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Research Article

STUDIES ON PRODUCTION OF DIFFERENT CHELATING GROUPS OF SIDEROPHORES BY MICROBIAL ISOLATES OBTAINED FROM RHIZOSPHERE SOIL

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ABSTRACT
Four different isolates were derived from the fungal and bacterial origin from the soil sample taken from rhizosphere for the detection of siderophores production. Biochemical characters were studied and the isolates were identified. Isolated colonies were confirmed as *E. coli, P. fluorescens, A. flavus,* and *Rhizopus* sp. The high amount of siderophores production was observed by *Pseudomonas fluorescens* from bacterial isolates, and from fungal isolates *Aspergillus flavus* showed more production and produces hydroxamate type of siderophores in high production compared to that of the catecholate. Thus, we can conclude that rhizosphere soil organisms which produce siderophores can be used to improve the plant growth.

Keywords: *Microbial Isolates, Siderophore, Catecholate, Hydroxamate*

INTRODUCTION
In rhizosphere, the obtainability of iron for microbial integration is extremely limiting (Buyer and Sikora, 1990; Loper and Buyer, 1991). For the survival in such environment, organisms secrete iron binding ligands called siderophore, also known as scavenger of iron from the environment with high specificity and make it accessible to the organism (Neilands, 1995; Leong, 1986). These small molecules which complexes with ferric iron and deliveries it to a cell by aiding in its transport across the plasma membrane. There are more than 500 different siderophores have been identified from microorganisms. Siderophores are small, relatively low molecular weight viz. about 400-1500 daltons ferric ion chelators secreted by many bacteria, fungi and grasses to obtain iron from the surroundings. The low solubility of outside iron presents a problem for microorganisms to active in an aerobic environment and having an absolute necessity for iron. To combat this lower solubility, most fungal and bacterial species have higher affinity iron transport system (Jikare and Chavan, 2014).
The production of Siderophores by bacteria is considered as an important factor of bacterial machinery for iron sufficiency and most important for survival and growth in the competitive soil environment, which is generally poor in soluble iron (Khan *et al.,* 2016). Pseudomonas species has studied to produce siderophores which can chelate any available iron. The role of siderophores is primarily to scavenge iron, and also form complexes with other elements (i.e. Mo, Mn, Co and Ni) or sometimes with K and Mg from the surrounding environment that serves as micronutrients, used for regulation of osmotic pressure and redox processes (Neilands, 1995; Bellenger *et al.,* 2008; Duhme *et al.,* 1998; Visca *et al.,* 1992; Parmar and Chakraborty, 2016).
Basically siderophore are considered to be two types, viz., secondary hydroxamic acid and catechol type (Neilands, 1981) but the bacteria could produce 4 different types of siderophores: hydroxamate, salicylate, carboxylate & catecholate which include *Escherichia coli, Salmonella, Enterobacter, Aerobacter aerogenes, Klebsiella pneumonia and Mycobacterium* species. Siderophores producing fungi includes *Penicillium chrysogenum, P. Citrinum, Mucor, Aspergillus nidulus, A. Versicolor, Saccharomyces cerivisiae, Rhizopus, Trametes* sp. *Nocardia and Streptomyces griseus* are some *Actinomycetes* which shows the high tolerance towards some metals. Its deficiency in plats can cause growth inhibition, inhibition of sporulation & changes in morphology. Iron is required for the biosynthesis of porphyrins, toxins, vitamins, antibiotics, cytochromes, pigments.

The present research work reveals the production of siderophore by the soil isolate bacterial and fungal species and their production strength of their particular type siderophore.

MATERIALS AND METHODS

***Sample Collection*:** Soil sample was collected from Victoria Reserve Forest Park from the rhizosphere soil and were brought in the sterile plastic bag for further examination.

Isolation of Bacteria and Fungi: 0.1ml of the serially diluted sample was taken from 10^{-4} to 10^{-8} dilution & was spread on nutrient agar plates and incubated at 37˚C for 24hr. After incubation, bacterial colonies were further identified and examined. 0.1ml of the serially diluted sample was taken from 10^{-3} to 10^{-5} dilution & was spread on potato dextrose agar and sabouraud's agar medium for 2-3 days at 28˚C. After incubation fungal colonies were detected and further selected for transferring on minimal medium.

Identification of Bacteria and Fungi: The characterization of the selected isolates were done by using the biochemical tests which include motility, MR-VP test, nitrate test, indole test, TSI slant test, sugar fermentation test: glucose, sucrose, lactose, mannitol, maltose, oxidase test, catalase test, sodium thioglycolate broth test. For bacterial identification, the results obtained were compared with Bergey's manual. The fungal isolates were mounted in Lactophenol cotton blue and were identified.

Analysis of Siderophores: Selected bacterial isolates of *E.coli, P. fluorescens* were further added to the standard succinate medium and were incubated in a shaking incubator for 48hr(28˚C) at 85rpm and Selected fungal isolates of *A. flavus & Rhizopus* sp. was inoculated to minimal medium & flasks were incubated on a shaking incubator for 48hr (28˚C) at 100rpm. Cultured bacterial and fungal cells were harvested and centrifuged at 10,000rpm for 15min. The supernatant was confirmed for siderophore production.

Detection of Chemical Nature of Siderophores: The 24hr old culture's supernatant was subjected to various tests for the detection of the iron-chelating function group. The various chemical nature groups were assessed by performing the following tests:

1. *Arnow's Assay:* For detection of catecholate's, 1ml of culture supernatant was mixed with 1ml of HCL, 1ml of nitrite molybdate (catechols produce yellow colour), 1ml of NaOH (colour change to red), add D/W to make up to 5ml. Take the absorbance at 500nm using 2, 3-dihydroxybenzoic acid as standard in U.V. spectrophotometer (Arnow, 1937).
2. *Tetrazolium Salt Test:* To a pinch of a tetrazolium salt, was added 1-2 drops of 2N NaOH & 0.1ml of the test culture supernatant. Instant appearance of a red to the deep-red colour indicated the presence of hydroxamate siderophores (Snow, 1954).

RESULTS AND DISCUSSION

In this study, the sample of rhizosphere soil from Victoria Park Reserved forest, obtained different isolates on nutrient agar and potato dextrose agar plate was identified as *E.coli, Pseudomonas fluorescenes, Aspergillus flavus, and Rhizopus* sp. respectively. The amount of siderophores produced from different isolates of Bacteria and Fungi. In succinate, medium was observed *E.coli* (39.14 mg) and *P. fluorescens* (47.49 mg) while in minimal medium was observed A. *flavus* (39.45 mg) and *Rhizopus* sp. (44.56 mg) (Figure 1).

The chelating functional group was determined by Arnow's and Tetrazolium test. All organisms showed the varied amount of hydroxamate and catecholate type of siderophore production. The highest amount 18.6 mm of catecholate type of siderophore was produced by bacterial isolate *P. fluorescens* and 21.3 mm produced from fungal isolate *Rhizopus* sp. while hydroxamate type of siderophore produced by 31.3 mm by *P. fluorescens* and 37.3 mm by *A. flavus* in high content (Table 1). The number of extracellular siderophores production by respective organisms shows production of catecholate type of siderophore in a lesser amount than comparing to that of hydroxamate type of siderophore. Siderophores were very important for soil and plants, play an important role in improving the rhizosphere establishment of the strain as well as in iron nutrition for plants (Vansuyt *et al.*, 2007) and secondarily promote plant growth by creating an destructive impact on phytopathogens (Chincholkar *et al.*, 2007).

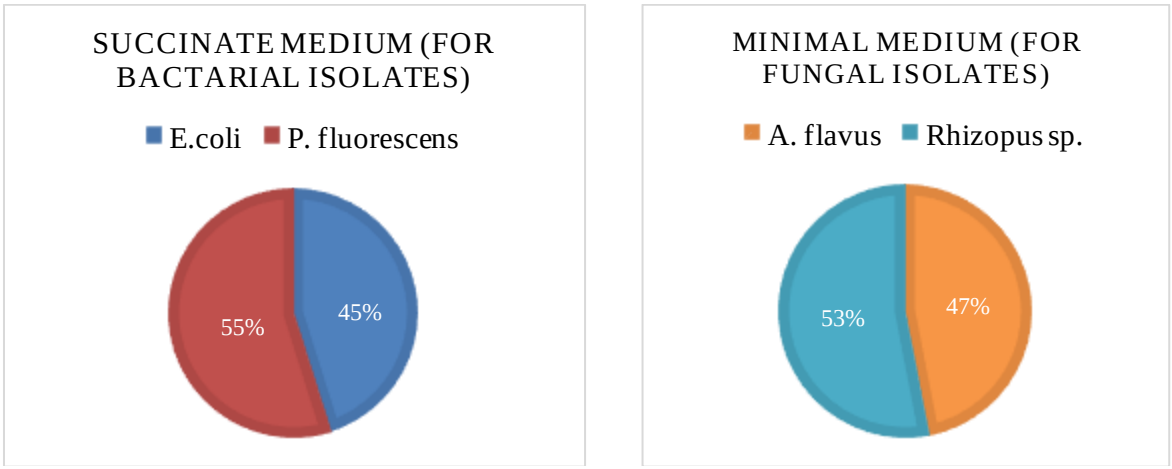

Figure 1: Siderophore Production by Bacterial and Fungal Isolates

Table 1: Chelating Functional Groups Produced by Bacteria and Fungi

No.	Test Organisms	Siderophore (mm)	
		Catecholate	Hydroxamate
1.	*E.coli*	10.2±1.3	20.3±1.5
2.	*P. fluorescens*	18.6±1.11	31.3±1.54
3.	*A. flavus*	21.2±1.5	37.3±0.5
4.	*Rhizopus. Sp.*	21.3±3.8	26.0±1.6

*Values are expressed as Mean ± SE

Conclusion

The present study concludes that the rhizosphere soil organisms produced siderophore in varying amount which can increase the affinity of plant growth, its Fe absorbing capacity and further these siderophores can be detected & used in different areas of medicine, bioremediation, environmental pollutants, petroleum hydrocarbons and can also work as an antimicrobial agents against clinical pathogens of human.

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