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Research Article

ISOLATION, CHARACTERIZATION OF SERRATIAMARCESCENS FROM MARINE SEDIMENTS AND EVALUATION OF POTENTIAL BIOLOGICAL APPLICATIONS

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ABSTRACT

Prodigiosin is a secondary metabolite and an alkaloid with a unique tripyrrole chemical structure, red in colour, isolated mainly from *Serratia marcescens* prodigiosin. The present study was focused to isolate prodigiosin pigment from Marine *Serratia marcescens* and to evaluate potentials importance. In the present investigation, prodigiosin producing microorganisms were isolated from the Marine sediment collected from Mandapam coast, Gulf of Mannar. The presumptive test for prodigiosin production was done with two morphologically orange to maroon coloured colonies, among only one isolate revealed positive result for prodigiosin production. The efficient prodigiosin production bacterium was identified as *Serratia marcescens*. The results were compared in accordance with the Bergey's manual of determinative bacteriology. The production of prodigiosin was formed in nutrient broth and peptone glycerol broth in both shaking and static condition. The production level was maximum at 72hrs. The total prodigiosin was found to be higher in nutrient broth compared to peptone glycerol broth. *Serratia marcescens* was evaluated for antimicrobial and antifungal properties. The effects of pH, temperature carbon and nitrogen sources on prodigiosin production were studied. It was found that sucrose and yeast extract were the best carbon and nitrogen sources for prodigiosin production whereas the maximum prodigiosin production was observed in pH 8 and temperature 28°C. Prodigiosin was found to be efficient against bacterial and fungal pathogens studied. The pigment produced by *Serratia sp.,* can be effectively used to dye textile material.

Keywords: Prodigiosin, Presumptive test, Optimization, Antimicrobial, Antifungal, Dye removal.

1. INTRODUCTION

Natural products either synthesized or secreted by organisms represent one of the critical sources of potential medicinal use. One of these smaller molecular weight natural products secreted by organisms and are having no demonstrable function on the secreting cells are known as secondary metabolite. These secondary metabolites include pigments, enzymes, steroids, and antibiotics. These products are being widely used recently for therapeutic treatment. Among the secondary metabolites, biopigments are obtained from two sources namely the plants and microorganisms [1].

Prodigiosin is a kind of secondary metabolite produced by *Serratia marcescens* and other bacteria. Prodigiosin has the structure containing pyrrolylpyrromethane skeleton with different alkyl substituents and these relatives have caused wide concern due to their ability to induced cell lines. Many prodigiosin derivatives with lower toxicity like G×15-075 have been used in clinic. Because of its low fermentation yield and high production cost, the price is up to Rs 500/mg. In light of its potential commercial values it is worth finding a strategy for getting inexpensive prodigiosin [2].

Prodigiosin is a red pigment isolated from a few species such as *Serratia, Pseudomonas* and *Streptomyces* [3]. *Serratia marcescens* is a rod-shaped, gram negative, facultative bacterium belonging to the Enterobacteriaceae family characterized by its ability to produce the red pigment prodigiosin. Although prodigiosin has no known defined role in the physiology of the strains in which it is produced, it has antifungal, antibacterial and anti-protozoal activities, and thus may have potential clinical utility [4]. Many types of differential and selective media have been used for *Serratia* growth and prodigiosin production. Regular prodigiosin production has been carried out in nutrient broth containing sesame seeds, maltose broth, peptone glycerol broth reported by [5].

Prodigiosin biosynthesis is a bifurcated process in which the monopyrrole 2-methyl -3-n-amylpyrrole and bipyrrole 4-methyl-2, 2-biprrole-5-carborcyaldehyde

precursors were synthesized separately and then the final step involves the condensing enzyme which condenses together and the genes found enclosed for biosynthesis was located on either cell chromosome or plasmid or both of them [6].

Serratia sp., are gram negative bacteria classified in the large family of Enterobactericeae and these bacteria grow well on standard media and produce a red to dark pigment that aids in its identification and the red colour pigment is so called prodigiosin. Prodigiosin is a multi-facted secondary metabolite. The prodigiosin group of natural product is a family of tripyrrole red pigment that contains as common 4-methyl bipyrrole ring system, Prodigiosin is a promising drug owing to its reported characteristics of having antibacterial, antifungal, antineoplastic, anti proliferative, anti-oxidant and anti-malarial activity [7]. The production of prodigiosin by *Serratia marcescens* is influenced by numerous factors including inorganic phosphate availability, medium composition, temperature, pH and natural components [8].

The marine isolates we described may be of common occurrence in sea water and sediments, but because of their cultural similarity to marine *Serratia* species, they may be difficult to handle in the laboratory conditions and their appearance to be minor different between strain of a single species. Hence the present study reports isolation of an efficient prodigiosin producing *Serratia marcescens* from marine sediments. Isolated microorganisms were screened with the culture conditions for enhanced prodigiosin productions, the efficient samples were extracted, estimated and purified. Beyond which study were extend to optimize (prodigiosin) producing organisms, with different pH, Temperature, carbon and nitrogen sources and to cultivate the microorganisms in different medium, study the crude extract of prodigiosin for antimicrobial, antifungal activity, analyze the effective of prodigiosin removal of dyeing property.

2. MATERIAL AND METHODS

2.1. Collection of Soil Sample

The marine sediment samples were taken from the Mandapam coast of Gulf of Mannar. The sediment samples were collected at a depth of 3-5 cm from the superficial layer. These samples were placed in a sterile plastic bag.

2.2. Isolation of Microorganisms from Marine Sediments

One (1) ml of the collected sediment samples were serially diluted in 99 ml of sterile distilled water. From 10^{-1} suspension, 1 ml of the diluted sample was transferred to 9 ml of sterile distilled water and subse-quently diluted upto 10^{-9} dilution. From the required dilutions, 0.1 ml suspensions were drawn and spread over the surface of nutrient agar medium (Himedia). The plates were incubated at 28°C for 24-48 hours (Plate 1). The morphologically distinct bacterial strains showing orange to maroon color colonies were selected for further study and were maintained on nutrient agar slant at 4°C.

2.3. Presumptive Tests for Prodigosin Production

Presumptive color tests for prodigiosin was carried out by scraping the pigmented growth on nutrient agar medium plates and suspended overnight in 95% ethanol at 28°C. Debris was removed from the suspension by centrifugation at 5000 rpm for 15 min. The clear solution was then divided into two portions. One part was acidified with a drop of concentrated HCl; the other part was alkalinized with a drop of concentrated ammonia solution. A red or pink color in the acidified solution and a yellow color in the alkaline solution indicated a positive presumptive test for prodigiosin [9].

2.4. Identification and Characterization of Isolated Prodigosin Producing Bacteria

Morphological characteristics such as abundance of growth, pigmentation, optical characteristics, size and shape were studied on Nutrient agar plates.

2.5. Biochemical Characterization

The isolate was further studied about characterization of gram staining, spore staining, and hanging drop method, similarly the biochemical characterization were performed the following tests like indole, methyl red, voges-proskauer test, citrate utilization test, triple sugar iron test, nitrate reduction test, gelatin hydrolysis test, starch hydrolysis test and carbohydrate fermentation test were studied[3].

2.6. Production of Prodigiosin

A loopful of culture was inoculated in a pre-sterilized 100 ml nutrient broth. The flask was kept in a shaker at 120 rpm for 16-18 h at 30°C. The culture broth was centrifuged at 10,000 rpm for 20 min. Cell suspension was prepared using sterile distilled water. 5 % of the above suspension was used as inoculum for the production of prodigiosin. Cultures were grown in both nutrient broth and peptone glycerol broth for prodigiosin production (Plate; 3). The production levels were estimated both at stationary and agitated phases to

investigate the effect of aeration on production. The levels of prodigiosin in these conditions were estimated after 0 hr, 24 hr, 48 hr and 72 hr.

2.7. Extraction of Prodigiosin

The cells were harvested by centrifugation at 10,000rpm for 10min. 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 min. The supernatant containing prodigiosin was transferred to the fresh vial [10].

2.7.1. Estimation of Prodigiosin

The absorption pattern of prodigiosin was checked at 499nm where prodigiosin absorbs maximally. At this wavelength the absorptions were recorded. The bacterial cell absorption prior to extraction was noted at every step.

Extracted prodigiosin was estimated using the following equation [11].

Prodigiosin unit/cell = ($[OD_{499}- (1.381xOD_{620})$ x $1000/OD_{620}$

Where OD_{499} = pigment absorbance

OD_{620} = bacterial cell absorbance

1.381 = constant

2.7.2. Partial Purification of Prodigiosin

Pigment produced by the bacterium was purified according to Pryce *et al.* [12], with some modifications. Equal volume of petroleum ether was added to the ethanol extract taken in a separatory funnel and mixed well. Equal volume of distilled water and concentrated solution of sodium chloride was then added to the separating funnel in order to enhance the phase separation. Slowly the pigment got transferred to the epiphase (petroleum ether phase). The hypophase with ethanol and water soluble impurities was removed. The petroleum ether phase is washed 4 or 5 times with distilled water to remove residual ethanol. The pigment collected from the petroleum ether phase was treated with 1 N HCl (9:1; v/v) and concentrated by evaporating the solvent in a 40°C water bath.

2.8. Optimization of Prodigiosin Production

2.8.1. Effect of Different Carbon Sources on Prodigiosin Production

To study the effect of different carbon sources on prodigiosin production, 0.5% of different sugars such as glucose, fructose, Mannitol, glycerol, sucrose, lactose and raffinose were added to the production medium. All the flasks were incubated at 28°C under shaking conditions for 72 hours and prodigiosin production was checked after 48 hrs.

2.8.2. Effect of Different Nitrogen Sources on Prodigiosin Production

The effect of different nitrogen sources on prodigiosin production was studied by replacing 0.1% of different organic and inorganic nitrogen sources such as ammonium chloride, yeast extract, peptone, beef extract, ammonium nitrate and sodium nitrate added to the production medium. All the flasks were incubated at 28°C under shaker conditions for 48 hrs and the prodigiosin production was checked.

2.8.3. Effect of pH on Prodigiosin Production

The optimum pH on prodigiosin production was studied with different pH values (7, 7.5, 8, 8.5 and 9.0) in the production medium. The flasks were incubated at 28°C under shaker conditions for 48 hours. The prodigiosin production was estimated after 48 hours.

2.8.4. Effect of Temperature on Prodigiosin Production

To determine the optimum temperature for prodigiosin production, the following temperatures were studied such as 20, 25, 28, 37 and 40°C. The prodigiosin production was estimated after 48 hours.

2.9. Antimicrobial activity of the extracted prodigiosin against selected pathogens by Kirby Bauer method

The 6 mm (diameter) discs were prepared from whatmann No.1 filter paper. The discs were sterilized by autoclaving at 121°C. After sterilization, the moisture of the discs was dried on hot air oven at 50°C. Then the solvent extract discs and the control discs were prepared.

2.9.1. Antimicrobial Susceptibility Testing by Kirby- Bauer Method

Antimicrobial activity of the prodigiosin extract was determined using a modified Kirby Bauer or disc diffusion method [19]. For the test, sterile petriplates of Muller Hinton agar was prepared. The test bacterial pathogens (*K. pneumoniae, Pseudomonas aeruginosa, E.coli, S.flexneri*) were swabbed onto the Muller Hinton agar

plates. Discs were impregnated with 25 µl of the test samples (prodigiosin extract), allowed to dry and placed onto inoculated plates (30 min incubation). The plates were incubated at 37°C for 24 hours, and then the diameters of the zone of inhibition were measured in millimeters. Each antimicrobial assay was performed in triplicate and the mean values were reported.

2.9.2. Antifungal Susceptibility Testing by Kirby- Bauer Method

Antifungal activity of the prodigiosin extract was determined using a disc diffusion method [20]. For that the test, sterile petriplates of Muller Hinton agar was prepared. The test fungal pathogens (*Aspergillus niger* and *Aspergillus flavus*) were swabbed onto the Muller Hinton agar plates. Discs were impregnated with 25 µl of the test samples (prodigiosin extract), allowed to dry and placed onto inoculated plates (30 min incubation). The plates were incubated at 37°C for 72 hours, and then the diameters of the zone of inhibition were measured in millimeters. Each antifungal assay was performed in triplicate and the mean values were reported.

2.9.3. Dyeing Effect of Prodigiosin

Three 5×5 cm cotton cloths were taken, one was kept as control, and in the second cloth 1 ml of crude prodigiosin methanolic extract was applied to the warm surface and was allowed to dry at room temperature for about 1 hr. In another piece of cloth, 1 ml of crude prodigiosin methanolic extract was applied to the warm surface and 0.5ml of thiourea was applied as a mordant and was allowed to dry at room temperature for about 1 hr. The third cloth was cut into four pieces, one was kept as control, second piece was washed in tap water containing detergent, third piece was washed in hot water containing detergent and fourth piece was washed in ice cold water containing detergent after 30 minutes the cloth pieces were washed with tap water and allowed to dry at room temperature.

3. RESULTS AND DISCUSSION

Prodigiosin is a non-diffusible tripyrrole that occurs naturally as a secondary metabolite which is mainly produced by *Serratia marcescens*. Prodigiosin is a promising drug due to its reported antifungal, immunosuppressive and anti-proliferative properties. Hence, the present investigation was focused with much attention to isolate prodigiosin pigments from marine *Serratia marcescens* and to evaluate antimicrobial and antifungal potentials.

3.1. Isolation of Microorganisms from Marine Sediments

In the present investigation, the sediment samples were collected from the Mandapam coast, Gulf of Mannar. The collected sediment samples were serially diluted and spread plated on nutrient agar plates and the plates were incubated at 28°C for 24 hours. After incubation, morphologically distinct orange to maroon colored colonies were selected (plate 1) and sub cultured on the same medium. Pure culturing of the isolates was performed by repeated streaking on Nutrient agar plates (plate2) and the cultures were maintained as glycerol stock and in nutrient agar slant for further use.

3.1.1. Presumptive Tests for Screening Prodigiosin Production

The presumptive test for prodigiosin production was done with two morphologically distinct orange to maroon colored strains. The two isolates screened, only one isolate revealed positive results in presumptive tests with red colour formation in acidified solution and yellow color in alkaline solution. This isolate was selected for further study.

3.2. Identification of the efficient Prodigiosin producing bacteria

3.2.1. Morphological and Biochemical characterization

On nutrient agar plate, the isolate produced orange to red colored colonies. The isolate was a gram negative, rod shaped motile organism. Further biochemical tests were conducted to ascertain the genus of the bacteria. The test results were depicted in (Table1 and Plate3& 4).

Table: 1 Biochemical Characterization of the Prodigosin Producing Microorganism of *Serratia Marcesens*

Test	Observation
Gram staining	Negative rod
Motility	Motile
Indole	Negative
Methyl red	Negative
Voges- proskauer	Negative
Citrate	Positive
Nitrate	Positive
Gelatin	Negative
Starch hydrolysis	Positive
Triple sugar iorn	Positive
Carbohydrate Fermentation	
Sucrose	Negative
Dextrose	Positive

Indole was not produced. Glucose was not oxidized with the production of high concentration of acid end products in methyl red test. The strain did not produce acetoin in voges proskauer test hence the result was negative. Citrate was utilized by the organism as the sole carbon and energy source.

Gelatin was hydrolyzed. Starch was not hydrolyzed by this organism. It ferments sugars like dextrose and not ferments the sugar sucrose. From the obtained results, this isolate was identified as *Serratia marcescens*. The results were compared with the Bergey's manual of determinative bacteriology [1].

The production of prodigiosin was formed in nutrient broth and peptone glycerol broth in both shaking and static condition. The production level was maximum at 72hrs. The total prodigiosin was found to be higher in nutrient broth rather compared with peptone glycerol broth. The results were noted in Table 2-6 and Figs. 1, 2 and Plate 5.

Table 2: Total Prodigiosin Estimated in Nutrient Broth and Peptone Glycerol Broth in Stationary and Agitated Phase

Hours of incubation	Total Prodigiosin in Nutrient broth Mg/l		Total Prodigiosin in Peptone glycerol broth (Mg/l)	
	Agitated	Stationary	Agitated	Stationary
24	0	0	0	0
48	1245.44	932.94	1218.33	1038.33
72	1734.06	1071.10	1328.04	1052.87
96	1392.01	810.11	1251.20	875.39

Table 3: Production of Prodigiosin in Nb+Sesame Powder Broth Agitated

S. No	Time (hr)	OD (499nm)	OD (620nm)	Value(mg/100ml)
1	24	0	0	0
2	48	1798.03	6701.10	1302.15
3	72	1935.10	6730.26	1508.70
4	96	1724.26	6214.07	1399.61

Table 4: Production of Prodigiosin in Nb+Sesame powder broth stationary

S. No	Time (hr)	OD (499nm)	OD (620nm)	Value(mg/100ml)
1	24	0	0	0
2	48	1684.91	6573.11	1209.71
3	72	1909.06	7261.33	1275.04
4	96	1715.83	6607.16	1217.43

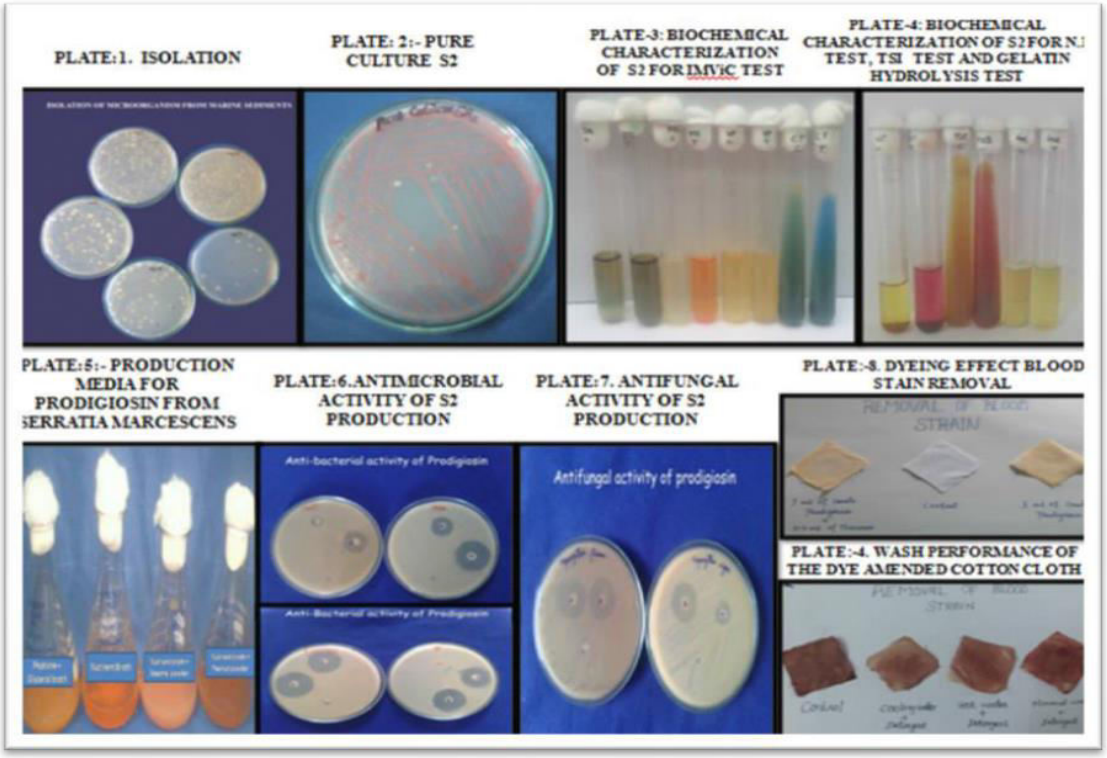
Table 5: Production of Prodigiosin in Nb+Peanut Powder Broth Agitated

S. No	Time (hr)	OD (499nm)	OD (620nm)	Value(mg/100ml)
1	24	0	0	0
2	48	1801.11	6607.11	1347.71
3	72	1957.14	6577.99	1613.90
4	96	1863.17	6697.03	1437.41

Table 6: Production of Prodigiosin in Nb+Peanut powder broth stationary

S. No	Time(hr)	OD(499nm)	OD(620nm)	Value(mg/100ml)
1	24	0	0	0
2	48	1795.01	6709.43	1298.11
3	72	1891.31	6809.11	1391.84
4	96	1759.49	6707.13	1230.94

Prodigiosin is a promising drug due to its reported antifungal, immunosuppressive and anti-proliferative properties. Hence, the present investigation was focused with much attention to isolate prodigiosin pigments from marine *Serratia marcescens* and to evaluate antimicrobial and antifungal potentials.



3.3. Optimization of Prodigiosin Production

Effect of different carbon sources on prodigiosin production were performed with glucose, glycerol, mannitol, sucrose, lactose, fructose and raffinose respectively. It was found out that an enhanced production of prodigiosin was recorded with sucrose, followed by glycerol and mannitol exhibiting 1147.32 mg/L, 1138.71mg/L and 994.38 mg/l (Fig.1).

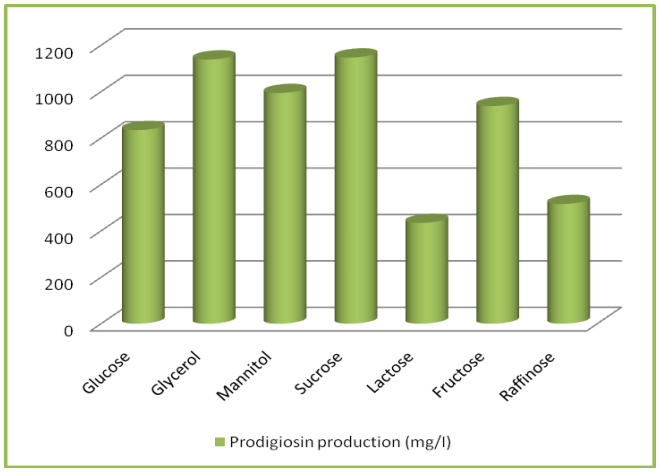


Fig.1: Effect of different carbon sources on prodigiosin production

The effect of different nitrogen sources on prodigiosin production were observed with NH_4Cl, NH_4NO_3, $NaNO_3$ Beef extract, yeast extract and peptone respectively. The results revealed that beef extract as nitrogen source enhanced the prodigiosin production. Moreover, yeast extract and peptone showed similar effects exhibiting 1078.38, 1129.01 mg/l of prodigiosin production. Simultaneously, the other nitrogen sources do not favor prodigiosin production (Fig. 2).

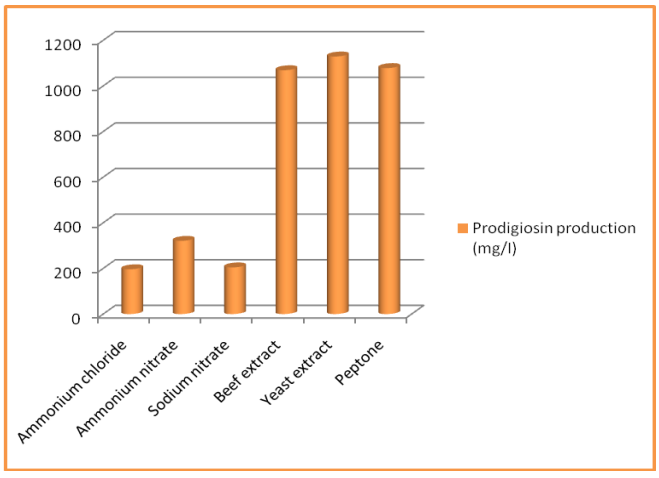


Fig.2: Effect of different Nitrogen sources on prodigiosin production

The effect of different pH on prodigiosin production was observed. Among which, pH 8 was found to be an optimum pH for an elevated prodigiosin production

exhibiting 997.02mg/l. Whereas when the pH was increased to 9.0 the production was found to be less. Which is probably due to *Serratiamarcescens* which posses strong buffering capacity (Fig. 3).

On analysis with treatment with different temperature to monitor prodigiosin production, an optimum temperature of 28°C revealed a better yield of prodigiosin with 884.72 mg/l which is followed by a temperature of 37°C led to a decreased yield of prodigiosin production (639.mg/l) (Fig. 4).

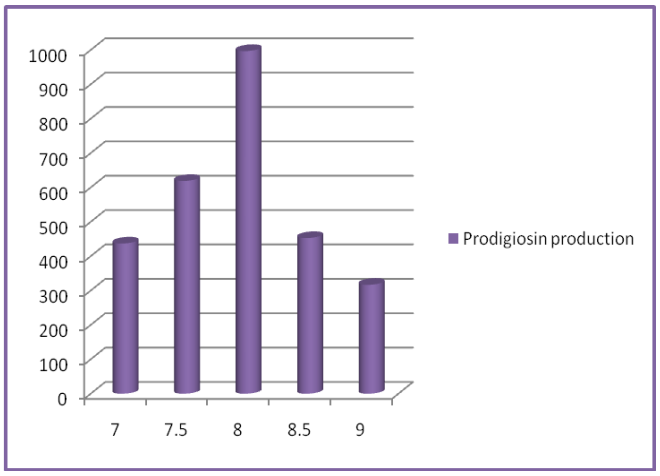


Fig. 3: Effect of different pH on prodigiosin production

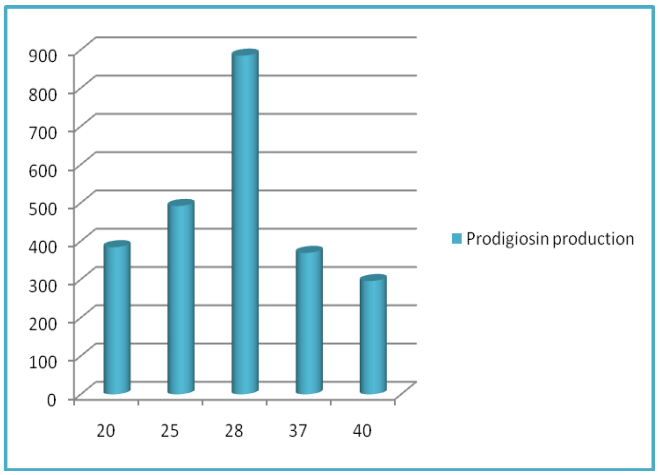


Fig. 4: Effect of different temperature on prodigiosin production

3.4. Antimicrobial and Antifungal activity of Prodigiosin

The studies pertaining to antimicrobial and antifungal activity of the extracted prodigiosin treated against nosocomial infections causing *K.pneumoniae, S.flexneri* and *E.coli* revealed zone of inhibition of 16mm, 39mm and 19mm, respectively. A treatmendous zone of inhibition was recorded for *P. aeruginosa* which causes pus in wound cells. Similarly, the effect of prodigiosin as drug was also evaluated against fungal isolates such as *Aspergillus niger* and *Aspergillus flavus.* A prominent Zone of inhibition with 14mm for *A. flavus* were observed followed by *Aspergillus niger* exhibiting its activity with 9 mm of zone of inhibition in diameter (Table 7 and plate 1 and 2).

Table 7: Antimicrobial and Antifungal Activity of the Extracted Prodigiosin against Pathogens

S. No	Pathogens	Zone of Inhibition in diameter (in mm)
1.	*E. Coli*	19
2.	*Shigellaflexneri*	39
3.	*Klebsiella Pneumoniae*	16
4.	*P.aeruginosa*	43
5.	*Aspergillus niger (Fungi)*	9
6.	*Aspergillus flavus (fungi)*	14

3.5. Dyeing potential of Prodigiosin

The pigment produced by *Serratia sp.,* can be effectively used to dye textile material (Plate3 and 4). Only prodigiosin applied with cotton cloth, did not bind effectively while the addition of mordant thiourea, enhanced the binding of the prodigiosin to the cloth. During the wash performance studies, it was found that when the cloth was washed with detergent in ordinary water the colour of the dyed cloth remained unchanged. Whereas, when it was washed in hot water and cold water containing detergent, there was a slight loss of pigment from the dyed cotton material. *Serratia marcescens* is a ubiquitous bacterium inhabiting water, soil, plants and vertebrates and it has various characteristics including the pigment prodigiosin. Prodigiosin were found protease resistance, also possess immunosuppressive activity. *Serratia marcescens* is a major producer of prodigiosin.

In the present study, one strain of *Serratia marcescens* was isolated from marine sediments noted for production of red pigments. Similarly our results coincides with Lewis and horth [10]. Serratia can also be isolated from nosocomial infections causing septi-cemia. Serratia isolated from infected adults, generally does not produce pigments.

Hence there is no prodigiosin production and it is found to be virulent where as colored pigmented prodigiosin found to be a virulent which was suggested by Staunton

and Wilkinson [13]. Hence, prodigiosin production can be safely carried out to isolate novel secondary metabolites leading to remarkable applications in pharmaceuticals.

In the present study, Gulf of Mannar is notable for vast biodiversity of marine microorganisms. Samples were collected, diluted and spread plated on nutrient agar plates, followed by morphological and biochemical characterization. In the present study, presumptive test for prodigiosin production were performed from different isolates but only on isolate revealed positive results.

The data from the morphological, physiological and biochemical characterization tests performed for the isolate in accordance with the methods described in the Berge's manual of determinative bacteriology and identified as *Serratia marcescens*.

Hence, prodigiosin production can be safely carried out to isolate novel secondary metabolites leading to remarkable applications in pharmaceuticals, pH of the media plays a very crucial role in the synthesis of secondary metabolites [14]. Therefore it affects the biosynthesis of prodigiosin. Maximum amount of prodigosin by *Serratia marcescens* was produced at an optimum pH of 8. Moreover, the production decreases at a low pH. Our results were in total conformity with the work of Kim *et al.* [15]. This suggests the importance of pH in the media.

Different sugars were added to the medium. The maximum amount of pigment production was in the presence of sucrose (1147.32mg/l). Moderate level of pigment production was observed in the medium with glucose, glycerol, mannitol, sucrose, lactose, raffinose and fructose. Similar result was obtained by Samrot *et al*. [16]. Our study revealed when sucrose was incurporated in the media resulted in enhanced production.

Studies pertaining to nitrogen sources on prodigiosin production revealed that *Serratia marcescens* proliferated and produced maximum pigment production with yeast extract (1129.01mg/l) whereas the organism failed to grow in the media supplemented with ammonium chloride, ammonium nitrate and sodium nitrate possibly indicating the toxicity of ammonium salts. Our results coincide with the work of [17].

In order to increase the potentiality of the bacteria to synthesize large quantities of the pigment, a comparative study of pigment production in different media was carried out. We have compared nutrient broth, peptone glycerol broth, nutrient broth supplemented with sesame powder and nutrient broth supplemented with peanut powder in both stationary and static conditions. An enhanced production of prodigiosin (1613.90 mg/100ml) was seen in nutrient broth supplemented with peanut powder in shaking condition. Similar results have been reported by Giri, *et al*. [3]. They had a maximum yield of prodigiosin (1613.90mg/100ml) in peanut seed broth. The enhanced production of prodigiosin in nutrient broth supplemented with peanut powder might be due to the presence of more saturated fatty acids and minerals. The yield of the pigment was found to be higher in all broths under shaking condition than at static condition. Our result was similar to Parekh, *et al*. [18] who obtained maximum yield of pigment at shaking condition of broths than stationary condition.

In the synthetic media, maximum prodigiosin production was obtained at the temperature of 28°C. The role of temperature on the pigment synthesis implies that these are important physical factors for prodigiosin production. Our results were coincides with Giri, *et al*. [3].

The antimicrobial activity by Kirby Bauer technique was performed in order to ascertain whether prodigiosin can inhibit the pathogenic bacteria and fungal isolates. The inhibitory zones of bacteria differed between 19-43 mm whereas the fungicidal activity was evident from the clear zones with 9-14mm in diameter. Prodigiosin possess antibacterial activity against *Klebsiella pneumonia, Shigella flexneri, E.coli* and *P.aeruginosa.*

The results obtained using prodigiosin of *Serratia sp*. against different genus of bacteria validate the broad antibacterial potentials of the red pigment. Our results are in agreement with the previous literature which revealed the inhibitory effect of prodigiosin against both Gram-positive and Gram-negative bacteria [19]. The results were shown higher inhibitory effect of prodigiosin against Gram-positive bacteria than Gram-negative bacteria. In the present study prodigiosin has higher activity against Gram- positive *p. aeruginosa* followed by Gram negative *Klebsiella pneumonia* [20] have reported that ethanol: HCl extract of *Serratia* has antibacterial activity and its zone of inhibition was higher against both Gram-negative (*E.coli* and *Klebsiella pneumoniae*). In our current research work, minimal antibacterial activity of prodigiosin against *E.coli* and *Klebsiella pneumonia* was observed. Papageorgiou, *et al*. [19] studied the antifungal activity of prodigiosin against plant pathogenic fungus *Didymellaapplanata*. They found

that the methanolic extract of prodigiosin from *S. marcescens* have the ability to suppress the vital functions of *D. applanata*. The antifungal activity of the crude prodigiosin was also tested against fungal pathogens, which showed the maximum inhibitory zone against *Helminthosporium sativum, Fusariumoxy sporium* and *Rhizoctonia solani* in decreasing order. In our study, we tested the antifungal activity of prodigiosin against *Aspergillus niger* and *Aspergillus flavus* and we found that prodigiosin was more effective against *Aspergillus flavus* than *Aspergillus niger* [18]. The harmful effects of synthetic dye and chemicals used at the time of dyeing have forced us to concern about alternative preparation of dye using natural sources. Pandey *et al*., [20] with similar test sample evaluated dyeing potential of red pigment from *Serratia* sp.BTWJ8 and blue pigment from *Janthinobavtreium lividium* on different textile cloths and rubber products. They observed that the color faded depending upon the material and they found that post treatment of pigment with thiourea reduced the fading. In this study, an attempt was made to explore the probable use of natural pigment produced by *Serratia sp*. for dyeing purpose in textiles.

4. CONCLUSION

In current research, findings deals with isolated prodigiosin pigment from Marine *Serratia marcescens* and to evaluated efficient microorganism. The wash performance studies with the cotton material treated with prodigiosin with thiourea, which is generally considered as a safe and effective mordant, showed slight loss of pigment during hot and cold water wash. This suggest, that with slight improvement in the binding capacity of pigment that there is ample scope for using this pigment as dye.

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