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ISOLATION AND STUDIES ON PRODIGIOSIN PRODUCING *SERRATIA SP.* FROM SOIL

Microbiology

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ABSTRACT

Natural pigments are great alternatives to synthetic colors. Microbial pigments are potential source of natural pigments as they are independent of seasonal variation, geographical distribution and can also be produced at a large scale with ease. Prodigiosin is a red colored multifaceted secondary metabolite contain a common 4-methoxy 2, 2 bipyrrole ring system with antibacterial, anti-fungal, anti-neoplastic, antiproliferative, antioxidant and antimalarial activity. This Prodigiosin is a natural pigment which is eco-friendly and sustainable as it is produced by bacterial biomass. Prodigiosin being from biological origin often called as 'Biocolor' and proved safe and edible coloring agent. In the current research studies, a red pigment producing bacterial sp. was isolated from soil its pigment was extracted and spectrophotometrically characterized as Prodigiosin. The pigment producing bacterial species was identified as *Serratia* sp. By phenotypic, biochemical and 16s rRNA sequence analysis. Physiological condition and nutritional parameters were optimized. The Prodigiosin producing *Serratia* species can be a better alternative source of natural pigment.

KEYWORDS

Prodigiosin, Serratia species, Microbial Pigments

INTRODUCTION

Bioactive pigments are obtained from plants, microorganisms and many other sources (Papageorgion *et al*., 1979) The pigment production from microorganisms is great alternative for natural pigment due to rapid growth of microorganism in the cost-effective culture medium, it is independent of weather conditions and shows diversity in color and source. Hence, microbial pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial applications. Prodigiosin, the bright red pigment produced by organisms of the genus *Serratia*, is among the more conspicuous pigments extant in the microbial world. The chemical nature of Prodigiosin continues to be the subject of extensive study and it has been defined as a tri-pyryl methene, the Prodigiosin pigments have intrigued organic chemists and pharmacologists and may yet play roles in the treatment of infectious diseases such as malaria and as immunosuppressive agents (Giri *et al*., 2004). The Prodigiosin being a colored compound can be said as Biocolor and can be used as coloring agent for coating of the tablets which will work for dual purpose i.e., as a Biocolor for pharmaceutical applications and as a potential drug to treat various infections. Prodigiosin producing *Serratia* sp. is a member of *Enterobacteriaceae.* The Prodigiosin pigment is a red colored multifaceted secondary metabolite contain a common 4-methoxy 2, 2 bipyrrole ring system. This Prodigiosin have antibacterial, anti-fungal, anti-neoplastic, anti-proliferative, antioxidant, anti-tumor (Castro *et al*., 1967) (Perez-Tomas *et al*., 2003), antiprotozoal (Croft *et al*., 2007), cytotoxic activities (Nakashima *et al*., 2005) and antimalarial activity. This Prodigiosin produced by this bacterium is a natural and eco-friendly and sustainable source of Biocolor'.

MATERIALS AND METHODS

Sample Collection And Enrichment

For isolation of Prodigiosin producing bacteria, random sampling was done in the month of June. Soil samples were collected in sterile container from different regions of District, Parbhani and stored at 4^0C prior to use. Enrichment of samples was done in Nutrient broth and incubated for 48h in presence of day light at room temperature.

Isolation And Screening

Isolation was done by serial dilution of enriched culture in physiological saline (0.85% NaCl) on nutrient agar plates (pH 7.0) and incubated for 48 h at 37^0C. After incubation, plates with isolated colonies with red pigmentation were selected as master plates and kept for further use. Screening of all pigment producing cultures was carried out based on bright red pigment production ability.

Phenotypic And Biochemical Characterization Of Isolate PS1

The phenotypic characterization of the red pigment producing bacterial isolate was done which include, morphological, microscopic, macroscopic features and culture characters. Biochemical characterization was done at laboratory level.

Identification of isolate based on 16s rRNA sequence and phylogenetic analysis

The 16s rRNA analysis was done from Agharkar Research institute, Pune, M.S.

Extraction And Analysis Of The Pigment Produced.

The 48h culture was centrifuged for 10 min. at 10,000 rpm the supernatant was discarded and pellet was washed with distilled water, then pellet was re-suspended in acidified ethanol (4% 1 M HCl in ethanol) to extract red pigment from the cells. Pellet was removed by a centrifugation after complete bleaching and the supernatant was separated as a crude pigment. Prodigiosin displays a characteristic absorption spectrum. The absorption maxima of the pigment were spectrophotometrically studied in visible range (400-600 nm) against ethanol as reference.

Standardization Of Physiological Conditions For Optimum Pigment Production

The effect of various culture conditions viz. different incubation temperature (20^0C, 25^0C, 30^0C, 35^0C, 40^0C and 45^0C), pH (6.5, 7.0, 7.5, 8.0, 8.5, 9.0, and 9.5) and nutritional parameters i. e. carbon source (Glucose, Lactose, Sucrose, Maltose, Mannitol) and different oils as carbon source. The pigment produced was determined in terms of optical density of crude pigment at 534 nm.

RESULTS AND DISCUSSIONS

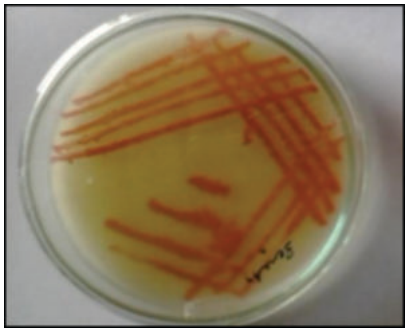

Figure:1 Isolate PS1 on nutrient agar

Isolation And Identification

Isolation and Screening of the pigment producing organisms from soil

sample was resulted in many of the isolates showing bright red coloration. The red pigment showing isolate PS1 was selected for the present study due to its bright pigmentation high yield among other isolates and the culture PS1 was maintained on nutrient agar plate at 4^0C for further studies (Fig.1). A Prodigiosin producing bacterium was isolated from soil and identified as *Serratia rubidaea.* Many strains of *Serratia* were studied for Prodigiosin include production include Nima (Qadri and Williams, 1972), B2 (Someya *et al.,* 2004), Swift-1 (SS-1) (Wei and Chen, 2005) and A3 (2) (Cerdeno *et al.,* 2001) and MKS, (Mohammed Hussain *et al*., 2012) Maximum absorption spectrum of Prodigiosin produced by *Serratia species* exhibited pigmentation in accordance with the literature (Williams *et al*., 1955).

Phenotypic, Physiological And Biochemical Characterization

PS1 was Gram- negative, motile, non-spore forming short rods approximately 1-2 µm in size. The colonies (1-2mm) appear with bright pigment on Nutrient agar with smooth, convex, opaque with butyrous consistency.

The bacterial isolate PS1 can grow with bright red pigmentation on Nutrient agar between temperature 20-30^{0}C, pH 7.0 to 8.0. Detailed biochemical characters are as per table-1

Table-1 Biochemical And Cultural Characters Of Isolate PS1

Sr. No.	Character	Observation	Sr. No.	Character	Observation
1.	Gram,s Nature	Gram negative	11.	Lactose	-
2.	Cell shape	rod	12.	Maltose	+
3.	Motility	motile	13.	Mannitol	+
4.	Colony color	red	14.	Glucose	
5.	Indole production	-	15.	Sucrose	+
6.	Methyl Red	+	16.	Nitrate reduction	+
7.	Voges-Proskaeur	-	17.	Lipase	+
8.	Citrate (Simmons)	+	18.	Catalase	+
9.	H_2S production	-	19.	Gelatin hydrolysis	+
10.	Oxidase	+	20.	Starch Hydrolysis	-

Legend: + Positive test; - Negative test

Identification Of Isolate By 16s r RNA Gene Sequence Analysis:

The 16s rRNA analysis report showed that isolate PS1 showed that closest neighbor as *Serratia rubidaea* JCM 1240T/AB004751 which confirms the isolate PS1 as *Serratia rubidaea* strain. The 16s rRNA partial sequence was given in figure 2 and phylogenetic affiliation was shown in figure 3.

ATTTGCCCATGGGCGCAAGCCTGCATGCNGCCATGCCGCGT
GTGTGAAGAACGCCTTCGGGTTGTAAAGCACTTTCAGCGA
GGAGGAAGGTGGTGANCTTAATACGTTCATCAATTGACGTT
ACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCC
GCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTG
GGCGTAAAGCGCACGCCAGGCGGTTTGTTAAGTCANATGT
GAAATCCCCGANCTTAACTTGGGAACTGCATTTGAAACTG
GCAAGCTAGAGTCTCGTATAGGGGGGTAGAATTCCAGGTGT
ACCGGTGGAAATGCGTACAAGATCTGGAGGAATACCGGTG
GCGAANGCNGCCCCCCTGGGACNAAAAACTGACGCTCAT
GTGCGAAAGCGTTGGGGAGCAAACCGGAATCACATACCCT
GGTCAATCCACGCCCGNTAAACGATGANCCATTTTGCGGA
GGTTGNNGCCCTTNGGAGGCGTGGGNTTCCGNGANCTAAC
ACTTAAAATCC

Figure-2 16s rRNA sequence from isolate Ps1

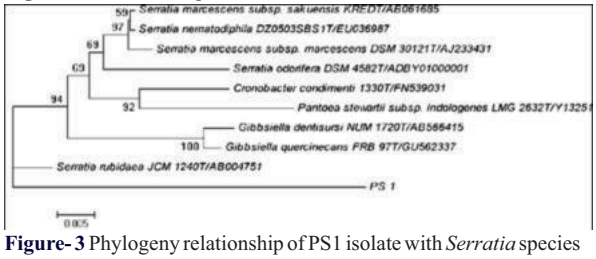

Figure- 3 Phylogeny relationship of PS1 isolate with *Serratia* species

Extraction And Spectrophotometric Analysis Of The Pigment Produced

The bright red pigment produced by isolate PS1 was identified as Prodigiosin depending on its spectral characters. In order to determine the wavelength at which maximum absorption occurs, spectrum scan was done using UV-Visible Spectrophotometer Systronics 119 model. The spectrum scan result showed that Prodigiosin molecule in acidic conditions showed an absorption maximum at 534nm (fig.4). The non-carotenoid nature of bright pigment produced may was confirmed by chemical test which showed absence of polyene structure in the pigment. During chemical test there was no blue ring at the interface of chloroform extract of pigment from dry pellet and concentrated H_2SO_4 (Mrak *et al*., 1949) (Karrer and Jucker, 1950). The similar test was done by Shatila *et al*., (2013) where chloroform extract of *Exiguobacterium aurantiacum* FH exhibited dark blue coloration upon addition of concentrated sulfuric acid which revealed the presence of Carotenoids.

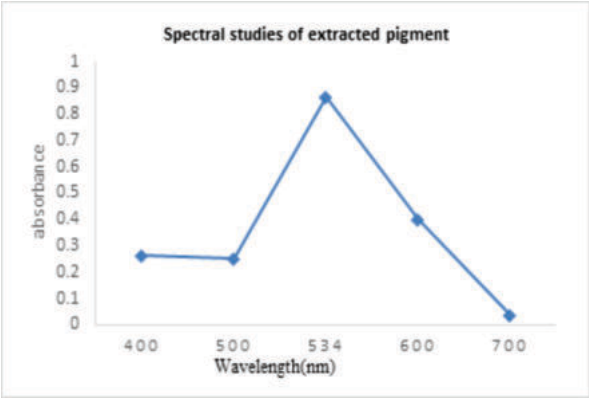

Fig.4- Spectral Studies Of Extracted Pigment

Standardization Of Optimum Conditions For Pigment Production

The production of Prodigiosin has been shown to be influenced by numerous environmental factors including media composition and pH (Weinberg *et al*., 1970; Williamson *et al*., 2005). The temperature is important physiological condition for optimum functioning of enzymes which affects the production of any metabolite in the cell. Here optimization of temperature was done to get maximum pigment production and maximum pigment production was found at 30^{0}C. The pigment production rises with rise in temperature up to 30^{0}C and there was decrease in pigment with rise in temperature beyond 30^{0}C (fig.5).

The optimum pH for pigment production was found as 7.0 which found as stringent condition for pigment production (fig.6). Prodigiosin, a secondary metabolite produced by *Serratia marcescens* (Samrot *et al.,* 2011) who reported that the optimal condition for pigment production i.e. nutrient broth at 28^{0}C and pH 7. In this study, the maximum pigment production was observed at 30^{0}C temperature and pH 7.0.

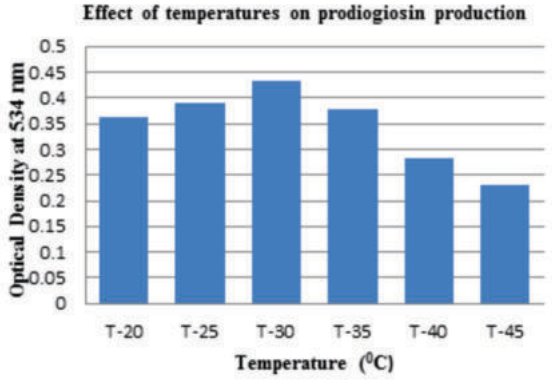

Fig.5 Effect Of Temperature On Prodigiosin Production

The maximum pigment production was found at shaking conditions on orbital shaker with 150rpm than stationary. At shaking conditions, the media and transfer of oxygen may be improved which improved the yield of pigment. The pigment production was improved by using lactose as carbon source and supplementation of mustard oil. The

Prodigiosin was found to be influenced by number of carbon sources where it was found that glucose inhibit the Prodigiosin production which may be due to catabolite repression whereas was observed that, pigment yield was more in response to lactose and sucrose (fig.7) Giri *et al.,* (2004) reported that powdered peanut broth had supported better growth of *Serratia marcescens* and higher yield of Prodigiosin when compared with the existing nutrient broth and peptone glycerol broth and they have concluded that the fatty acid supplementation in media improves cell growth and Prodigiosin production when used as carbon source. According to Kim *et al.,* (1998) oils are superior carbon and nitrogen sources than fatty acid Containing seeds. Similar findings are reported in recent studies where, more Prodigiosin production was found with the Mustard oil (Fig.8).

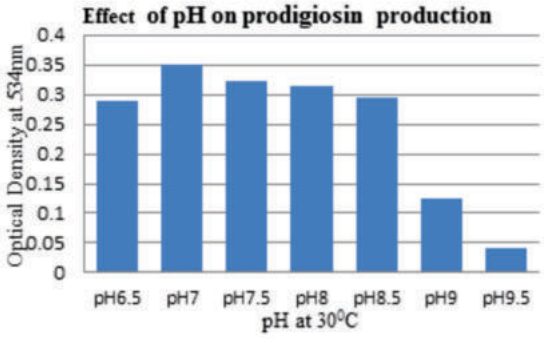

Fig. 6 Effect of pH on Prodigiosin production by

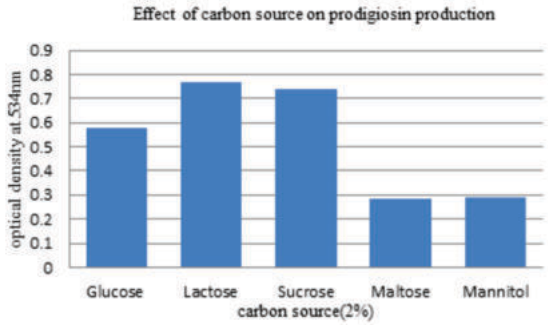

Fig.7 Effect Of Carbon Source On Prodigiosin Production

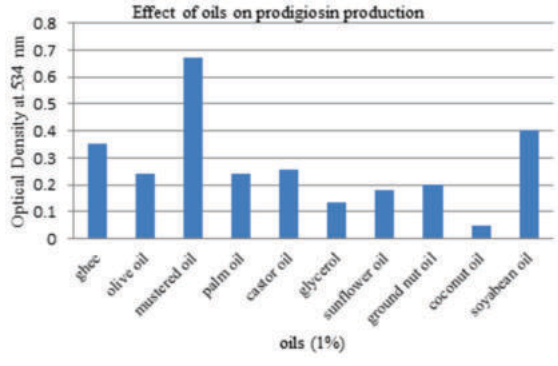

Fig.8 Effect Of Oil On Prodigiosin Production

Nutrient broth containing lactose and mustered oil showed maximum production of Prodigiosin and less production in nutrient broth at 45^{0}C. The lactose containing nutrient broth, mustered oil supplemented nutrient broth supported the Prodigiosin production at 30^{0}C. The addition of sugars showed slight enhancement of Prodigiosin production in nutrient broth. From this, it can be concluded that lactose acts as a better carbon source for enhancing Prodigiosin production in nutrient broth. Among 10 different oils, the mustered oil showed high pigment Production from this, Soybean oil, Ghee, and Castor oil as substrate were more efficient in inducing pigment production than other oils.

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