

Isolation and Characterization of Red Pigment Producing *Serratia* sp., from Soil Sample

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ABSTRACT

The approach of the present study was aimed at isolating pigment producing microorganism from soil sample. The red pigment producing microorganism was isolated and characterized through various biochemical tests. The red pigment prodigiosin production was screened through DNase agar and DNase agar with malachite green. The pigment was extracted from the isolate and quantitated. The prodigiosin production was found to be more in the nutrient broth at 72hrs when compared to peptone glycerol broth.

Key words: *Serratia* sp., *Prodigiosin*, *Red pigment*.

INTRODUCTION

Pigments have a role in respiration and possess antagonistic properties. It is presumed that pigment biosynthesis acts as a protective mechanism in unfavourable conditions when the growth of cells is delayed. The red pigment prodigiosin was isolated from *S. marcescens* way back in 1902^[1] by Kraft. *Serratia* sp., are gram negative bacteria classified in the large family of Enterobacteriaceae and these bacteria grow well on standard media and produce a dark red pigment that aids in its identification and the red color pigment is so called prodigiosin^[2]. The prodigiosin group of natural product is a family of tripyrrole red pigment that contains a common 4-methoxy 2, 2 bipyrrrole ring system. The biosynthesis of the pigment is a bifurcated process in which mono and bipyrrrole precursors are synthesized separately and then assembled to form prodigiosin². Hence an attempt was made to isolate microbes producing red pigment from soil and to study its characteristic feature.

METHODOLOGY

Sample collection

Different soil samples were collected from different locations. From this red pigment producing

bacteria was isolated and the bacterial culture was used for the present study.

Isolation of the pigment producing microorganism

Soil contains a large number of different groups of pigment producing bacteria. Out of this only red colour pigment producing bacteria was isolated. For their isolation serial dilution technique was used³. Colonies with different morphology and individual isolates were picked up separately and purified by quadrant streaking in Nutrient agar plates for the isolation of individual bacterial respectively³. The bacterial isolates were incubated at 37 °C for 24 hours⁴.

Identification of bacterium

Red colour pigmented bacterial colonies were isolated from nutrient agar and selective media trypticase soy agar. The strain was identified microscopically by Gram staining and various biochemical tests⁴.

Screening test

DNase test agar ^[5]: The test was carried out using DNase agar for screening the production of red pigment by the microorganism. The DNase agar plates were prepared and inoculated with the bacterial culture and

incubated for 24 hours at 37°C. After 24 hours of incubation the surface of the plates was flooded with 1N HCL to detect the zone of hydrolysis.

DNase Agar containing malachite green [6]: In this test malachite green was added to the DNase agar to give green colouration to the medium. The DNase agar plate was inoculated with the organism and incubated for 24 hours at 37°C. After the incubation, The plates were observed for the zone of hydrolysis.

Prodigiosin production [7]: Cultures were grown in nutrient broth and peptone glycerol broth which are the most opted media for prodigiosin production. 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 0hr, 24hrs, 48hrs, 72hrs, 96 hrs, 120hrs, and 144hrs.



Fig. 1 : Trypticase soy agar medium showing red colour pigmented colonies



Fig. 2 : Nutrient agar medium showing red pigmented colonies

Extraction of prodigiosin pigment[8]:

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 minutes. The supernatant prodigiosin was transferred to the fresh vial and its spectroscopic estimation was carried out at 499nm.

Estimation of prodigiosin [8]

The pigment absorption maxima were at 499nm where prodigiosin also absorbs maximally. At this wavelength the absorptions were recorded. The bacterial cell absorption prior to extraction was noted at every step. Isolated prodigiosin was estimated using the following formula:

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



Fig. 3 : DNA hydrolysis shown by *Serratia* sp., on DNase agar media

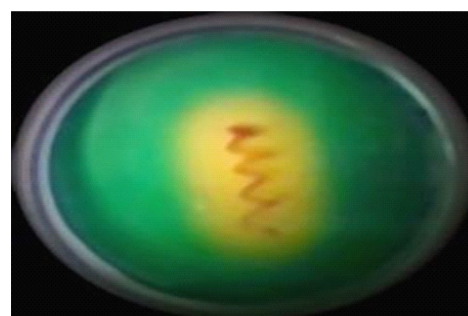


Fig. 4 : DNA hydrolysis shown by *Serratia* sp., with malachite green

OD- Optical density; OD499 – Pigment absorbance; OD620 – Bacterial cell absorbance, 1.381 – Constant

RESULTS AND DISCUSSION

Isolation of pigment producing organism

Various pigment producing bacteria was isolated from different soil samples by serial dilution and plating technique.

Identification of bacterium

Morphological study

Streak plate method: On nutrient agar surface and trypticase soy agar the isolate was found to be convex, circular, mucoid producing red pigmentation (Fig. 1) and (Fig. 2).

Microscopy:

Gram’s staining technique: The isolate was found to be gram negative, rod shaped organism.

Biochemical test: The organism was identified by using various biochemical tests

Organism	I	M R	V P	C	G	L	S	M	TS I	U
<i>Serratia sp.</i> ,	-	-	+	+	A	-	A	A	A K/ A	-

From the above biochemical results the organism was conformed to be *Serratia sp.*

DNase test agar: A clear zone surrounding the colony indicated DNase activity (Fig. 3). Production of DNase resulted in the hydrolysis of the DNA into nucleotide fractions. Similar pattern of hydrolysis was reported in the earlier work done by Jeffries *et al.*, 1957^[6].

DNase test agar containing malachite green:

DNase resulting in freeing of the malachite green with the production of a clear zone surrounding the colonies was observed (Fig.4). The production of DNase was detected based on decolorization of malachite green as DNA, after reaction with malachite green forms a clear zone and when the DNA is hydrolyzed the area becomes colorless. Larger the colorless area around the colony, larger is the DNase production. Similar results were reported in the earlier work done by Jeffries *et*

al., 1957^[6] and ^[1]Kraft *et al* 1902.

Estimation of prodigiosin pigment: Prodigiosin production was nil after 24hrs in stationary phase which was determined spectrophotometrically at 499nm. Prodigiosin production in nutrient broth and peptone glycerol broth with shaking after 24hrs was nil. Sufficient production was observed after 48hrs in nutrient broth and peptone glycerol broth. Maximum production in both the media was observed in 72hrs. Production was seen to decrease from 92hrs in nutrient broth and peptone glycerol broth respectively. Hence aeration resulted in enhanced levels of prodigiosin. This result indicated that the maximum amount of prodigiosin production was in nutrient broth at 72hrs (Fig.5). Similar pattern of pigment estimation was done earlier by ^[9] Mekhael and Yousif, 2009.

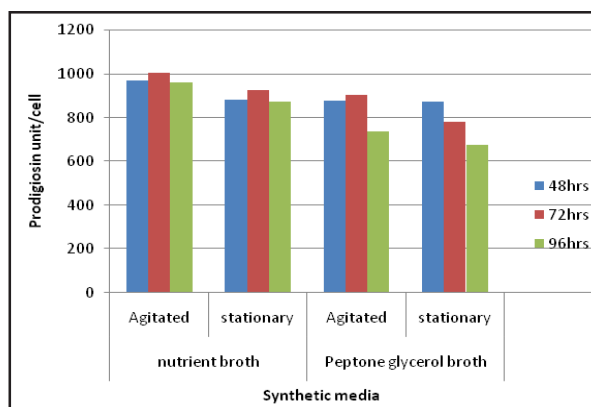


Fig. 5 : Estimation of Prodigiosin pigment

CONCLUSION

In the current study, an attempt was carried out to isolate the pigment producing *serratia sp.*, from the soil sample. The red pigment producing *Serratia sp.*, was isolated, characterized and its pigment producing ability was screened through various tests. Further, optimization of various parameters needs to be carried out for the maximal production of the pigment so that the pigment could be exploited in future for various application like textile colouring. In addition to dyeability, the large scale production of the pigment will make it a potential alternate for the chemical dyes. Further the antimicrobial ability of the pigment can be looked for so that it could of great use to the mankind.

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