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Studies on Antifungal Activity of Siderophores Produced by *Rhizobium spp* Isolated from Groundnut (*Arachis hypogaea*)

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Abstract

Many of plant growth promoting rhizobacteria (PGPR) have ability to produce the siderophores, among this the Rhizobial *spp* have more capable of producing siderophores under iron limiting conditions. In the present study *Rhizobium spp* were isolated from root nodules of groundnut and characterized morphologically, the isolates were grown under iron limiting conditions to produce siderophore. The siderophore were extracted, purified and characterized. Siderophore produced by the isolates are dihydroxymate type and they showed anti-fungal activity on charcoal rot disease caused by Aspergillus *spp*.

Keywords: Siderophore; *Rhizobium*; PGPR; Antifungal activity

Introduction

In general, siderophores are low molecular weight compounds that can chelate ferric iron from many insoluble compounds in the environment. Ranging in size from 500-1500 daltons, they are synthesized by many microbes when growing under low iron conditions. Siderophores can be divided into three main classes depending on the chemical nature of the functional group or groups used for Fe (III) coordination. These classes are the catecholates (*sensu stricto*, catecholates and phenolates; better termed as "aryl caps"), hydroxamates and the (α-hydroxy-carboxylates [1]. A fourth group, designated as "Mixed type", is comprised of those siderophore that use a combination of any of the above types to chelate iron.

Generally, each of the rhizobial species can produce at least one siderophore. However, it is also true that in some genera such as *Bradyrhizobium* siderophore production is much less widespread. Examples of some rhizobial siderophores are the trihydroxamate vicibactin that was first isolated from *R. leguminosarum* [2]; Schizokinen and rhizobactin 1021, dihydroxamates isolated from *R. leguminosarum* [3] and *Sinorhizobium meliloti* [4] respectively; and a carboxylate called rhizobactin found in *S. meliloti* [5].

Rhizobia have been reported to inhibit significantly the growth of pathogenic fungi such as *Macrophomina phaseolina*, *Rhizoctonia spp*, *Fusarium* sp., and *Pythium spp*. in both leguminous and non-leguminous plants. Such biocontrol bacteria have different mechanisms or combinations of mechanisms which may be involved in the suppression of different plant diseases; for example, the inhibition of the pathogen by antimicrobial substances (antibiosis); production of diverse microbial metabolites such as siderophore, rhizobitoxin); induction of plant resistant mechanisms; inactivation of pathogen germination factors present in seed and root exudates and degradation of pathogenicity factors of the pathogen such as toxins; parasitism that may involve production of extracellular cell wall degrading enzymes, for example, chitinase that can cause pathogen cell walls lysis or plant growth enhancement through IAA production [6].

Materials and Methods

Well-developed pinkish nodules were separated from the black gram plants with a portion of root attached to them and washed with water to remove soil. The nodules were surface sterilized with 0.1% mercuric chloride solution, then were transferred to sterile petridish containing 95% ethyl alcohol for 1 minute. The nodules were then crushed and transferred in Erlenmeyer flask. The well mixed suspension of each soil sample was subjected to 10-fold dilutions ranging from 10^{-4} to 10^{-7}. 0.1 mL of suspension from each dilution was transferred aseptically to petriplates and melted YEMA medium containing 0.2 mL of Congo red was poured in each petriplate. Then, they were rotated in clockwise and anticlockwise direction for uniform distribution and incubated at 37°C. After incubation period, the colonies were picked up and subcultured for further study.

Characterisation of *Rhizobium* isolates

The isolated cultures were tested for their morphological and Standard biochemical test were performed for each isolate and they were identified using Bergey's classification of bacteri

Estimation of hydroxamate siderophores

To 0.5 mL of culture supernatant 0.5 mL of 6 M H_2SO_4 was added and the mixture was autoclaved in a glass tube. 1 mL of sulphanilic acid (1% v/v) in 30 per cent acetic acid (v/v) and 0.5 mL of 1.3 per cent iodine in 30 per cent acetic acid (w/v) were added to autoclaved chemicals. The excess of iodine was destroyed by the addition of 1 mL of 2 per cent (w/v) sodium arsenate solution. 1 mL of solution of α-naphthylamine (0.3 per cent in acetic acid) was then added and the total volume was made upto 10 mL with distilled water. After 30 min, the absorbance at 526 nm was measured. Hydroxlyamine hydrochloride was used as standard and 1.0 µmol of the compound gave an absorbance of 0.1.

Biocontrol activity of *Rhizobium* isolates against the collor rot disease caused by *Sclerotium rolfsii* in groundnut plant

A loopful culture of each *Bradyrhizobium* isolates was transferred aseptically to the centre of PDA plates which have been pre-inoculated with *Sclerotium rolfsii*. Then, the plates were incubated at 28 ± 2°C for

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7 days. Afte the incubation period, the diameter of zone of inhibition against *Sclerotium rolfsii* was measured.

Results and Discussion

All the three isolates RM-1 to RM-3 exhibited the characters of *Bradyrhizobium viz*., circular, entire colonies, 3.1 mm in dia., whitish pink on solid yeast extract mannitol agar plates and gram negative, motile and non-spore former [7] (Tables 1-3). The siderophore, recorded with the rhizobial isolates *viz*., RM-1 followed by the isolate RM-2 production has a comparative range which is already been reported [8]. Sekar reported the interstrain difference *Azospirillum brasilense* for siderophore production [9]. The three *Rhizobium* isolates, *viz*., RM-1 to RM-3, obtained from the rhizosphere of rainfed groundnut plant, were screened and bio-control ability against *Sclerotium rolfsii*. It was observed that all the three isolates of *Rhizobium* were able ZOI against *S. rolfsii* but with a marked variation among them. The present study also confirmed with the earlier findings of Madhaiyan et al. regarding the biocontrol ability of *Methylobacterium* isolates (zone of inhibition) against *S. rolfsii*, available [10].

Characters	*Rhizobium*
Morphology	
Colony pigmentation	Whitish pink
Diameter of colonies (mm)	1
Cell width (µm)	0.4-0.5
Cell length (µm)	1.3-1.9
Yeast Extract Mannitol Agar	+
Nutrient agar	+
Temperature (°C)	20-30
Optimum (°C)	28
pH range	6.0-8.0
Optimum	7
D-Glucose	+
D-Arabinose	+
L. Rhamnose	-
D. Raffinose	-
Inulin	-
Sodium acetate	-
Sodium citrate	+
Sodium sorbate	+
Sodium pyruvate	+
L.Theronine	+
L-Cysteine	-
L-Arginine	+
L-Alanine	-
L. Phenylalanine	+
Ampicilin (50)	-
Sterptomycin (5)	+
Erythromycin (50)	+
Gentamycin (50)	+
Chloroamphenicol (50)	+

Table 1: Characterization of *Rhizobium* isolates.

Isolates	Siderophore production (µg mL^{-1})
	Hydroxylamine hydrochloride
RM-1	4.28 ± 0.35
RM-2	4.20 ± 0.30
RM-3	3.55 ± 0.41

Table 2: Siderophore production of *Rhizobium*.

Isolates	Zone of inhibition against (mm in dia)
RM-1	12.2
RM-2	8.1
RM-3	7.2

Table 3: Antifungal activity against the Collor Rot Disease caused by *Sclerotium rolfsii* in groundnut plant.

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