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Antibacterial Potentials of Red Pigment Extracted From Soil Isolate Serratia Sp.

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Abstract: *Bacterial pigments are the secondary metabolites produced by many bacterial strains. Bacterial pigments have grabbed attention since the demand for alternative of standard antibiotics has risen due to the resistance adapted by pathogens. The aim of the present study was to isolate pigment producing bacteria from soil, extraction and identification of the isolate and further to test antibacterial potential of the pigment. Pigment producing bacteria was isolated from soil and the isolate was characterized and identified based on morphology and biochemical analysis as* ***Serratia sp.*** *. Antibacterial assay of pigment carried out on bacterial pathogens Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa well diffusion method against ampicillin as standard. The present study revealed that red pigment extracted from the soil isolate has antibacterial activity.*

Keywords: *Serratia sp., Antibacterial, Bioactivity, bacterial pigment*

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I. 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 is dependent on compounds obtained naturally. 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). Biopigments are the secondary metabolites produced by many plants, animals and microorganisms (Alihosseini *et al.*, 2008). Biopigments contain a functional group called as chromatophore responsible for color formation. Pigments produced by microorganisms are more preferable than plant pigments.

Wide range of bacterial species producing pigments including carotenoids, flavonoids, quinines, prodigiosins and rubramines etc. (Krishna et al., 2013). Microbial species like *Streptomyces coelicolor* (prodigiosin and actinorhodin), *Chromobacterium violaceum* (violacein) and *Thialkalivibrio versutus* produces natronochrome and chloronatronochrome etc. produce pigments (Ahmad et al., 2012),. *Psedomonas aeruginosa* produces blue colored pyocyanin and red colored aeruginosa (Abu et al., 2013). These pigments have gained huge interest in pharmacological field as they are believed to serve as alternatives for synthetic drugs apart from their ability to dye the substances.

Demand for replacement of synthetic substances by natural ones has emerged huge opening for study of biopigments. Many bacterial pathogens have acquired resistance against antibiotics hence discovery of new bactericidal agents is growing trend in the field of pharmacology. Bacterial pigments can become the new potent drug in treating life threatening microbial diseases as alternative of synthetic drugs which have many adverse effects on living beings. Since pigments have commercial applications in the field of pharmacology and dyeing materials acquired the interest to study in detail. The present study involves isolation and characterization of pigment producing bacteria and characterization of the isolate. Furthure of antimicrobial activity of the pigment was tested on bacterial pathogens .

Materials:

Chemicals and reagents:

Nutrient agar, Peptone, Beef extract , Sodium chloride ,Gram's iodine, Crystal violet, Safranin .Hydrogen peroxide 3%, Kovac's indole reagent, MRVP broth, Dipotassium hydrogen phosphate, Methyl red pH indicator ,α- naphthol ,Potassium hydroxide, Simmon's Citrate agar, Methanol ,Silica gel, Ethyl acetate ,Hydrochloric acid, Ammonia solution, Spectrophotometer, pH meter, Hot air oven, Centrifuge, Chromatography chamber ,Rotary evaporator

Isolation of pigment producing bacteria

Collection of soil sample:

Soil samples were collected from different sites of Garden of P. C. Jabin Science College, Hubballi labeled the samples as S1, S2, S3 respectively.

Isolation identification and characterization of pigment producing bacteria

Serial Dilution Method:

Pigment producing bacteria was isolated by serial dilution method using 0.8% saline solution. The experiment was carried out in three different sets of dilution aliquots using 1gm of soil from each sample collected. 0.1ml of sample from soil dilutions 10^{-5}, 10^{-6}, 10^{-7} was inoculated on nutrient agar plates by spread plate method and incubated at 37°C and room temperature for 72 hours.

Subculture of pigment producing bacteria

Bacterial colonies appearing red in color were picked and subcultured on Nutrient agar plates by quadrant streaking method and incubated at room temperature for 72 hours. The pure, isolated colony from previously streaked plate is inoculated on Nutrient agar slants and in Nutrient broth and grown on optimum conditions.

Identification of pigment producing bacteria

Bacterial colonies were identified based on their colour and further characterized by Gram's staining, Biochemical analysisCatalase test Indole Test, Methyl Red Test, Voges Proskauer Test, Citrate Utilization Test.

Extraction of pigment

Isolate *Serratia p.* produces pigment in manners extracellularlly. The pigment collected should be devoid of bacterial cells and media components. Hence solvents like methanol, ethanol, acetone, choloroform have been used to achieve aim of collecting the pure pigment. These solvents dissolve the pigment extracting from bacterial cells and solvents can be evaporated to concentrate the pigment into solid substance. In the present study methanol solvent was preferred as the solubility of pigment was comparatively superior than in other solvents and pigment extracted was purer as observed.

Procedure for extraction of pigment from bacterial cells involves the steps like pure and isolated colony of pigment producing bacterial colony was inoculated in Nutrient broth of 300 ml and was incubated at room temperature for four to five days. Four to five days old cultured broth was centrifuged at 10,000 rpm for 10 minutes; pellet was suspended in 95% methanol and centrifuged again at 10,000 rpm for 10 minutes. Supernatant was collected which red in colour, is filtered using 0.2µm Whatmann paper. Filtrate was concentrated by rotary evaporator. Extract was added with 5 ml of 95% methanol and transferred to a petridish, dried in oven (Ahmad *et al.,* 2012).

Antibacterial Activity of Prodigiosin

Red pigment extracted from isolate-*Serratia sp.* was tested to have an antibacterial activity. Dried prodigiosin extract was dissolved in 95% methanol. The stock of pigment was prepared as 10mg/ml and created various concentration ranging 20µg/ml, 40µg/ml and 80µg/ml. Bacterial pathogens against which the activity was detected in the form of zone of inhibition *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* have been purchased from NCIM, Pune Test microorganisms were sub cultured in nutrient broth and incubated at 37°C for 24 hours.

The activity was done based on well diffusion method and carried out in triplicates. Suspension of test micro-organisms was spread on nutrient agar plate with the help of sterile glass spreader. Each petriplate was made with five wells where one well loaded with methanol and one with ampicillin, a standard antibiotic as controls. The stock of ampicillin was prepared as 10mg/ml and loaded in the concentration 40µg/ml. Each well loaded with 50µl of all samples - controls and different concentration of prodigiosin, incubated at 37°C for 24 hours. Zone of inhibition was measured to analyze the activity quantitatively.

II. Results

To isolate pigment producing bacteria serial dilution technique was carried out using 0.8% saline solution for soil samples collected, S1, S2 and S3. At the end of incubation period of 72 hours red colored colonies were observed in the nutrient agar plates isolated from soil sample S2 in the dilution 10^{-6}. In order to get pure strains isolate was subcultured in a nutrient agar plate by quadrant streaking. Then from the previously streaked plate a pure, isolate colony was selected and inoculated in nutrient broth. Based on morphological and biochemical characterization the bacterial strain was identified as *Serratia p.*

Antibacterial activity test of prodigiosin has given good results; zone of inhibition has been seen for all the concentrations of prodigiosin tested. Antibacterial activity of prodigiosin on three bacterial pathogens *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* was tested. The concentrations of prodigiosin used were 20µg/ml, 40µg/ml and 80µg/ml taken from the stock prepared 10mg/ml using methanol.

Standard ampicillin was the control used. Zone of inhibition measured to analyse the results. At 80µg/ml concentration the zone was bigger when compared with both 20µg/ml, 40µg/ml.

III. Discussion

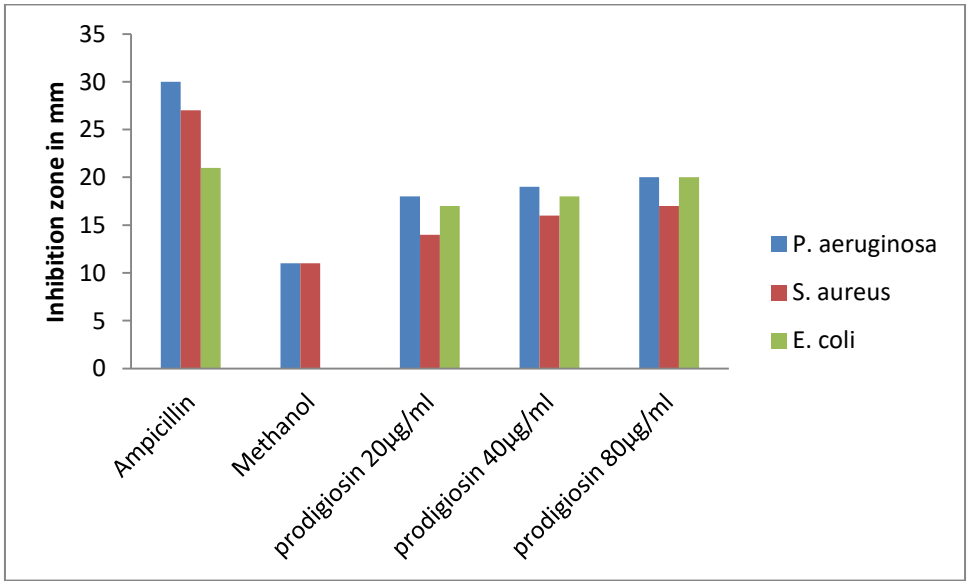
In the present study soil isolate *Serratia p.* was inoculated in nutrient broth, incubated in different temperatures 28^{o}C and pH 7. The nutrient broth supplemented yeast extract and 1% glucose as nitrogen and carbon source respectively

Antibacterial activity of red bacterial pigment has been examined on *Pseudomonas aeruginosa* which exhibited the zone of inhibition measuring 18, 19 and 20 mm at concentrations 20, 40 and 80µg/ml respectively. In case of *Staphylococcus aureus* at concentrations 20, 40 and 80µg/ml the zone of inhibition measured as 14, 16 and 17 mm respectively. The zone of inhibition produced by prodigiosin against *Escherichia coli* measured as 17, 18 and 20 mm in diameter Graph 1.

Some recent studies done on the antimicrobial activity were the red antibacterial pigment was produced by a marine bacterial isolate *Serratia marcescens* IBRL USM 84 intracellularly, inhibited 13 out of 18 tested bacteria (Ibrahim et al., 2014). The red pigment produced by a novel strain of *Bacillus* species had inhibitory effect on both Gram positive and Gram negative bacteria and the isolated strain was resistant to different antibiotics (Goswami et al., 2014). Antimicrobial activity of pigment *Serratia marcescens* NCIM 5061 was observed against test organism from which *Candida albicans* & *Candida utilis* shows maximum zone of inhibition i.e.2.5 and 2.7 cm respectively. The antimicrobial activity of pigment was found to be more against Gram positive test organism than Gram negative test organism (Pore et al., 2016).

IV. Conclusion

In the present study pigment producing bacterial strains was isolated of from soil by serial dilution technique and isolated strains was identified by morphological characters and biochemical characterization . The bacterial strain was identified as *Serratia sp.* The extracellular pigment was extracted using 95% methanol and was concentrated by evaporating the solvent using rotary evaporator.. Antibacterial assay has been carried out to examine the susceptibility of *P. aeruginosa, S.aureus* and *E. coli* to red pigment by well diffusion method, the zone of inhibition measured to quantify the assay. The present study revealed that red pigment extracted from the soil isolate has antibacterial activity.



Graph. 1. Antibacterial activity of soil isolate *Serratia sp.*

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