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PRODUCTION, PURIFICATION, IDENTIFICATION OF PRODIGIOSIN FROM SERRATIA SP. AND ITS ANTIMICROBIAL ACTIVITY

Tejasvini S. Pore, Ashwini B. Khanolkar, Naiem H. Nadaf*

Department of Microbiology, Shivaji University, Vidyanagar, Kolhapur 416004, INDIA

ABSTRACT

A red pigment produced and identified as prodigiosin from *Serratia* sp. The optimum conditions for pigment production in Peptone glycerol broth found to be at 30°C and pH 7. 87% glycerol gave a better yield of pigment. Prodigiosin, a secondary metabolite produced by *Serratia* sp which was found to be anti-microbial and anti-yeast in nature. Antimicrobial activity was observed against test organism from which *Candida albicans* & *Candida utilis* shows maximum zone of inhibition i.e. 2.5 & 2.7 respectively. The antimicrobial activity of pigment was found to be more against Gram positive test organism than Gram negative test organism. Purification of pigment was carried out by TLC and it shows 84 R_f value. The further analysis of pigment reveals that it shows maximum absorption at 534nm and have 323 molecular weight.

KEY WORDS: *Serratia*, prodigiosin, Red pigment, Antimicrobial

***Corresponding Author: Dr. Naiem. H. Nadaf Ph.D.**

Department of Microbiology, Shivaji University, Vidyanagar, Kolhapur (MS) 416004.

*Email Address: dr. nhnadaf@gmail.com

1.INTRODUCTION

Microbial natural products are known to have the richest sources of chemical diversity and potential in therapeutics. Even though there is a huge availability of alternative sources provided by the research and development, nearly 30% of the drugs sell is dependent on compounds obtained naturally mostly secondary metabolites. These metabolites of natural include pigments, antibiotics and immunomodulators, antidiabetic and anti-cancer compounds. Bacteria are the more promising group of natural resource to obtained these metabolites (Grabley and Thiericke, 1999). Microorganisms are known to produce differnt pigments like carotenoids, melanins, flavins, quinones, prodigiosins and more specifically monascins, violacein or indigo (Dufosse, 2009). From all above chemical compounds, one of the same kind product is nothing but prodigiosin. It's naturally occurring red colored pigment (secondary metabolite) frequently occurs in *Serratia marcescens* and other bacterial sp. (Gerber, 1975; Parachuri and Harshey, 1987; Pandey et al., 2003). *Serratia* are Gram-negative bacteria which are found in water, soil, plants and air (Grimont and Grimont, 1978). Chemically, prodigiosin group of natural compounds were included in tripyrrole red pigment family that contain a common 4-methoxy, 2-2 bipyrrole ring system. The prodigiosin biosynthesis is a bifurcated process in which mono and bipyrrole precursors are synthesized separately and then they assembled to form pigment (Boger and Patel, 1988). A detailed study done on prodigiosin demonstrated its antimicrobial, antimalarial and antitumor properties and induction of T and B lymphocytes apoptosis was also studied. Due to these all properties of pigment there is an increased interest of researchers in the fields of medicine, pharmaceutics and different industries (El-Bondkly et al., 2012). The rising in bacterial resistance to antibiotics and other drugs is recognized as a major health problem throughout the world. There are different reason for the drug resistance in microorganism that include increased use and misuse of antibiotics and other drug molecule in human, veterinary medicine and in agriculture has further provoked these problem (Todar, 2004). There is a greater need of alternate chemotherapeutics because In spite of great research in the modern chemotherapy, the exploitation of new antimicrobial agents from natural resources is considered as an important task particularly in developing countries where the threat of drug resistance in microorganism is more (Paarakh, 2009). prodigiosin was seen to be a promising agent in these regard as the sensitivity to prodigiosin demonstrated by oxacillin resistant *Staphylococcus aureus* (Hemaiswarya et al., 2008). So the

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present study was aimed to extraction, purification and characterization of the red pigment from *Serratia marcescens* sp. and evaluation of its antibacterial activity against different microorganism.

2. MATERIAL AND METHODS

a) Production Microorganism:

Serratia marcescens NCIM 5061 obtained from NCIM (National Collection of Industrial Microorganisms), India. Grown and maintained on Peptone glycerol agar.

b) Microorganism used for antimicrobial activity:

Test organism used are- *Bacillus subtilis* NCIM 2635, *Bacillus cereus* NCIM 2703, *salmonella* sp NCIM 2501, *Shigella* sp NCIM 5265 *Candida albicans* NCIM 3466, *Candida utilis* NCIM 3469. Were obtained from NCIM (National Collection of Industrial Microorganisms), India. All bacterial cultures were maintained on nutrient Agar and for maintenance of yeast Culture Glucose Yeast extract Malt extract medium was used.

Microorganisms and culture conditions

The microorganisms employed for the production of prodigiosin production is *Serratia marcescens* NCIM 5061. These microorganism was grown on peptone glycerol agar at 30°C for 24 h. and maintained on same medium at 4°C until its use.

production of pigment

For production of prodigiosin pigment the inoculum of *Serratia marcescens* NCIM 5061 was added in sterile cooled peptone glycerol broth medium and incubated at room temperature on shaker for 30°C for 24 hour.

Pigment extraction

Pigment was extracted by using ethyl acetate solvent. Broth with grown mass of *Serratia marcescens* NCIM 5061 was centrifuged at 8000 rpm for 15 min in cold centrifuge tube (4°C). Supernatant was collected and mixed with 1:1 proportion of ethyl acetate & kept in separating funnel for 3 hours with intermediate shaking after every 30 min. Two layers were separated in two different containers. The red colored ethyl acetate extract was taken to remove moisture by using sodium sulphite. After removal of trace water these extract was kept for drying overnight. After evaporation of solvent, plate was kept in hot air oven at 55°C so as to get dry powder of pigment.

Pigment was dissolved in methanol & sterilize by filtration through sterile syringe filter having pore size 0.02 micrometer before its antimicrobial activity.

Purification of pigment by TLC

For purification of the pigment the powder of pigment was dissolved in chloroform at 25 µg/ mL concentration and used as a sample for TLC the sample was monitored for TLC by using a solvent system as follows methanol: ethyl acetate: chloroform.(6:3:1)..

Effect of p^H on prodigiosin production:

To find out effect of pH on Prodigiosin production, different p^H (6.5, 7.0, 7.5, 8.0, 8.5, 9.0, and 9.5) were adjusted using 1N NaOH in Peptone glycerol broth. Defined volume of the production microorganism was inoculated and incubated at 30⁰C under shaking conditions for 24 h. The initial p^H at which maximum production of prodigiosin was observed was chosen and maintained in the following studies.

Effect of temperature on prodigiosin production:

To study the effect of different temperature on Prodigiosin production, defined volume of the bacterial isolate was inoculated in Peptone glycerol broth and incubated at different temperature (20⁰C, 25⁰C, 30⁰C, 35⁰C, 40⁰C and 45⁰C) under shaking conditions for 24 h. The temperature at which maximum production of prodigiosin was observed was chosen and maintained in the following studies.

Characterisation of pigment

Purified pigment characterised by UV/VIS spectrophotometer and Gas chromatography & mass spectroscopy.

Antimicrobial activity

Sterile methanolic extract of pigment used to determine antimicrobial activity. The activity was carried out by agar well method. Suspension of test micro-organisms was spread on nutrient agar plate with the help of sterile glass spreader. Test organism used are *Bacillus subtilis* NCIM 2635, *Bacillus cereus* NCIM 2703, *salmonella* sp NCIM 2501, *Shigella* sp NCIM 5265 *Candida albicans* NCIM 3466, *Candida utilis* NCIM 3469. After even spreading of test suspension wells were bored with the help of sterile cork borer. 100 micro-liters of methanolic extract added to wells by micro-pipette. Plates were kept in refrigerator for 10 min. for diffusion of pigment extract. Then plates were incubated at 37^oc for 24hours. After incubation plates were observed for zone of inhibition.

3. RESULTS AND DISCUSSION

Production of Pigment:

When the organism was allowed to grow in various media, the organism was found to produce more prodigiosin in peptone glycerol broth containing 1.5 ml 87% glycerol, usually Prodigiosin production done in nutrient broth (Pryce and Terry, 2000) or peptone glycerol broth (Jonas *et al.*, 1993). *Serratia marcescens* also found to produces maximum prodigiosin in maltose containing medium Sundaramoorthy et al (2009). There are other reports for the use of substrate to produce prodigiosin like, Nakamura (1986) has used triolein and reported a moderate yield prodigiosin.

Effect of pH and temperature on pigment production:

It was found that the organism produced more prodigiosin at 30°C at pH 7 (Figure 1 and 2) the rate of pigment production was reduced as pH and temperature increases above 30°C at pH 7. There is an diversity in strain of *serratia marcesence* and their optimum conditions of ptdigiosin production. Williams and Quadri (1980) reported maximum prodigiosin production at 27°C and that of no prodigiosin was produced when cultures were incubated at 38°C. in other study complete block in prodigiosin was observed at 37° C reported by (Pryce and Terry, 2000). These results suggested that the antibacterial red pigment production is growth dependent and slight fluctuation in growth parameters influence the prodigiosin production. In anather finding (Darah et al., 2014) showed that, the slight increased in pH of the medium from 7.5 at the beginning of the cultivation to 9.6 at 72 hours of cultivation, showing that the bacterial cells need an alkaline condition to produced the antibacterial activity significantly.

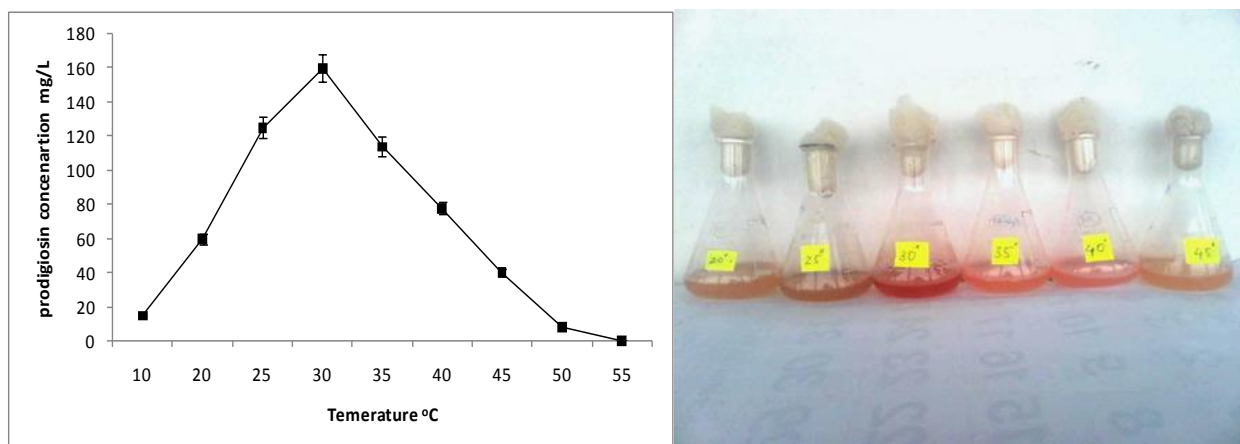


Figure 1: *Serratia sp* showing pigmentation at different temperature

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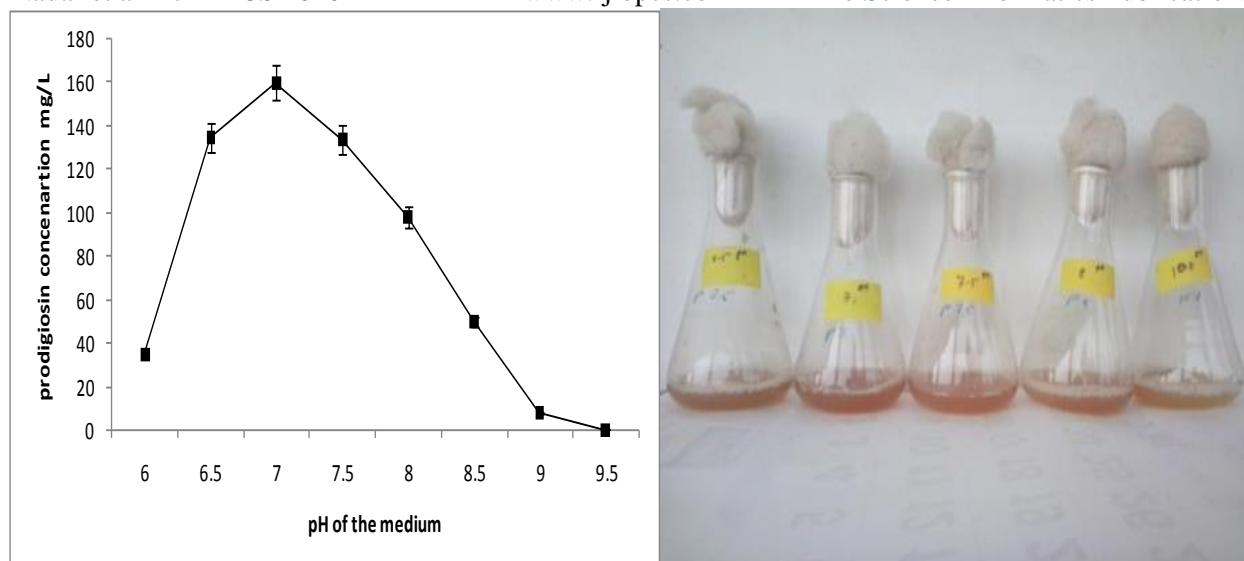


Figure 2: *Serratia sp* showing pigmentation at different p^H

TLC of extracted pigment:

The isolated pigment was subjected for TLC. The prodigiosin was analyzed and the R_f value of fraction is 0.84 (Figure 3). Song et al (2006) has extracted the red pigment directly from the internal adsorbent using acidified methanol and phase separation. And he has purified by silica gel chromatography and high performance liquid chromatograph (HPLC). This purified fraction was monitored for the characterization by UV/ Vis Spectrophotometer and GCMS.

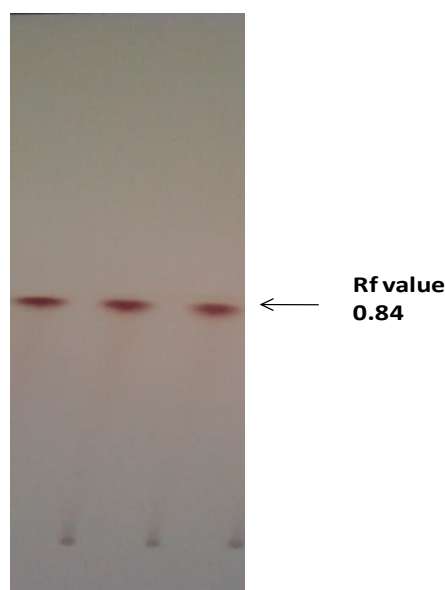


Figure 3: TLC of extracted pigment:

Result of UV/ VIS and GC-MS analysis:

Prodigiosin has tri pyrrol ring structure which is soluble in organic solvent so methanol was used for extraction the UV/VIS characterization of pigment shows maximum absorbance at 530 nm as shown in fig No. The pigment produced by *S. marcescens* was identified and characterized using UV-Vis spectrophotometry and mass spectrophotometry, respectively. It was observed that the pigment shows maximum absorption maxima at 530 nm and the calculated mass by GC/MS is near about 323 (figure. 4 and 5). According to Silva et al. (2012), the substance produced by *S. marcescens*, which has absorbance at 534 nm and a molecular weight of 323 m/z, is characterized as prodigiosin. Moreover, Yang et al. (2013) describe a red pigment produced by *Microcystis aeruginosa*, the molecular weight of which is 323 m/z, as being prodigiosin.

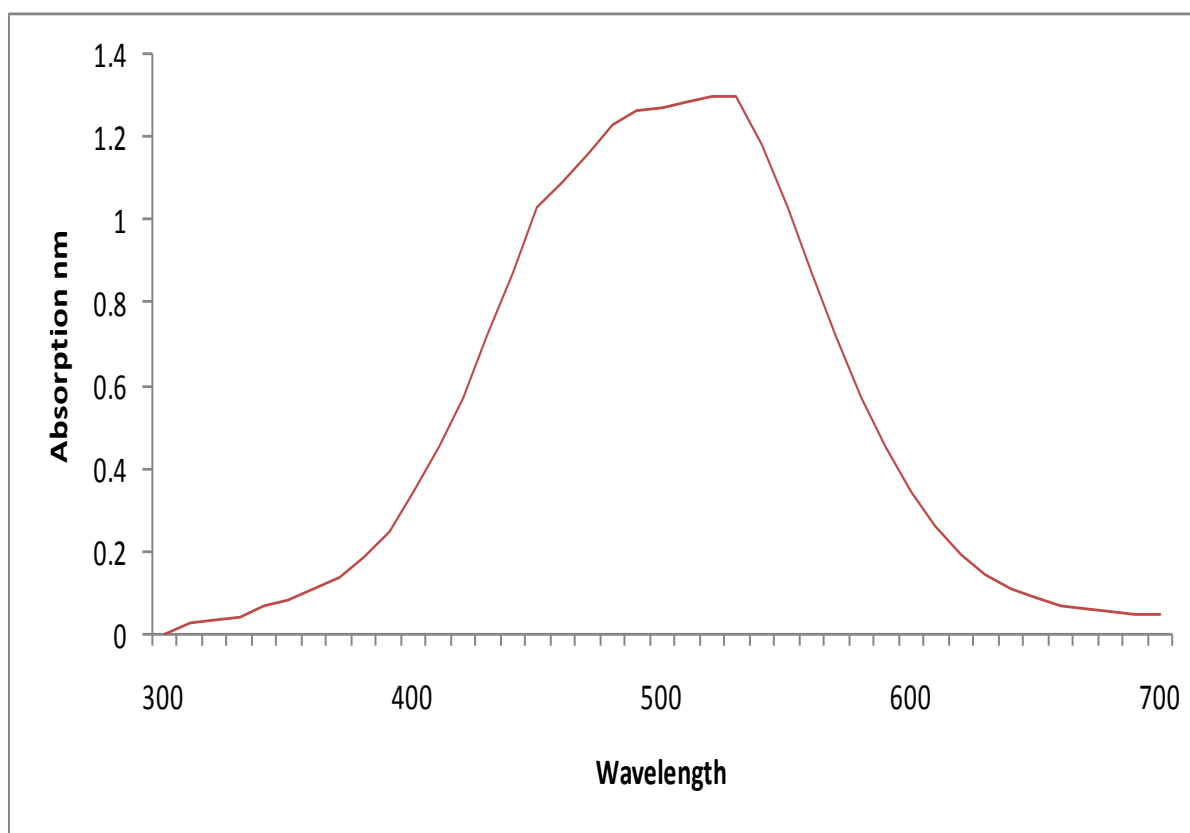


Figure 4: UV/Vis spectrophotometric analysis of prodigiosin pigment

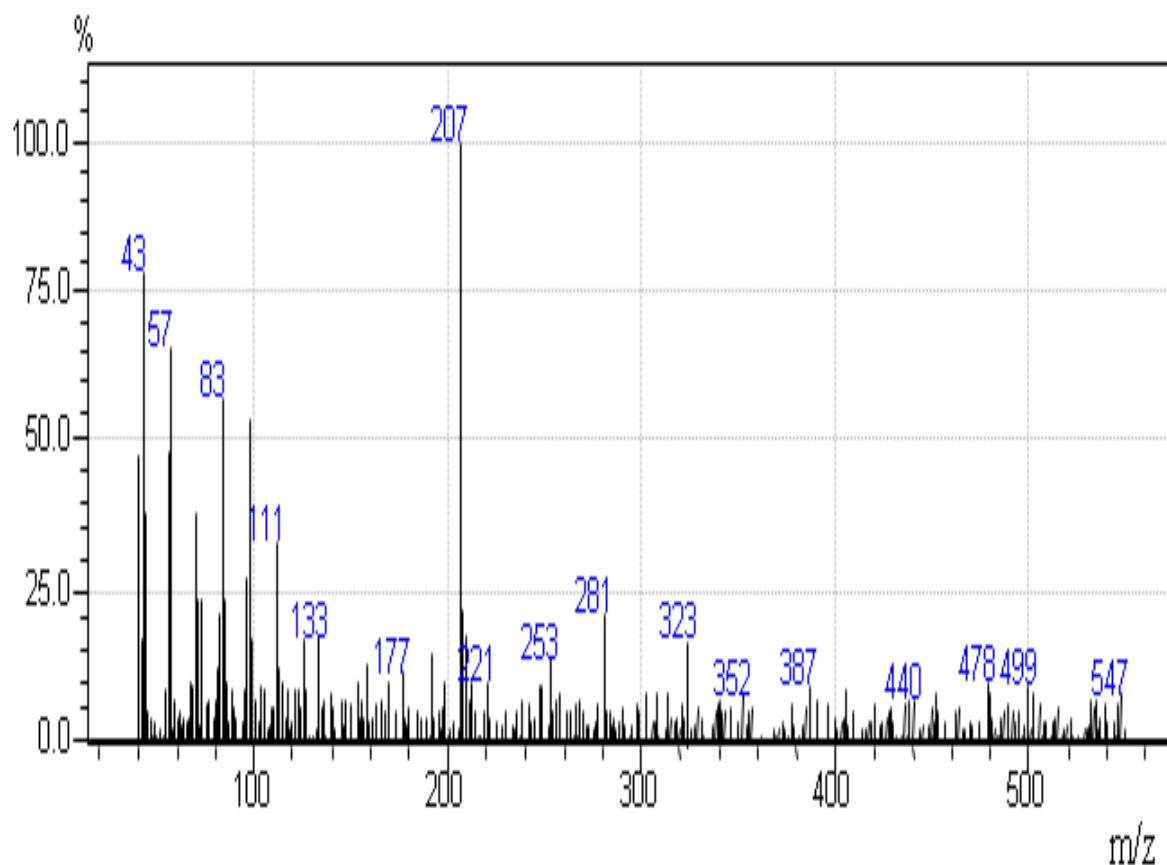


Figure 5: Mass spectrometry (GC/ MS) of prodigiosin

Antimicrobial activity of pigment:

The extracted pigment shows antimicrobial against - *Bacillus subtilis* NCIM 2635, *Bacillus cereus* NCIM 2703, *salmonella* sp NCIM 2501, *Shigella* sp NCIM 5265, *Candida albicans* NCIM 3466, *Candida utilis* NCIM 3469. as shown table 1. According to Chandni Gulani et.al (2012) the prodigiosin was a potent inhibitor against Gram positive bacteria like *Staphylococcus aureus* and *Bacillus cereus* and fungal pathogens like *Candida albicans*, *Cparapsilosis* and *Cryptococcus* sp.

Table 1: Zone of inhibition against test organisms.

Test organisms	Zone of inhibition in cm.
<i>Bacillus cereus</i> NCIM 2703	1.8
<i>Bacillus subtilis</i> NCIM 2635	2.4
<i>salmonella</i> sp NCIM 2501	1.5
<i>Shigella</i> sp NCIM 5265	1.2
<i>Candida albicans</i> NCIM 3466	2.5
<i>Candida utilis</i> NCIM 3469	2.7

4. CONCLUSION

323 molecular weight bearing pigment shows maximum absorbance at 530 nm. A red pigment produced and identified as prodigiosin from *Serratia marcescens* NCIM 5061. The optimum conditions for pigment production in nutrient broth found to be at 30°C and pH 7. 87% glycerol gave a better yield of pigment in half strain nutrient broth. For the synthesis of pigment, lipid source is required & 87% glycerol serve as precursor for production of pigment. Prodigiosin, a secondary metabolite produced by *Serratia sp* which was found to be anti-microbial and anti-yeast in nature. Different zone of inhibition was observed on test organism from which *Candida albicans* & *Candida utilis* maximum zone of inhibition i.e.2.5 &2.7 respectively. Zone of inhibition against Gram positive test organism is comparatively larger than Gram negative test organism.

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