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ISOLATION, SCREENING AND PRODUCTION OF (PRODIGIOSIN) NOVEL DRUG FROM MARINE SEDIMENTS

Soma Prabha, A., Jayachitra, A.

Research Scholar, School of Biotechnology, Madurai Kamaraj University, Madurai-21, Tamilnadu, India.
Assistant Professor, School of Biotechnology, Madurai Kamaraj University, Madurai-21, Tamilnadu, India.

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ABSTRACT

*Serratia marcescens*is a motile, short rod-shaped, Gram-negative, facultative anaerobic bacterium. It is commonly found in the moist regions of coastal areas and grows in room temperature at pH 5-9. S. marcescens is noted for production of secondary metabolites called prodigiosin, a bioactive compound having antibacterial, antifungal, anti-neoplastic, anti-proliferative, anti-oxidant and anti-malarial properties.Inthe present investigation, the marine sediment samples were collected from Gulf of Mannar, Mandapam coast. The samples were serially diluted and spread plated on Nutrient agar medium to isolate the efficient prodigiosin producing bacteria. The efficient prodigiosin producing bacteria was identified as Serratia marcescens based on morphological and biochemical characterization methods. Optimization studies production of prodigiosin by *S.marcescens* is influenced by numerous factors such as inorganic phosphate availability, medium composition, temperature, pH, and natural components. The production of prodigiosin were extract, estimate and partially purified (crude) prodigiosin was used as a antimicrobial, anti-fungal, Anti-inflammatory and dyeing effect was carried out. Specially, it has an inhibiting and destructive effect on the production and stability of prodigiosin. FTIR analysis were studied. The prodigiosin has known define role in the physiology of the strains in which it is produced, it has antifungal, antibacterial and antiprotozoal activities, and thus may have potential clinical utility.

Corresponding author
Soma Prabha, A.
Research Scholar,
School of Biotechnology,
Madurai Kamaraj University,
Madurai-21, Tamilnadu, India.
Somabt2012@gmail.com

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INTRODUCTION

Prodigiosin(5[(3-methoxy-5-pyrrol-2-ylidene-pyrrol-2-ylidene)-methyl]-2-methyl-3-pentyl 1*H*pyrrole) is a red pigmented secondary metabolite alkaloid with a unique tripyrrole chemical structure that contains a common 4-methoxy, 2-2 bipyrrole ring system. These pigments are emerging as a novel group of compounds having distinct biological activities like antibacterial, antimycotic, immunomodulating, anti-diabetic, algalicidal,anti-tumor and antimalarial (Bennett and Bentley, 2000*).* Hence, they are desired for several medical applications. Among them,*Serratiamarcescens*is a major producer of prodigiosin. *S. marcescens* is a rod-shaped, Gram negative, facultative bacterium belonging to the *Enterobacteriaceae* family which forms beautiful pillar box red colonies. Pigment production is highly variable among species and depends on many factors such as species type incubation time, temperature and nutrient source (Pandey*et al.,* 2009; Kim *et al.*, 2009).Although prodigiosin has now known defined role in the physiology of the strains in which it is produced, it has antifungal, antibacterial, antimalarial, anti-diabetic, algalicidal, anti-inflammatory and antiprotozoal activities, and thus may have potential clinical utility (Moraes*et al.*, 2009).

Prodigiosin (2-methyl-3-pentyl-6-methoxyprodigiosin) is a linear tripyrrole typical secondary metabolite, appearing only in the later stages of bacterial growth. Prodigiosin may conform to the classical description of a secondary metabolite, having no essential role in the growth or survival of the cell, but instead acting as an overflow for 'waste products from primary metabolism. (Qadri, *et al*.,1972).

As recently shown in the study of prodigiosin is regulated by a proposed interspecies quorum sensing (QS) signalling system,in some *Serratia* strains. Quorum sensing (QS) is a mechanism whereby bacteria can regulate gene expression in response to the population cell density via detection of a diffusible signaling molecule.(White head *et al*., 2001).Recently, *Serratia marcescens*, has been a subject of researchinterest by the scientific community because of its developing therapeutic potential.(Pandey*et al*., 2009).

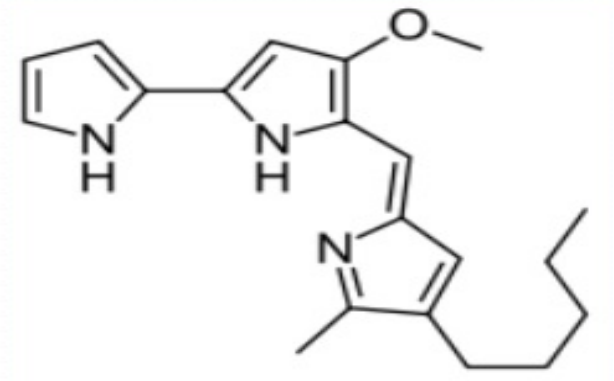


Figure 1. Chemical structure of prodigiosin.

Hence the present investigation was found with much attention to isolates an efficient prodigiosin producing *Serratia marcescens* from marine sediments(Gulf of Mannar,Mandapam). Optimize the culture conditions for enhanced prodigiosin production. In addition, the antibacterial, anti-fungal, anti-inflammatory and dyeing effect of the potential extracted prodigiosin pigment will be evaluated.The prodigiosin production from different media was studied. The FTIR analysis of crude prodigiosin was carried out.

MATERIALS AND METHOD

Isolation of *S. Marcescens*from Marine Sediments

Marine sediment samples were collected from Mandapam coast of Gulf of Mannar. 1 ml of the collected sediment samples were serially diluted and spread over the surface of nutrient agar medium (Himedia). The plates were incubated at 28^{o}C for 24-48 hours. The morphologically distinct bacterial strains showing orange to maroon colour colonies were selected for further study and were maintained on nutrient agar slants at 4^{o}C.

Presumptive Tests for Prodigiosin Production

Presumptive colour tests for prodigiosin was carried out by scraping the pigmented growth on nutrient agar medium plates and suspended overnight in 95% ethanol at 28^{o}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 colour in the acidified solution and a yellow colour in the alkaline solution indicate a positive presumptive test for prodigiosin.

Identification of the Efficient Prodigiosin Producing Bacteria

The isolated prodigiosin producing bacterial strain was identified based on morphological and biochemical characterization methods.

Optimization Studies

Effect of Temperature

The Pigment production was carried out at , 28, 30, 35 and 37^{o}C keeping all other conditions at their standard levels and then assayed for prodigiosin. Cultivation temperatures on prodigiosin production have been carried out by incubating the nutrient broth at different temperatures. The optimum temperature achieved by this step was fixed for subsequent experiments.

Effect of pH

To determine the effect of pH of the nutrient broth on prodigiosin production, experiments were performed with media of different pH. While optimizing the pH of the basal medium, the pH of aqueous solution was varied from 5.0 to 9.0 with 0.1 M NaOH or 0.1 N HCl and then assayed for prodigiosin. The optimum pH achieved by this step was fixed for subsequent experiments.

Production of Prodigiosin using Different Media

The four different media such as Nutrient broth, peptone glycerol broth, nutrient broth amended with sesame powder and Nutrient broth supplemented with peanut powder were used for prodigiosin production.The pH of all the above media were maintained at 7.0. 5% of fresh pigmented inoculum was added to each of the broth and incubated at 28°C. The production levels were estimated both at stationary and agitated phases The levels of prodigiosin in these conditions were estimated after 24hr, 48hr, 72hr and 96hr of experiment.

Extraction of Prodigiosin

Extraction of the pigment was done according to Slateretal.,(2003) with slight modification. The broth was centrifuged at 10,000 rpm for 15 min. The cell pellets resuspended in 1ml ethanol and then centrifuged at 10,000 rpm for 15 min. The supernatant was transferred into fresh vials and the pellets were discarded.

Estimation of Prodigiosin

The transferred supernatant during extraction was taken and O.D was observed at 499nm by scanning in a UV-Visible spectrophotometer (Shimadzu, Japan) and cells were taken and O.D observed at 620nm was substituted in the below equation:

$$\{[O.D_{499} - (1.381 \times O.D_{620})] \times (100 \div O.D_{620})\}$$

Where OD_{499} = pigment absorbance, OD_{620} = bacterial cell absorbance
1.381 = constant, 100=Volume in ml.

Partial Purification of Prodigiosin

Pigment produced by the bacterium was purified according to Song *et al.* (2006), with some modification. 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 separatory 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 40ºC water bath.

Antimicrobial Activity of the Extracted Prodigiosin against Selected Pathogens by Kirby Bauer Method

Antibacterial activity of the prodigiosin extract was determined using a modified Kirby Bauer or disc diffusion method. Each antibacterial assay was performed in triplicates and the mean values were reported.

Antifungal Susceptibility Testing by Kirby- Bauer Method

Fungal pathogens (*Aspergillusniger* and *Aspergillusflavus*) were swabbed onto the Muller Hinton agar plates Each antifungal assay was performed in triplicate and the mean values were reported.

Anti-inflammatory effect

Anti-inflammatory activity was evaluated by formalin induced paw oedema method. Three small albino mice were taken, one mice was injected with prodigiosin methanolic extract it was given under the plantar uponeurosis of the right hand paw second mice was kept as a formalin control third mice was kept as a control. The mice were kept undisturbed for 30 minutes then first and second mice were injected with 1ml of 1% formalin under the plantar uponeurosis of the right hand paw third mice which was kept as a control was undisturbed. Then the oedema was measured for every 1hr until three hours.
Then the percentage of inhibition was calculated in this formula.

% Inhibition = {(Inflammation of control-Inflammation of test) / (Inflammation of control)}*100

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.

Sample Preparation for FTIR

Prodigiosin production by *Serratia marcescens* species was collected after 20 days of incubation. The prodigiosin reduce was air dried and used for FTIR analysis. Sample for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride (salt).

RESULT

The present investigation was focused with much attention on production of prodigiosin from marine *Serratia sp*. Prodigiosin is a non-diffusible tripyrrole that occurs naturally as a secondary metabolite which is mainly produced by *Serratia species* known to posses therapeutic application.

In the present investigation the sediment sample were collected from the Mandapam coast, Gulf of Mannar. The collected sediment samples were serially diluted and spread plated on nutrient agar plate and the plates were incubated at 28°C for 48-72 hours. After incubation, morphologically distinct orange to maroon coloured colonies were selected and sub cultured on the same media. Pure culturing of the isolate was performed by repeated streaking on nutrient agar plates (Plate:1).

Presumptive test for Screening Prodigiosin Production

The presumptive test for prodigiosin production was carried out with two morphologically distinct orange to maroon coloured isolates. One isolate revealed the positive results in the presumptive test with the red colour in the acidified solution and yellow colour in alkaline solution exhibit in Plate:2. The isolate was selected for further study.

Morphological and Biochemical characterization

On nutrient agar medium, the isolate produced orange to red colured colonies. The isolate was a Gram negative, rod shaped and motile. Further biochemical tests were conducted to ascertain the genus of bacteria. The isolate was negative for indole production. Glucose was not oxidized with the production of high concentration of acid end products in methyl red tests. The strain produced acetone and butane diol in Voges-Proskauer test. Citrate was utilized by the organism and as its sole carbon and energy source. Nitrates were reduced to nitrites. Gelatin was hydrolyzed and starch was not hydrolyzed by this strain. From the obtained results, these isolate was identified as *Serratia sp.* The results were compared in accordance with the Bergey's manual of determinative bacteriology.(Table:1).

Comparative Analysis of Prodigiosin Production by *Serratia sp.* in Different Medium

The production of prodigiosin was performed in different media viz., nutrient medium, peptone glycerol broth, nutrient broth supplemented with sesame powder broth and nutrient broth supplemented with peanut powder broth was tested in both shaking and static conditions. The production levels of prodigiosin were measured at every 24 hrs upto 96 hrs. The production of prodigiosin with nutrient broth in agitated condition was estimated periodically (24, 48, 72, and 96h) (Fig-1 and Plate:3). Prodigiosin production expressed as mg/100ml, at 72hrs was found to be 139.19 mg/100ml. Production ceased towards 96hrs, whereas, the production of prodigiosin in nutrient broth (static conditions) at 72hrs was found to be optimum exhibiting 118.67 mg/100ml. Whereas, in the agitated phase determined maximumproduction of 131.19 mg/100ml.

Similarly, the production of prodigiosin with peptone glycerol broth in agitated condition revealed 131.90 mg/100ml of prodigiosin in static condition expressed 105.23 mg/100ml of prodigiosin. Nutrient broth containing sesame powder in shaking condition produced 150.70 mg/100ml of prodigiosin and in static condition expressing 127.04 mg/100ml at 72 hr.

Supplementation of peanut powder in nutrient broth(shaking conditions) resulted in drastic increase in the production of prodigiosin 161.9 mg/100ml at 72 hrs. Similarly, in stationary phase the production was comparatively less (139.84 mg/100ml) at 72 hr of incubation.Comparatively all the media showed a better prodigiosin production under shaking condition.(Fig-:2).when compared to static condition. Among the various broth tested for prodigiosin production, the maximum yield was observed in nutrient broth supplemented with peanut powder in shaking (161.9 mg/100ml) followed by stationary (139.84 mg/100ml).

Optimization Studies

Effect of pH on prodigiosin production:

The selected potential prodigiosin producing *Serratia marcescens* was subjected to various pH ranging from 5 to 9. The results were presented in Fig.3. The prodigiosin was found to be highest in the neutral pH-7 with 155.97 mg/100ml. Less prodigiosin production were noticed in the alkaline pH (8 and 9) and acidic pH (5 and 6).

Effect of Temperature on prodigiosin production:

In the present study the effect of Temperature on prodigiosin production was studied with different temperatures such as 27^{o}C, 30^{o}C, 35^{o}C and 37^{o}C respectively. The maximum yield of prodigiosin was observed at 30^{o}C for nutrient broth with 148.75mg/100ml. above the temperature (35^{o}C and 37^{o}C). The yield was found to be decreased with 137.90 mg/100ml and 128.88mg/100ml respectively. The results were noted in Fig.4.

Antibacterial Activity of Prodigiosin:

It was performed in order to ascertain the role of prodigiosin against pathogens. The maximum zone of inhibition was formed against *Bacillus subtillus*(13mm) followed by *Klebsiella pneumonia* (7mm). The least activity was observed in *E.coli* (2mm) and *Pseudomonas aeurogenosa*(1mm) (Plate:4). The standard antibiotics used was Streptomycin.

Antifungal activity of Prodigiosin

Similarly the antifungal activity of prodigiosin against *Aspergillus niger* and *Aspergillus flavus* was tested. The maximum zone of inhibition was observed *Aspergillus flavus* (9mm). While *Aspergillus niger*havea zone of inhibition of 5mm.(Plate:5).

Anti-inflammatory study

Anti-inflammatory activity was evaluated by formalin induced paw oedema method. The paw size of the control mice remained constant. The second mice which was treated as formalin control exhibited 2.3 cm paw size at the time of formalin injection and after 1 hour the paw size was found to be 3.1. Consecutively, after two hours the paw size was 3.2 and after 3 hours the paw size remained unchanged (3.2). The third mice which received prodigiosin extract, 30 minutes prior to formalin injection was found to have paw size of 2 cm at the time of formalin injection after 1 hour the paw size was 2.7, after 2 hour and 3 hour the paw size was found to be reduced as 2.5 and 2.3 cm respectively. The results revealed maximum oedema formation in formalin control mice. And the oedema formation was found to be less in prodigiosin injected mice the percentage of inhibition of oedema was found to be 66.7%. It was also noted that, prodigiosin inhibited the jumping and licking response in mice whereas in control (formalin treated) mice produced paw licking and paw jumping.(Plate:6 and Table:2 and3).

Dyeing Potential of Prodigiosin

The pigment produced by *Serratia sp.*, can be effectively used to dye textile material. On the addition of prodigiosin alone to 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 hotwater and cold water containing detergent, there was a slight loss of pigment from the dyed cotton material. The results recorded in Plate:7 and 8.

Characterization of prodigiosin by Fourier Transform infrared Spectroscopy

FTIR is reliable rapid economical technique. The present study is conserned with production of prodigiosin of marine sediments infrared region in mid IR (400-4000cm^{-1}) is the most commonly used region for analysis since the molecules characteristic absorbance frequencies and primary molecules in this range. The chemical changes in the functional group of prodigiosin present in marine sediments were monitored using fourier transform infrared spectroscopy. This functional group present in molecules tend to absorb IR radiation to the wave number less of the other structure in the molecules. Hence there is a correlation between IR band position and chemical structure in the molecules.

The results of FTIR studies indicated a predominant peak of 3362.07cm^{-1} indicated OH stretching band due to vibration of functional group molecular bands in protein and mucopolysaccarides. Another prominent peak of 2937cm^{-1} exhibited C-H stretching of methylene groups. For monitoring the FTIR spectra the peak of1809.31cm^{-1} indicated C=O a carbonyl strong band appeared due to red pigmentation. Simultaneously another peak with the wave length of 1544.08cm^{-1} revealed to pyrrole group. On examining the another peak of 1651.14cm^{-1} indicated C=C streching vibrations. From the spectrum, the main functional groups spectrum of prodigiosin showed bands at 1047.39cm^{-1}. The results showed in Fig:5.

Similarly another study of prodigiosin production using a different substrate peanut powder study of prodigiosin production using a different substrate peanut powder exhibited a peak of 3409.33cm^{-1},occured due to aliphatic alcohols. Similarly the FTIR spectra exhibited another peak 2932.89cm^{-1} revealed a strong alkane structure C-H. Similarly another peak 1653.07cm^{-1} revealed aromatic stretch followed by similar peak with a characteristic feature of C=O stretching. The FTIR spectrum for prodigiosin observed with the peak value of 3409cm^{-1}. The results revealed in Fig:6.

Table:1. Morphological and Biochemical Characterization of Marine Isolates.

Test	Observation
Gram Staining	Negative rod
Motility	Motile
Indole test	Negative
Methyl Red test	Negative
VP test	Positive
Nitrate reduction test	Positive
Citrate utilization test	Positive
Gelatine Hydrolysis test	Positive
Starch hydrolysis test	Negative
Triple sugar iron test	Positive
CarbohydrateFermentation	
Fructose	Positive
Sucrose	Negative

Table-2:Evalution of anti-inflammatory effect of methanolic extract of prodigiosin –induced paw oedema in small albino mice.

Sl.no	Time interval (h)	Paw size of control mice(cm)	Paw size of formalin control mice(cm)	Paw size of prodigiosin injected mice (cm)
1	0	2	2.3	2
2	1	2	3.1	2.7
3	2	2	3.2	2.5
4	3	2	3.2	2.3

Inflammation = (Paw size of the mice at the zeroth hour) – (Paw size of the mice at the respective hour).

Table-3: Size of inflammation in small albino mice.

S.no	Time interval (h)	Paw size of control mice(cm)	Paw size of formalin control mice(cm)	Paw size of prodigiosin injected mice (cm)
1	0	0	0	0
2	1	0	0.8	0.7
3	2	0	0.9	0.5
4	3	0	0.9	0.3

%Inhibition after 3H =(Inflammation in control mice–Inflammation in test mice)x100
(Inflammation in control mice)
= ([(0.9-0.3)/0.9] × 100)
= 66.67.

Plate:1. Pure Culture of Serratia Marcescens

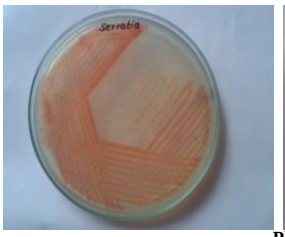


Plate:2. Phase separation of prodigiosin and petroleum ether

Plate:3. Different Production Media for Prodigiosin in Shaking Condition

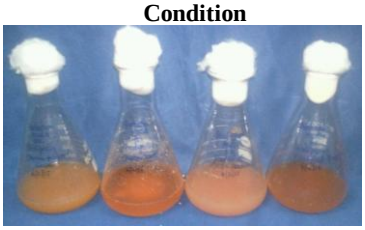


Plate:4. Antibacterial Activity of the Extracted Prodigiosin against Bacterial Pathogens

Plate:5.Anti-Fungal Activity of Prodigiosin

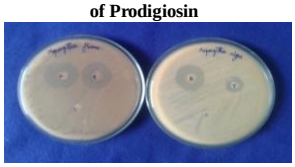


Plate:6.Formalin induced paw oedema in mice

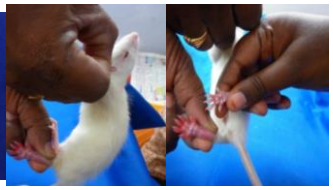


Plate:7.Dyeing Property of prodigiosi

Plate.8. Wash performance of the dyed cotton cloth

Fig. 1:Comparative Analysis for Prodigiosin Production in Different Media in Shaking Condition

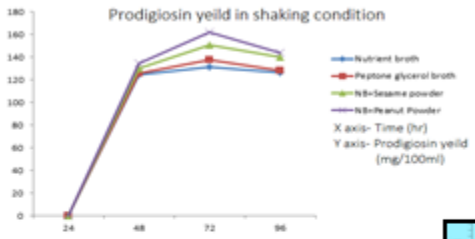


Fig. 2: Comparative Analysis for Prodigiosin Production in Different Media in static Condition

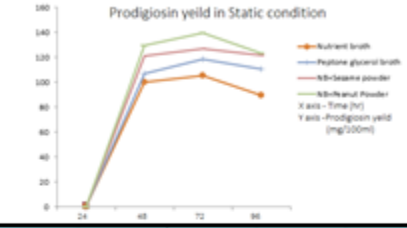


Fig.3.Effect of pH on prodigiosin production

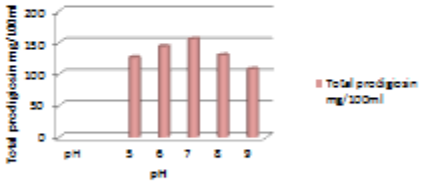


Fig.4. Effect of Temperature on prodigiosin production

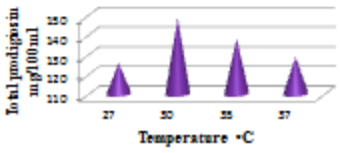


Fig.5: - FTIR Analysis of prodigiosin Production

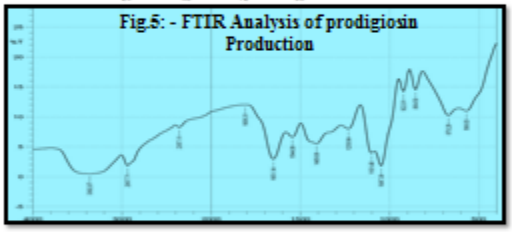
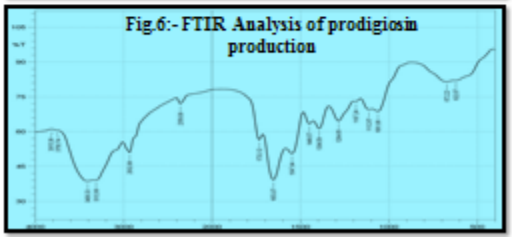


Fig.6:- FTIR Analysis of prodigiosin production



DISCUSSION

Prodigiosin or prodigiosin like pigments have not been reported for any other genera of gram negative rods other than genus *Serratia* (Brisou, 1955) .The results of our biochemical test was found to be similar to that of biochemical results of *Serratia* by and which confirmed that the isolated strain was a member of genus *Serratia.* 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 (161.9mg/100ml) was seen in nutrient broth supplemented with peanut powder in shaking condition.

Similar results have been reported by Giri*et al*., (2004). They had a maximum yield of prodigiosin (161.9mg/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 who obtained maximum yield of pigment at shaking condition of broths than stationary condition.

The results obtained using prodigiosin of *Serrataia 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 (Mekhael and Yousif., 2009; Samrot*et al.,*2011). Mekhael and Yousif (2009) have shown higher inhibitory effect of prodigiosins against Gram-positive bacteria than Gram-negative bacteria. In the present study prodigiosin has higher activity against Gram- positive *Bacillus subtilis* followed by Gram negative *Klebsiella pneumonia*. Samrot et al. (2011) 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 *Pseudomonas sp*.). We observed contradictory results were minimal antibacterial activity of prodigiosin against *E. Coli* and *Pseudomons auerоginosa* was observed.

These reports are in agreement with the present finding. This might be due to the fact that the terminal step in prodigiosin biosynthesis i.e., condensing of mono and bipyrrolemoieties was temperature sensitive. Williams, et al., (1965). The maximal amounts of prodigiosin were synthesized in either minimal or completed medium after incubation of cultures at 27^{o}C for 7 days which was reported by Williams *et al.,* (1971).

Investigation on this study revealed that on enhanced prodigiosin production was observed at pH 7.0 with the yield of 155.97 mg/100ml. The influence of external pH on the regulation of biosynthesis of secondary metabolites has been described previously Shah *et al.*,(1991). In some cases, the optimum for biomass yield was different from that for metabolite production. James *et al*., (1991).

Antimicrobial potential of the pigment may aim at the possible future usage of prodigiosin as a therapeutic. Duzhak*etal.,* (2012) 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 by against fungal pathogens, which showed the maximum inhibitory zone against *Helminthosporiumsativum, Fusariumoxysporium* and *Rhizoctoniasolani* 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.*

This is the first time to the best of our knowledge that the anti-inflammatory property of prodigiosin was evaluated using formaline induced paw oedema method in small albino mice in-vitro. This was carried out based on the results of *in–silico* molecular docking analysis of prodigiosin and cycloprodigiosin as COX-2 inhibitors by pabba *et al.*,(2013).

In our study using mice model, we observed 66.7% inhibition of odema in paw. Hence it was suggest that fully processed prodigiosin can be used to replace the rofecoxib and valdecoxib which are widely used as anti-infalmatory drugs but they have increased risk of heart attacks and strokes hence fully processed prodigiosin can replace this risky drugs. 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.Tested dyeing potential of red pigment from *Serratia* sp.BTWJ8 and blue pigment from *Janthinobavtreiumlividium*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. 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. FTIR spectroscopy provided information for comparing the spectra of functional groups of prodigiosin. The FTIR spectra of red pigment showed that it has several degree of similarity to the spectra of prodigiosin proposed by Song *et al.,* (2006). This study demonstrated a successful optimization of the cultural parameters that facilitated the enhanced production of the prodigiosin.

CONCLUSION

Serratia marcescens, gram negative facultative anaerobic bacterium. It was isolated from marine sediment sample, gulf of mannar, mandapam, Ramnad. D.t. The isolates were extracted for pigment production and to screen for prodigiosin a novel secondary metabolite known to possess wide application antineoplastic, antibacterial, antifungal activity. Production of prodigiosin was performed in different media on Peptone glycerol broth, Nutrient broth also toenhance production, it was supplemented with sesame broth, peanut powder, was tested in two different shaking and static condition. Prodigiosin production at 72 hrs was found to be 139.19mg/100 ml. Whereas agitated medium exhibited an increased level 131.19 mg/100ml. Further extension lead to ceasing of enzyme production. It was unfortunate to note that supplementation of peanut powder enhanced the yield of enzyme to 161.9mg/100ml.at 72 hrs. Optimization studies pertaining to pH 7 were studied. The effect of Temperature on prodigiosin production was maximum yield of prodigiosin was observed was 30^{o}C. Prodigiosin revealed Antibacterial activity against selected pathogen. Antifungal activity was performed against selected *Aspergillus niger, Aspergillus flavus.* Besides the study was extended to assess the prodigiosin for wash performance test in order to elucidate, the drug can be used an detergent. The functional groups changes monitored in FTIR revealed a broad IR spectrum is indicative vibrations of molecular bonds in proteins. The mice model, we observed 66.7% inhibition of odema in paw. Hence it was suggest that fully processed prodigiosin can be used to replace the rofecoxib and valdecoxib which are widely used as anti-inflammatory drugs but they have increased risk of heart attacks and strokes hence fully processed prodigiosin can replace this risky drugs. In future study prodigiosin is a promising drug owing to its reported characteristics of having antibacterial, antifungal, antineoplastic, anti proliferative, anti-oxidant and anti-malarial activity. Prodigiosins are strong therapeutic molecules especially for their immunosuppressive properties and anticancer properties. It was also show to involve in apoptosis of haematopoietic cancer cell. The treatment of urinary tract infections in patients with indwelling catheters or after prostatic surgery in difficult of *Serratia* species, the presumptive drug of choice currently is gentamicin, unless sensitivity tests show the organism is sensitive to a less toxic agent. For treatment of asymptomatic bacteria due to serratia when all catheters have been removed and surgical wounds are healed, nalidixic acid may be useful and perhaps should be tried even when disc sensitivity studies show resistence, since drug levels may reach 50 to 200 mg/ml, so we are highly recommended to increase the quantity and quality of prodigiosin contains application in the field of medicines.

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