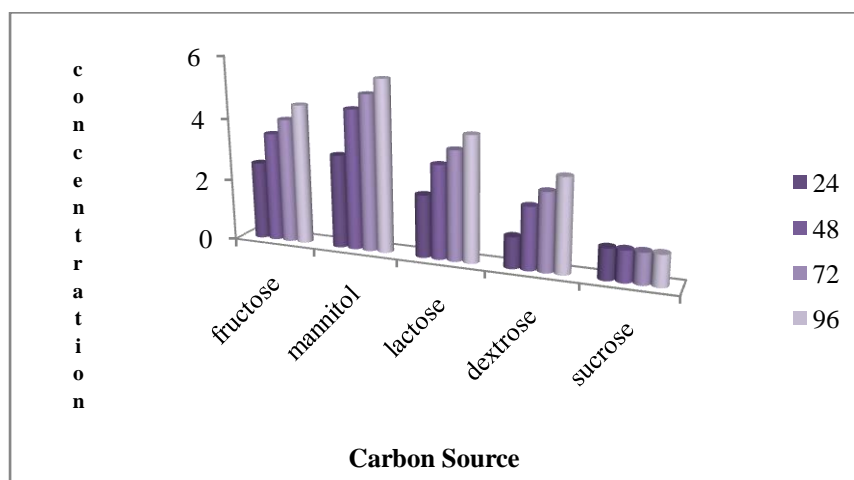


Fig 3.5.3. Effect of sugar substrates on prodigiosin production

### 3.6 Effect of physico chemical factors on prodigiosin production

#### 3.6.1. Effect of pH

The pH affects the production of prodigiosin pigment. The maximum production of prodigiosin pigment is at pH 7 (1332.82 mg/L). There was less prodigiosin pigment production at pH 6 followed by pH 8, PH 9, pH 10 (Fig 3.6.1).

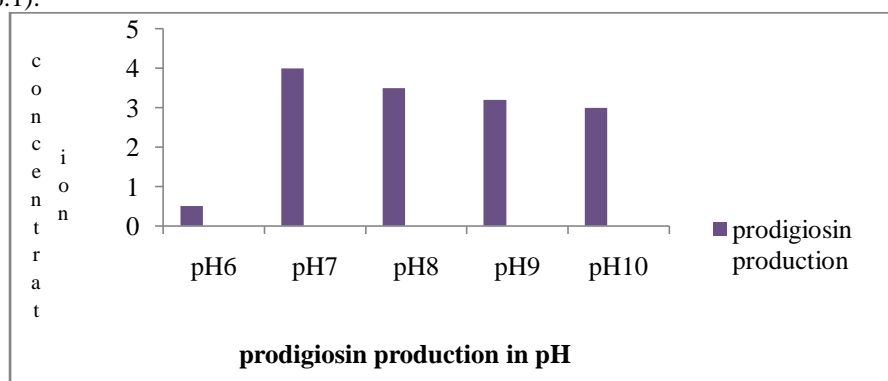


Fig 3.6.1. Effect of pH on prodigiosin production

**3.6.2. Effect of temperature**

The highest yield of prodigiosin pigment production was observed at 28°C followed by 30 °C, 37 °C, 39 °C, 40 °C. The prodigiosin yield obtained was represented in pie chart (Fig 3.6.2)

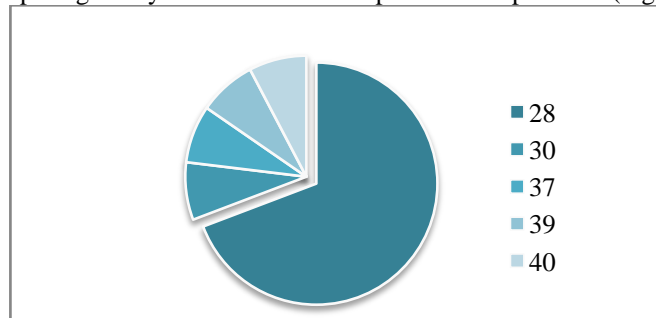


Fig 3.6.2. Effect of temperature on prodigiosin production

**3.6.3. Effect of nitrogen source**

Peptone supported the maximum pigment production and the yield was 2006.04 mg/L at 96 hours of incubation. The media supplemented with urea showed least prodigiosin production followed by yeast extract, beef extract, tryptone (Fig 3.6.3).

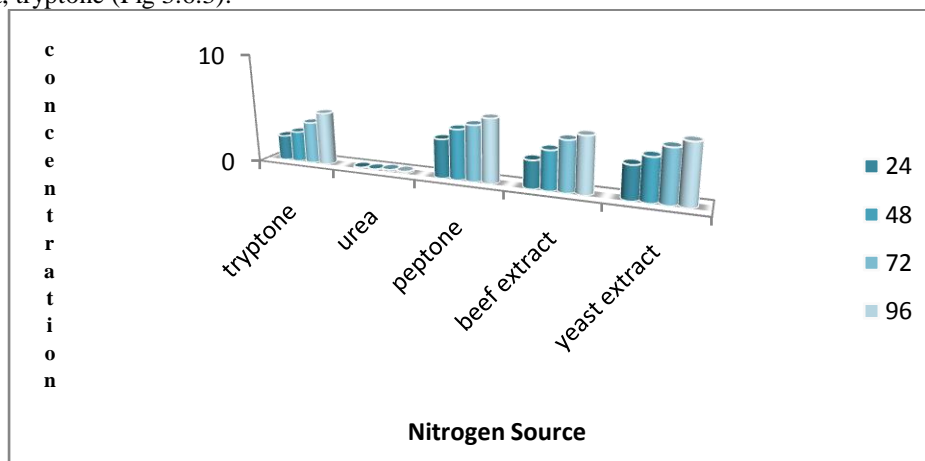


Fig 3.6.3. Effect of nitrogen source on prodigiosin production

**3.7 Extraction of the prodigiosin pigment**

The supernatant obtained from the repeated centrifugation of bacterial cultures was extracted with ethyl acetate, petroleum ether and methanol. None of the extracts gave residual crude pigment. Then the pellet was extracted with acetone which yielded a residual crude pigment. Thus acetone was found to be best for the extraction of pigment from the pellet.

**3.8 Estimation of prodigiosin pigment**

The results were studied after 24, 48, 72 and 96 hours time intervals. The bacterial cell absorption prior to pigment extraction as noted at every step. The prodigiosin pigment was estimated using the following formula

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

OD= optical density; OD499=Pigment absorbance; OD 620=Bacterial cell absorbance; 1.381 =constant [34].

**3.9 Purification of prodigiosin pigment**

**3.9.1. Thin Layer Chromatography**

In the present study, the pigment purification was done by using thin layer chromatography. After purification, the RF value of this pigment was 0.78.

**3.10 Applications of prodigiosin**

**3.10.1. Antibacterial activity**

Antibacterial activity of prodigiosin pigment extracted from *Serratia marcescens* was assayed against five bacterial strains such as *Staphylococcus aureus*, *Bacillus spp.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus spp.*, *Pseudomonas aeruginosa* and *Vibrio spp.* From this assay, *Staphylococcus aureus* and *Escherichia coli* showed highest zone of inhibition (Table 3.10.1).

Table 3.10.1. Antibacterial activity of prodigiosin pigment

S.No.	Bacterial isolates	Zone of inhibition (mm)
1.	<i>Staphylococcus aureus</i>	12
2.	<i>Bacillus spp.</i>	9
3.	<i>Escherichia coli</i>	15
4.	<i>Klebsiella pneumoniae</i>	9
5.	<i>Proteus spp.</i>	6
6.	<i>Pseudomonas aeruginosa</i>	8
7.	<i>Vibrio spp.</i>	-

### 3.10.2. Antifungal activity

Antifungal activity of the prodigiosin pigment was assayed against *Aspergillus flavus*, *Goetrichum spp.* and *Candia albicans*. From these *Aspergillus flavus* showed highest zone of inhibition (Table 3.10.2).

Table 3.10.2. Antifungal activity of prodigiosin pigment

S.No.	Fungal isolates	Zone of inhibition (mm)
1.	<i>Aspergillus flavus</i>	6
2.	<i>Candia albicans</i>	2
3.	<i>Goetrichum spp.</i>	3

### 3.10.3. Potential effect of prodigiosin on dyeing

Bio synthesis of colorants for textile applications attracted increased interest in recent years. Nature produces many biocolorants from various resources including plants, animals and microorganisms, which are possible alternatives to synthesis dyes and pigments some natural colorants, especially anthraquinone in addition to providing bright colour Which could serve as functional dyes in producing coloured in textiles. The red pigment prodigiosin extracted from the *Serratia marcescens* was applied on cotton cloth, which showed good colour tone and the colour did not change while washing but it was sensitive to sunlight drying (Fig 3.10.3).



Fig 3.10.3. Prodigiosin pigment on cotton cloth

## IV. Discussion

In the present study, the pigment prodigiosin produced by the bacterium *Serratia marcescens* has many applications such as dyeing, antibacterial and antifungal 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 [38]. Frequently Prodigiosin production was done in nutrient broth [39] and peptone glycerol broth [40]. Protein was found to be highest in nutrient broth followed by powdered sesame seed broth and peanut 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 [41]. Peptone does not contain some minerals and vitamins. 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 [42]. 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 medium is also used



for the prodigiosin production. It serves as the best medium [43]. The chief producer of prodigiosin pigment is *Serratia marcescens* and in this production carbon source may well play a critical role [44] [19]. There is a proof representing that the bacterium *Serratia marcescens* grow well on artificial media using different compounds as a sole carbon source [45]. 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 [46] [19]. In the prodigiosin pigment production there is diversity in *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 [11]. In another study, prodigiosin pigment production was found to be stopped up at 37° C [39]. Both organic and inorganic nitrogen sources were used, from that yeast extract supported the growth and pigment production resultant in the highest biomass of 3.4g/L and 31 mg/L of prodigiosin pigment. Hence, yeast extract is a good choice for the biomass and the pigment production [47]. Cerdeno et al. (2001) and Furstner et al. (2003) reported that prodigiosin pigment is a natural compound, it has antifungal, antibacterial, algicidal, antiprotozoal, antimalarial, cytotoxic, anticancer and antiproliferative properties [48] [44]. Darah et al., 2014 suggested that the bacterial cells need an alkaline condition to produce the antibacterial activity [49]. Prodigiosin has antibacterial activity as it inhibits various species of gram positive and gram negative bacteria [50]. Mordants are applied on the textile fabrics to gain altering colours and to enhance the dye uptake, to get better the colour fastness performance of several natural dyes [51]. Many types of mordants used on the natural dye may darken, make brighter, or significantly modify the colour of the dye mordant solution and control the darkness of the final product which may be a required effect or an unwanted occurrence [52]. The fading of coloured textiles upon exposure to light is a well-known phenomenon [53].

## V. Conclusion

The textiles are one amongst in the rapidly growing industries worldwide, which utilizes enormous amounts of synthetic dyes. The effluent from these textile industries possess serious threat to the environment, which is often very difficult to treat and dispose. Based on the study, an attempt was carried out to isolate the pigment producing *Serratia marcescens* from the soil samples. The red pigment producing bacteria was isolated and characterised using nutrient broth. The highest yield of the pigment was obtained from the nutrient broth, but peanut seed medium was found to be the best and cheapest for the prodigiosin pigment production from *Serratia marcescens*. The pigment was dyed on cotton cloth which exposed good colour tone. The pigment also has antimicrobial activity. In large scale production, the pigment will make it an alternate to chemical dyes and further study can carried out in anticancer activity in human cervix carcinoma cells.

## VI. Acknowledgement

The authors are thankful to prof. Dr. M. karunanithi, Chairman and Secretary, Vivekanandha Educational Institutions, and Dr. B.T. Suresh Kumar, Principal, Vivekanandha College of arts and sciences for women, Elayampalayam, Tiruchengode, Namakkal District, Tamilnadu for providing all the facilities for our research work.

## References

- [1]. A.J. Papageorgion and H.J. Winkler, Influence of dissolved oxygen levels on prodigiosin production, *Applied Microbiology*, 19(5), 1979, 800-805.
- [2]. C.H. Kim, S.W. Kim, S.I. Hong, An integrated fermentation separation process for the production of red pigment by *Serratia spp.* KH-95, *Process Biochemistry* 35(5), 1999, 485-490.
- [3]. S. Parekh, V.A. Vinci and R.J. Strobel, Improvement of microbial strains and fermentation processes, *Applied Microbiology and Biotechnology*, 54(3), 2000, 287-301.
- [4]. Y.J. Cho, J.P. Park, H.J. Hwang, S.W. Kim, J.W. Choi and J.W. Yun, Production of red pigment by submerged culture of *Paecilomyces sinclairii*, *Letters in Applied Microbiology*, 35(3), 2002, 195-202.
- [5]. C. Tibor, Liquid Chromatography of natural pigments and synthetic dyes, *Journal of Chromatography Library*, 71, 2007, 1-591.
- [6]. S. Pattnaik , V.R. Subramanyam , M. Bapaji , C.R. Kole 1997, Antibacterial and antifungal activity of aromatic constituents of essential oils, *Microbios*, 89(358), 39-46.
- [7]. Paul singleton, Diana sainsbury, *Dictionary of Microbiology and Molecular Biology* (John Wiley and Sons Ltd, 3rd Edition, 2006).
- [8]. P. Auwaerter, *Serratia species*, Point-of-Care Information Technology, ABX Guide, Johns Hopkins University, 2007.
- [9]. A.K.P. Harris, N.R.H. Williamson, H.A. Cox, S. Abbasi, F.I. Slater, H.T. Simonsen, F.J. Leeper, G.P.C. Salmond, The *Serratia* gene cluster encoding biosynthesis of the red antibiotic, Prodigiosin,

- shows species –and strain –dependent genome context variation, *Microbiology*, 150(11), 2004, 3547 – 3560.
- [10]. N.N. Gerber, Prodigiosin like pigments, *CRC Critical Reviews in Microbiology*, 3(4), 1975, 469–485.
- [11]. R.P. Williams and S.M. Quadri, The pigments of *Serratia*, Edited by A. Von Graevenitz and S. J. Rubin. In the genus *Serratia* Boca Raton, *CRC Press*, 1980, 31–75.
- [12]. T. Azuma, H. Watanabe, H. Yagisawa, M. Hirata, M. Iwamura and Y. Kobayashi. Induction of apoptosis of activated murine splenic T cells by cyclo Prodigiosin hydrochloride a novel immunosuppressant, *Immunopharmacology*, 46(1), 2000, 29-37.
- [13]. S.B. Han, S.H. Park, Y.J. Jeon, Y.K. Kim, H.M. Kim, K.H. Yang, Prodigiosin blocks T cell activation by inhibiting interleukin - 2Rα expression and delays progression of autoimmune diabetes and collagen induced arthritis, *Journal of Pharmacology Experimental Therapeutics*, 299(2), 2001, 415-425.
- [14]. George W. Gokel and Natalie Barkey, Transport of chloride ion through phospholipid bilayers mediated by synthetic ionophores, *New Journal of Chemistry*.33(5), 2009, 947-963.
- [15]. A.J. Castro, Antimalarial activity of prodigiosin, *Nature*, 213, 1967, 903-4.
- [16]. R. Perez-Tomas, B. Montaner, E. Llagostera, V. Soto - Cerrato, The prodigiosins, proapoptotic drugs with anticancer properties, *Biochemical Pharmacology*, 66(8), 2003, 1447–52.
- [17]. S.L. Croft, K. Seifert and A.A. Duchene Deorukhkar, R. Chander, S.B. Ghosh and K.B. Sainis, Doxorubicin resistant carcinoma (GLC4/ADR) cell line to prodigiosin through apoptosis activation, *Anticancer Drugs*, 16, 2007, 393-399.
- [18]. T. Nakashima, T. Tamura, M. Kurachi, K. Yamaguchi and T. Oda, Apoptosis-mediated cytotoxicity of prodigiosin-like red pigment produced by gamma- Proteobacterium and its multiple bioactivities, *Biological and Pharmaceutical Bulletin*, 28(12), 2005, 2289– 2295.
- [19]. A.V. Giri, N. Anandkumar, G. Muthukumar, G. Pennathur, A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil, *BMC Microbiology*, 4(11), 2004, 1-10.
- [20]. P.S. Variyar, R. Chander, S.R. Venkatachalam and D.R. Bongirwar, A new red pigment from an alkalophilic *Micrococcus species* *Indian Journal of Chemistry*, 41, 2002, 232-233.
- [21]. Amit A. Deorukhkar , Ramesh Chander, B. Sukhendu, Ghosh , B. Krishna, K.B. Sainis, Identification of a red-pigmented bacterium producing a potent anti-tumor N-alkylated prodigiosin as *Serratia marcescens*, *Research in Microbiology*, 158(5), 2007, 399-404.
- [22]. R. Pandey, R. Chander, K.B. Sainis, Prodigiosins: A novel family of immune suppressants with anticancer activity, *Indian Journal of Biochemistry and Biophysics*, 44(5), 2007, 295-302.
- [23]. C. Campas, M. Dalmau, B. Montaner, M. Barragan, B. Bellosillo, D. Colomer, G. Pons R. Perez-Tomas and J. Gil, Prodigiosin induces apoptosis of B and T cells from B-cell chronic lymphocytic leukemia, *Leukemia*, 17(4), 2003, 746-750.
- [24]. E. Llagostera, V. Soto-Cerrato, R. Joshi, B. Montaner, P. Gimenez-Bonafe, R. Perez Tomas, High cytotoxic sensitivity of the human small cell lung doxorubicin-resistant carcinoma (GLC4/ADR) cell line to prodigiosin through apoptosis activation, *Anticancer Drugs*, 16(4), 2005, 393-9.
- [25]. S.A.S. Maphari, K.F. Nielsen, T.O. Larsen, J.C. Frisvad, A.S. Meyer and U. Thrane, Exploring fungal biodiversity for the production of water soluble pigments as potential natural food colorants, *Current Opinion in Biotechnology*, 16(2), 2005, 231-238.
- [26]. D. W. Hines, P. N. Saurugger, G. M. Ihler, and M. J. Benedik, Genetic analysis of extracellular proteins of *Serratia marcescens*, *Journal of Bacteriology*, 170(9), 1988, 4141–4146.
- [27]. J. Monreal, and E.T. Reese, The Chitinase of *Serratia marcescens*, *Canadian Journal of Microbiology*, 15(7), 1969, 689-696.
- [28]. R. L. Fuchs, S. A. McPherson and D. J. Drahos, Cloning of a *Serratia marcescens* gene encoding chitinase, *Applied and Environmental Microbiology*, 5(3)1, 1986, 504–509.
- [29]. Antony V.Samrot, K. Chandana, P. Senthikumar and G. Narendra Kumar, Optimization of prodigiosin production by *Serratia marcescens* SU-10 and evaluation of its bioactivity, *International Research Journal of Biotechnology*, 2(5), 2011, 128-133.
- [30]. R.kavitha, S.Aiswariya, Chandana. M.G. Ratnavali, Anticancer activity of red pigment from *Serratia marcescens* in Human cervix carcinoma, *International Journal of PharmTech Research*, 2(1), 784-787.
- [31]. FJ. Francis, Anthocyanins and betalains composition: composition and applications. *Cereal Foods World*, 45(5), 2000, 208–213.
- [32]. L. Dufosse, Pigments, *Encyclopedia of Microbiology*, 4, 2009, 457-471.
- [33]. A. Keneni, and V.K. Gupta, Characterization of a Red bacterium Strain isolated From Root Nodule of a Faba Bean (*Vicia faba. L.*) for Growth and Pigment production, *Journal of Advanced Laboratory Research in Biology*, 2(3), 2011, 138-146.

- [34]. R. Mekhael, and S.Y. Yousif, The role of red pigment produced by *Serratia marcescens* as antibacterial and plasmid curing agent, *Journal of Duhok University*, 12(1), 2009, 268-274.
- [35]. R. Kanchana, A. Fernandes, B. Bhat, S. Budkule, S. Dessai and M. Reshma, Dyeing of textiles with natural dyes - an eco-friendly approach, *International Journal of Chem Tech Research*, 5(5), 2013, 2102-2109.
- [36]. W.A. Ahmad, W.Y.W. Ahmad, Z.A. Zakaria and N.Z. Yusof, Application of bacterial pigments as colorant (Springer Briefs in Molecular Science), 2012, 57-74.
- [37]. V. Narayanaswamy, N.K.N. Gowda and R. Sudhakar, Natural dye from the bark of *Casuarina equisetifolia* for silk, *International Journal of Pharmaceutical and BioSciences*, 4(3), 2013, 94-104.
- [38]. N. Sundaramoorthy, P. Yogesh, and R. Dhandapani, Production of prodigiosin from *Serratia marcescens* isolated from soil, *Indian Journal of Science and technology*, 2(10), 2009, 32 – 34.
- [39]. L.H. Pryce, F.W. Terry, Spectrophotometric assay of gene expression: *Serratia marcescens* Pigmentation, *Bio science*, 26(), 2000, 3-13.
- [40]. D. Jonas, B. Schultheis, C. Klas, P.H. Krammer and S. Bhakdi, Cytocidal effects of *Escherichia coli* hemolysin on human T lymphocytes, *Infection and Immunity*, 61(5), 1993, 1715-1721.
- [41]. J.A. Lindquist, Nutrition and Cultivation of Bacteria – Appendices d.1 and E.1 in General Microbiology – A Laboratory Manual. 31999 McGraw- Hill/Primis Custom Publishing ISBN 0-07-235906-4.
- [42]. J.C. Lapenda Lins, C.C.S. Maciel, H.S. Xavier, C.A. Alves da Silva and G.M. Campos-Takaki, Production and toxicological evaluation of prodigiosin from *Serratia marcescens* UCP/WFCC1549 on Mannitol solid medium, *International Journal of Applied Research in Natural Products*, 7(2), 2014, 32-38.
- [43]. B. Pradeep, F.S. Pradeep, J. Angayarkanni and M. Palaniswamy, Optimization and Production of Prodigiosin from *Serratia marcescens* MBB05 using various natural substrates, *Asian Journal of pharmaceutical and clinical Research*, 6(1), 2013, 34-41.
- [44]. A. Furstner, Chemistry and biology of roseophilin and the Prodigiosin alkaloids: A survey of the last 2500 years, *Angewandte Chemie International Edition*, 42(31), 2003, 3582-3603.
- [45]. Y.H. Wei, and W.C. Chen, Enhanced production of Prodigiosin - like pigment from *Serratia marcescens* SMdeltaR by medium improvement and oil supplementation strategies, *Journal of Bioscience and Bioengineering*, 99(6), 2005, 616 -622.
- [46]. Anuradha V Giri, Nandini Anandkumar, Geetha Muthukumaran and Gautam Pennathur, A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil, *BMC Microbiology*, 4(11), 2004 1-10.
- [47]. K. Vijayalakshmi and K. Jagathy, Production of Prodigiosin from *Serratia marcescens* and its antioxidant and anticancer potential, *International Journal of Advanced Research in Biological Sciences*, 3(3), 2016, 75-88.
- [48]. A.M. Cerdano, M.J. Bibb, G.L. Challis, Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3 (2): new mechanisms for chain initiation and termination in modular multi enzymes, *Chemistry and Biology*, 8(8), 2001, 817-829.
- [49]. Darah Ibrahim, Teh Faridah Nazari, Jain Kassim and Sheh-Hong Lim, Prodigiosin - an antibacterial red pigment produced by *Serratia marcescens* IBRL USM 84 associated with a marine sponge *Xestospongia testudinaria*, *Journal of Applied Pharmaceutical Science*, 4(10), 2014, 001-006.
- [50]. G.S. Kalesperis, K.V. Prahlad and D.L. Lynch, Toxicogenic studies with the antibiotic pigments from *Serratia marcescens*, *Canadian Journal of Microbiology*, 21(2), 1975, 213 – 220.
- [51]. A.K. Samanta and P. Agarwal, Application of natural dyes on textiles, *Indian Journal of Fibre and Textile Research*, 34(4), 2009, 384-399.
- [52]. G.U. Mohammad, Effects of Different Mordants on Silk Fabric Dyed with Onion Outer Skin Extracts, *Journal of Textiles*, 2014, 1-8.
- [53]. P. Thiagarajan and G. Nalankilli, Improving light fastness of reactive dyed cotton fabric with antioxidant and UV absorbers, *Indian Journal Fibre Textiles Research*, 38(2), 2013, 161-164.