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Research Article

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Studies on siderophore production by microbial isolates obtained from rhizosphere soil and its antibacterial activity

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ABSTRACT

Sample was collected from rhizospheric soil. Among this study, two bacteria and two fungi were selected for siderophore production. The siderophore producing organisms were identified based on cultural, morphological and biochemical characteristics. Hence, the isolated colonies were confirmed as E.coli, Pseudomonas fluorescence, Rhizopus sp and Aspergillus flavus. By this study, analysis and nature of siderophore production was determined using these organisms. Pseudomonas fluorescens and Rhizopus sp showed the maximum catechol type of siderophore produced followed by E.coli and A.flavus minimum catechol type of siderophore. Among these, Pseudomonas fluorescens and Aspergillus flavus showed the maximum hydroxamate siderophore, followed by Rhizopus and E.coli minimum hydroxamate type of siderophore produced. Whereas, the optimization of medium for siderophore production maximum in bacteria when compared with fungi. The antibacterial activity was performed by well diffusion assay against clinical bacterial pathogens such as Streptococcus mutans, Salmonella typhi, Staphylococcus aureus and Klebsiella pneumoniae. High frequency of antibacterial activity was observed in Pseudomonas fluorescens are more efficient than the fungi Aspergillus flavus. Thus we concluded that rhizosphere soil organism producing siderophore to improve the plant growth and good antibacterial activity for clinical pathogens.

Keywords: *Pseudomonas fluorescens, E.coli, Aspergillus flavus, Rhizopus,* Siderophore, Anti bacterial activity.

INTRODUCTION

Siderophores (from the Greek: iron carriers) are defined as relatively low molecular weight, ferric ion specific chelating agents synthesized by bacteria, actinomycetes, fungi and certain algae growing under low ironic stress. Chemically siderophores are iron binding proteins with molecular weight ranging from 400 - 1500 Da. The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cell. Siderophores have been related to virulence mechanisms in microorganism pathogenic to both animals and plants. In addition, they have applications in clinical, agricultural and environmental fields. At present, nearly 500 siderophores are reported from selected microorganisms.

Siderophores are low molecular weight bio-molecules secreted by microorganisms in response to iron starvation for acquisition of iron from insoluble forms by mineralization and sequestration [1]. Although some siderophores are known to chelate other ions, their specificity and avidity for iron is the most consistent feature [2]. Siderophores produced by rhizosphere inhabitants has been studied well and it has been reported that ability to produce

siderophores not only improve rhizosphere colonization of producer strain but also play an important role in iron nutrition of plant [3] and antagonism against phytopathogens.

Bacteria produce four types of siderophores: hydroxamate, catecholate, salicylate and carboxylate. These siderophores play an important role in the extra cellular solubilization of iron from minerals or organic substances. Some important siderophore producing bacteria includes *Escherichia coli, Salmonella, Klebsiella pneumoniae, Vibrio cholerae, Vibrio anguillarum, Aeromonas, Aerobacter aerogens, Enterobacter, Yersinia* and *Mycobacterium species.*

Fungi are the important siderophore producing microorganisms next to bacteria. Some important siderophore producing fungi includes *Aspergillus nidulans, A. versicolor, Penicillium chrysogenum, P. citrinum, Mucor, Rhizopus, Trametes versicolor, Ustilago sphaerogina, Saccharomyces cerivisiae, Rhodotorula minuta* and *Debaromyces species.*

Actinomycetes are aerobic gram positive filamentous bacteria with high guanine + cytosine (G+C) content and form asexual spores. Mostly they are saprophytic in nature which prefer complex substrate for their growth and able to tolerate certain metals at high concentrations. Siderophore producing actinomycetes includes *Actinomadura madurae, Nocardia asteroids and Streptomyces griseus*. Actinomycetes produce both hydroxymate and salicylate types of siderophores. Few algae also reported as siderophore producers. Schizokinen, a dihydroxamate type of siderophore, produced by *Anabaena sp* reported to facilitate iron uptake. *Anabaena flosaquae* and *Anabaena cylindrica* produce siderophores which accumulate copper.

EXPERIMENTAL SECTION

Sample collection: Rhizosphere soil sample was collected from Thirumangalakkottai, Orathanadu Taluk, Thanjavur District, Tamil Nadu and South India. The collected soil sample was brought to the laboratory in sterile polythene bag aseptically and maintained at the laboratory for further study.

Isolation of bacteria and fungi: 0.1 ml of serially diluted sample was taken from 10^{-4} – 10^{-7} dilution and was spreaded over the nutrient agar plates and incubated at 37^{o}C for 24hrs. After incubation, bacterial colonies were formed and observed on the plates.

0.1 ml of serially diluted sample was taken from 10^{-2} – 10^{-5} dilution and were spreaded over the prepared potato dextrose agar plate medium and the plates were incubated at 28^{o}C for 2-3 days. After incubation, fungal colonies were formed and observed on the plates [4].

Analysis of siderophore: Siderophore production was studied in standard succinate medium and minimum medium [5-6].

P.fluorescens and *E.coli* was inoculated to standard succinate medium and the flasks were incubated on a shaking incubator for 48h (28°C) at 85 rpm. After 48 hrs of incubation, cultured bacterial cells were harvested by centrifuging at 10,000 rpm for 10 minutes. The supernatant was subjected to Neiland's spectrophotometric analysis to confirm siderophore production.

A.flavus and *Rhizopus* sp was inoculated to minimum medium and the flasks were incubated on a shaking incubator for 48 hrs (28°C) at 100rpm. After 48hrs of incubation, cultured fungal cells were harvested by centrifuging at 10,000 rpm for 15 minutes. The supernatant was subjected to Neiland's spectrophotometric analysis to confirm siderophore production.

Detection of nature of siderophore: The presence of catechol nature of siderophore was tested by Arnow method [7]. Then followed by hydroxamate nature of siderophore was tested for Czsaky method [8].

Optimization of medium: The components in medium have a great effect on the amount of siderophore production. So the rhizosphere soil *E.coli* and *Pseudomonas fluorescens* were grown on Succinate medium and *Aspergillus flavus* and *Rhizopus sp* were grown on minimal medium. The amount of siderophore was measured by Czsaky assay for comparative study of siderophore production.

Antibacterial activity: The clinical pathogens such as *Streptococcus mutans, Salmonella typhi, Staphylococcus aureus,* and *Klebsiella pneumoniae* were collected from clinical laboratory (Thamarai Laboratory, Muthupet). These bacterial cultures were used for well diffusion method. One well of 5mm size was made in the agar plates with the help of sterile cork borer under aseptic condition. The wells were loaded with 5 µl of siderophore extract of two bacteria and two fungi with the help of a micropipette. The bacterial plates were incubated at 37^{o} C for 24 hours [9]. The bacterial plates were observed after 24 and 48 hours respectively for the clear zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone.

RESULTS AND DISCUSSION

The rhizosphere soil sample was serially diluted and the colonies were observed on nutrient agar plates and potato dextrose agar plate after 24 hours and 2-3 days. Uninoculated plate with medium was maintained as control.

Table-1 Catecholate nature of siderophore production by bacteria and fungi

S.No	Test Organisms	Catecholate Siderophore(mm)
1	*E.coli*	11.6 ± 1.248
2	*Pseudomonas fluorescens*	18.6 ± 1.113
3	*Aspergillus flavus*	21 ± 1.581
4	*Rhizopus sp*	21.3 ± 3.735

Values are expressed as Mean ±SD

Table-2 Hydroxamate nature of siderophore production by bacteria and fungi

S.No	Test Organisms	Hydroxamate Siderophore(mm)
1	*E.coli*	20.3 ± 1.528
2	*Pseudomonas fluorescens*	31.3 ± 1.536
3	*Aspergillus flavus*	37.3 ± 0.578
4	*Rhizopus sp*	26 ± 1.581

Values are expressed as Mean ±SD

Table-3 Antibacterial activity of siderophore extract

S.No	Organisms	Zone of Inhibition (mm)			
		E.coli	*P.fluorescens*	*A.flavus*	*Rhizopus sp*
1	*S.mutans*	2.3 ± 2.01	9.3 ± 8.6	3.6 ± 3.2	5 ± 5.1
2	*S.typhi*	3.3 ± 2.16	9.8 ± 7.5	10.3 ± 8.9	2.8 ± 2.4
3	*S.aureus*	11 ± 10.8	18.6 ± 17.4	13.3 ± 13.0	15.3 ± 14.6
4	*K.pneumoniae*	5.0 ± 4.9	14.4 ± 14.0	5.0 ± 4.0	12.5 ± 12.9

Values are expressed as Mean ±SD

The isolated organisms were identified as *E.coli, Pseudomonas fluorescens, Aspergillus flavus* and *Rhizopus sp*. These organisms were confirmed according to the Bergey's manual of systematic bacteriology and Manual of soil fungi.

The isolated and identified *E.coli*, *Pseudomonas fluorescens, Aspergillus flavus* and *Rhizopus* were screened for siderophore production. The nature of siderophore was determined by Arnow and Czsaky method. All isolates showed hydroxamate and catecholate nature of siderophore but the amount varied with organisms [table 1 and 2]. Our study correlated to the hydroxamate type of siderophore is produced by fungi and bacteria [10].

The results of siderophore production and optimization conditions were examined the extracellular siderophore production by *E.coli, P.fluorescens, A.flavus* and *Rhizopus* species.

The result obtained in siderophore production by succinate medium was observed in *E.coli* (42.0 mg) and *P.fluorescens* (48.0 mg) then followed by minimal medium was observed in *A. flavus* (40.3 mg) and *Rhizopus sp* (45.3 mg). Our study correlated with the siderophore production and optimization condition is in agreement with result obtained by [11].

The antibacterial potency of siderophore extract were tested against clinical pathogenic bacteria was quantitatively assessed for the presence or absence of zone of inhibition. The results relative to antibacterial activity was observed by measuring the diameter of the zone of inhibition [table 3]. Our studies agreed with finding of [12].

CONCLUSION

The present study deals with the collection of rhizosphere soil sample from Thirumangalakkottai, Orathanadu Taluk for the isolation and identification of bacteria and fungi used to study on siderophore production and its antibacterial activity of clinical pathogens. The present study concludes that the rhizosphere soil organism producing siderophore to improve the plant growth and good antibacterial activity for clinical pathogens of humans.

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