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In-vitro production of Fluorescein pigment by *Pseudomonas fluorescens* and its antibacterial activities

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Abstract

Bacterial strains also produce fluorescence like *Bacillus* spices, *Pseudomonas aeuroginosa*, *Pseudomonas fluorescens* etc., the fluorescent pseudomonads are prevalent in soil environment, especially in root tubers like potato soil sweet potato soil. The present study deals with the isolation of pigment producing bacteria *P.fluorescens*. The organism was collected from rhizosphere soil of potato fields from Nilgiris. It was also tried in other areas like paddy fields, groundnut fields but a fluorescent organism was not isolated. The isolated strains were confirmed by biochemical characterization. The strain was grown in YEPG broth for fluorescent pigment and after the fluorescent pigment was extracted with acetone. The extracted fluorescent pigment was tested against 4 taxa of clinical important. Out of 4 taxa *E.coli* showed some sensitivity.

Keywords: Soil samples, Flurescence, Pigment production, Antibacterial activity.

1. Introduction

There are a few fragmentary reports of the widely distributed members of Bacillus that produce yellow green diffusible fluorescent pigment. The early reports [1] who isolated two fluorescent Pseudomonas bacteria from water and soil environment and named them *Pseudomonas fluorescens* and *Pseudomonas putida*. These bacteria and many other strains of the fluorescent group of Bacillus were reclassified and renamed as fluorescent Pseudomonads. Suggested that *Pseudomonas* may excrete water-soluble fluorescent pigment into the culture media.

This property is sometimes used as a taxonomic classification of different *Pseudomonas* [2]. Biological activity of cultures and extracts of *Pseudamonas fluorescens* and production and purification of these inhibitory substances and the characterization of a metabolite responsible for significant proportion of antibacterial activity [3, 4]. *Pseudomonas fluorescens* Pf –5 and CHAD produce pyototeroin, pyrrolnitrin, 2,4-diacetyl phloroglvanol, hydrogen cyanide and various pyoverdine siderophores to suppress plant diseases caused by the pathogenic fungi [5, 6].

The present study deals with the production of fluorescent pigment from *Pseudomonas fluorescens*, soil isolated extraction of the pigment and its antibacterial activity is tested against various clinically important pathogens.

2. Materials and Methods

2.1. Sample Collection

Soil sample was collected from Nilgris hill station from potato Rhizosphere soil.

2.2. Isolation and identification of *Pseudomonas fluorescens*

The collected sample is serially diluted upto 10-6 dilution. The dilution is made with blank, sterile distilled water. Then the diluted sample was inoculated in special media called kings 'A' and King 's 'B' Media. The isolated strain was identified by morphological and Biochemical characteristics by Bergry's Manual of Determinative Bacteriology.

2.3. Biochemical characterization of *Pseudomonas fluorescens*

After isolation the organism was subjected to the biochemical test for confirming the organism.Test like Indole, MR, VP, citrate, TSI, urease are carried out for the organism isolated from soil

2.4. Pigment Extraction

The Bacterial culture grown for pigment production is taken along with YEPG broth. Then the Pseudomonas fluorescens cells are washed with acid (Hcl) of 30%. Then the washed cells placed in glass flask containing 150 ml of 80% cold acetone (40C). Then the mixture was agitated for 1 min in shaker. Then allow for 5 mins to settle the cells. Then the pulp of cells was vacums filtered with Whatmann no-1 filter paper and the supernatants were saved.

The bacterial biomass was washed twice with 100 ml of 80% acetone. The acetone extract were combined and concentrated to approximately 150 ml under vacuum in a rotary flask. All this volume, the bright red color of the mixture, which fluoresced yellow, turned to brown and a yellow precipitate formed. The slurry was centrifuged at 5000 rpm and then it was kept for 10 minutes at 4^0C. Then the non-fluorescent supernatant was discarded and the pellet was saved and re suspended in 100 ml of 100% acetone giving a bright orange color. Finally yellow pellet fluoresced orange obtained.

2.5. Antibacterial Activity

A Sterile Disc (HiMedia) 6mm were impregnated with different concentration 10µl, 20µl and 30µl of the extract to obtain 100 µg, 200 µg and 300 µg/disc and allowed to dry at room temperature. [8].

3. Results

3.1. Isolation of *Pseudomonas fluorescens* from soil

After incubation the organism *Pseudomonas fluorescens* present in potato rhizosphere soil will develop on the Kings A media plates and Kings B media plates (selective media for *Pseudomonas fluorescens* for pigment production). After incubation *Pseudomonas fluorescens* organism produced pigment in YEPG broth. YEPG broth is specially made for the pigment production (pyoverdine) yellow precipitate was formed and finally yellow pellet fluoresced orange colors was observed in eppendroff. The isolated strain was identified by morphological and Biochemical characteristics (Table 1).

Table 1- Morphological and Bio-chemical test results

Organisms	Morphological Characteristics			Biochemical-test					
	Gram Staining	Motility	Colony Morphology	Indole	MR	VP	Citrate	TS1	Urease
Pseudomonas fluorescens	G(-) Rod	Motile	Small, round, greenish colonies.	-	-	-	+	A/A H2S –	-

3.2. Antibacterial Activity

The antibacterial activity of *Pseudomonas fluorescens* pigment was determined against four bacterial pathogens (Table 2). The highest activity in *Salmonella typhi* and *Escherichia coli* in (11 mm) 300 µg. followed by *staphylococcus aureus* (5mm) no activity in *Pseudomonas aeruginosa*.

Table. 2 Antibacterial Activity of *Pseudomonas fluorescens* pigment

S. No	Pathogens	Zone of inhibition in mm.		
		100 µg	200 µg	300 µg
1	*Escherichia coli*	5	9	11
2	*Staphylococcus aureus*	-	4	5
3	*Pseudomonas aeruginosa*	-	-	-
4	*Salmonella typhi*	7	9	11

4. Discussion

The used common laboratory media NA, TSA, PCA, PsAF and added egg white protein canalbumin to bind all iron to the media and made them efficient diagnostically isolated a Gram positive Bacilli with fluorescence. The organism resembled the Bacillus bacteria and produce chloranthomycin, a fluorescent, chlorinated, penta cyclic pyrene [9, 10].

Pseudomonas, Bacillus sps, also fluoresced yellow under long wavelength UV light on several common culture media .while *Pseudomonas* sps emit green light ,*Bacillus* gives out yellow light Both of them are isolated from Agricultural soil. In *Bacillus* the pigment is characterized as chloroxanthomycin pyrene compound. While *Pseudomonas*, it is pyoverdine unlike the fluorescent compound produced by *Bacillus*. The green fluorecent pyoverdine produced by *Pseudomonas fluorecens* diffuses in to the medium, from young cells to aged one [11].

Antibiotic compound from *Pseudomonas fluorescens* and purified it by using thin layer chromatography. They tested this antibiotic for antibacterial activity. A similar attempt was made here to test the antibacterial activity of fluorescin [12].

The present study deals with the isolation of pigment producing bacteria *P.fluorescens*. The organism was collected from rhizosphere soil of potato fields from Nilgiris. It was also tried in other areas like paddy fields, groundnut fields but a fluorescent organism was not isolated. The isolated strains were confirmed by biochemical characterization. The strain was grown in YEPG broth for fluorescent pigment and after the fluorescent pigment was extracted with acetone. The extracted fluorescent pigment was tested against 4 taxa of clinical important. Out of 4 taxa *E.coli* showed some sensitivity

Conflict of Interest

No conflicts of interest.

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