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Research Paper

PGPR and Antagonistic Activities of Bacteria Isolated from Rhizosphere of *Sesamum indicum*

Biological Science

KEYWORDS : Sesame, rhizosphere, indole acetic acid, siderophore, M. Phaseolina

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ABSTRACT

S. indicum is a well known plant cultivated for its seed and oil. Seeds of the plant contain high level of protein and other nutrients. Owing to its nutritive importance efforts are needed to increase growth and yield of Sesame plants. Utilization of PGPR's is one such approach. In present study bacteria isolated from rhizosphere of Sesame were evaluated for their PGPR activities. 11 bacteria out of a total of 22 isolates exhibited IAA production, 12 exhibited HCN production and 14 exhbited siderophore production. Many of the bacteria isolated also exhibited antagonistic activity against M. Phaseolina, a potent pathogen of Sesame.

Introduction

Sesamum indicum L. belongs to family Pedaliaceae is commonly called as Sesame and have been cultivated since ages (from around 2350 B.C.) for edible oil and food. The plant is widely cultivated in regions of Asia, Africa and South America (Anilakumar *et al* 2010, Haruna *et al* 2011). Sesame is an annual shrub which bears flowers which are white or purplish and bell shaped (Martin and Leonard, 1967). Each plant bears about 15-20 fruits and each fruit contains around 70-100 seeds.Tropical climate, sandy well drained soil with moderate rainfall is most preferred climatic conditions for sesame. Sesame have been reported to contain several biologically important phytoconstituents such as Linoleic acid, palmitic acid, steoric acid, lignans like Sesamin, sesamol, sesaminol, high protein content and elements such as phosphorous, sulphur and calcium (Van Raheen 1973, Panhwar, 2005).

Several studies conducted have revealed utilization of Sesame for several medicinal purposes (Frank 2005, Kapadial *et al* 2002, Chen *et al* 2005, Kaur and Saini 2000). Health benefit of Sesame has been reported in several traditional system of medicine such as Ayurveda (Renound Indian medicine system), In Thebes Medicinal Papyrus (1552 B.C) which in an old text of Egypt, etc. Antioxidant, anti-inflammatory, serum lipid lowering and antiaging are most important biological activities of Sesame (Namiki 2002).

Nutritive importance, medicinal and health benefits have popularized utilization of Sesame seed as well as Sesame oil for several purposes. This inturn has resulted in high demand of Sesame seeds. Routine cultivation practices utilized for cultivating Sesame often falls short to supply demand (Mahrous *et al* 2015). Several factors such as rainfall, ordinary cultivation practices, limited use of certified seeds, insects, fungal pathogen problems associated with harvesting limit the possible net productivity (Osman 1985, Khidir 1981,1997). Hence, there is requirement to follow improved cultivation practices which results in enhance and improved plant growth, provide protection from potent pathogens which will ultimately result in enhanced productivity. In several studies conducted on several plants utilization of PGPR's have been found to significantly increase productivity of plants. Hence, the present study was conducted to evaluate effectiveness of PGPR and the potential PGPR activities possessed by bacteria isolated from rhizosphere of Sesame plants.

Material and methods

S. indicum plants grown in Haridwar region were utilized as study material for present study. Bacteria isolated from Rhizosphere of Sesame plants (Jaisingh *et al* 2016) were taken as test sample and were subjected to evaluate their ability to produce IAA, siderophore and HCN.

Gordon and Paleg (1957) method was utilized for analyzing IAA activity. Each isolated bacteria was inoculated in nutrient broth and incubated for about 14 hrs. after which cultures were centrifuged for 15 min at 1000rpm. Supernatant was collected for further analysis. 2 ml salper's reagent was added drop wise with mixing and test tube were incubated in dark for 30 minutes. Production of pink colour indicated IAA production.

Bakker and Schippers (1987) methodology was utilized for determining HCN production. Petriplates containing nutrient agar supplemented with glycine were inoculated with bacterial isolates. Whatman filter soaked in 2 % w/v Na_2CO_3 in 0.5% picric acid was gently placed inside the lid of Petri plate and plates were incubated at 28-30ºC for 3-4 days. Change in colour of filter paper from yellow to reddish brown indicated production of HCN by respective bacterial culture.

For analyzing siderophore production chrome azurols medium was prepared. Each culture was streaked onto separate plate and incubated at 35±2 ºC for 1-3 days. Appearance of hollow yellow zones around bacterial colonies indicated production of siderophore (Manivannan *et al* 2012, Husen E 2003).

The isolated bacteria were screened for their antagonistic activity against *Marphomina Phaseolina*. Firstly the fungi was grown on potato dextrose agar (PDA) plates. Fresh PDA plates were taken and the culture of fungi was inoculated in the centre of the medium. Culture of test bacteria were inoculated about 3 cm apart from fungi. Separate plate was prepared for each bacterial isolate and petri plates were incubated at 35±2 ºC for 4-7 days. The distance between fungi and bacterial growth indicated antagonistic activity of respective bacteria (Manivannan et *al* 2012)

Results and discussion

A total of 22 bacteria were isolated from rhizosphere of *S. indicum* and were labeled as SI01-SI22. In earlier study Jaisingh *et al* 2016 identified three out of these three bacteria SI04, SI06 and SI18 to belong to genus *Pseudomonas*, *Azotobacter* and *Bacillus Spp.* respectively. Each bacterium was maintained as pure culture on NAM and were evaluated for different PGPR activities.

A maximum of 11 isolated bacteria (SI01,SI03,SI04,SI06,SI07,SI11,SI13, SI18,SI19,SI21,SI22) exhibited IAA production (Table 1). Cultures namely SI04, SI06, SI11 and SI19 were found to produce

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higher amount of IAA. Rest of the cultures did not produced IAA. Also IAA production by these bacteria was stimulated without addition of any tryptophan in the culture. This indicates that these bacteria were naturally involved in indole acetic acid. However some bacteria require addition of tryptophan into culture medium for production of IAA. In a study conducted by Park *et al*, 2009 *Pseudomonas fluorescens* was reported to synthesize IAA but only in presence of L-tryptophan. Other studies have also reported production of IAA by bacteria. Production of IAA by *B. pumilus* and *B. licheniformis* have been reported by Gutierez- Manero *et al*.,2001, Patten and Glick (1996) have stated that about 8% of bacteria isolated from rhizosphere of different plant are capable of producing indole acetic acid. In most of the studies conducted related to production of plant hormones by bacteria IAA remains the most common hormone produced by bacteria found in rhizosphere.

14 out of 22 isolated bacteria exhibited siderophore production as indicate by appearance of yellow-orange zones around colonies. SI01, SI04, SI05, SI06, SI08, SI11, SI12, SI13, SI15, SI16, SI18, SI19, SI20, SI22 were found positive to produce siderophore whereas other isolates were negative for siderophore production. Cultures SI02, SI04, SI05, SI06, SI08, SI10, SI13, SI14, SI16, SI18, SI19 and SI21 were found to be positive for HCN production. Among these SI06, SI10 and SI19 exhibited higher level of HCN production as compared to other cultures producing HCN. Other bacterial isolates (SI01, SI03, SI07, SI09, SI11, SI12, SI15, SI20 and SI22) were found negative for HCN production.

M. phaseolina is a potent fungal pathogen of Sesame and is known to cause significant damage to Sesame crops hence effective control of pathogen becomes must to obtain maximum yield from sesame crop. Bacterial isolates SI02, SI03, SI04, SI06, SI08, SI09, SI10, SI13, SI14, SI15, SI16, SI17, SI18, SI19, SI20, SI22 exhibited antagonistic activity against *M. Phaseolina* (Table 2) .Among the above mentioned cultures SI08 displayed maximum antagonistic activity against the pathogen. The extent of antagonistic activity of all bacterial isolates was found to increase with corresponding increase in incubation time. Several other reports are available in which different bacteria have been reported to inhibit growth of several pathogenic fungi. Farhan *et al.,* 2010 reported antagonistic activity of *Pseudomonas putida* 2 and *Pseudomonas fluorescens* 3 against *Fusarium* fungi. Their study also reported better results when both the *Pseudomonas* species were utilized as consortium. In an another study (Ziedan *et al.*, 2011) imycorrhizae was reported to effectively restrict the growth of *Fusarium oxysporum* and *Marphomina phaseolina*, the two most common pathogen of sesame. As already stated SI04, SI06 and SI18 were identified to belong to *Psuedomonas, Azotobacter* and *Bacillus spp*. based upon morphological and biochemical properties. All the three species exhibited antagonistic activity against *M. phaseolina*. Beside this all the three bacteria were found to be positive for IAA, siderophore and HCN production.

Conclusion: From results obtained it can be concluded that utilization of PGPR can be significantly beneficial for growth and productivity of Sesame plants as many of the isolates were found to produce IAA, siderophore and HCN and antagonistic activity , all of which are known to enhance plant growth in one or the other way.

Table 2: Antagonistic activity of bacterial isolates against *M. phaseolina*

Bacterial Isolates	IAA	Siderophore	HCN
SI01	+	+	-
SI02	-	-	+
SI03	+	-	-
SI04	++	++	+
SI05	-	+	+
SI06	++	+	++
SI07	+	-	-
SI08	-	+	+
SI09	-	-	-
SI10	-	-	++
SI11	++	+	-
SI12	-	+	-
SI13	+	+	+
SI14	-	-	+
SI15	+	+	-
SI16	-	+	+
SI17	-	-	+
SI18	+	++	+
SI19	++	++	++
SI20	-	++	-
SI21	+	-	+
SI22	+	+	-

Bacterial isolates	M. Phaseolina	Zone of inhibition after 48hrs (mm)
SI01	-	-
SI02	+	**4.8±0.2**
SI03	++	**5.1±0.3**
SI04	+	**3.6±0.5**
SI05	-	-
SI06	++	**6.7±0.4**
SI07	-	-
SI08	+++	**7.5±0.4**
SI09	+	**3.9±0.3**
SI10	++	**4.5±0.2**
SI11	-	-
SI12	-	-
SI13	++	**4.6±1.0**
SI14	-	-
SI15	+	**4.4±0.7**
SI16	-	-
SI17	++	**5.4±0.3**
SI18	++	**5.6±0.6**
SI19	++	**6.4±0.5**
SI20	+	**4.1±0.6**
SI21	-	-
SI22	+	**4.0±0.2**

(No response), + (positive, moderate response), ++ (enhanced response), +++(extensive response)

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