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Research Article

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Isolation and characterization of PGPR from rhizosphere of *Sesame indicum* L.

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Abstract

Sesamum indicum is a well known commercially cultivated crop for its seed and oil. Biochemical, nutritive and medicinal importance of sesame oil have generated an ever increasing demand for enhanced production of sesame. Several environmental factors are known to adversely affect yield and productivity of Sesame. Hence there is need to develop and promote cultivation practice which enhance sesame productivity. One such approach is utilization of PGPR's. In the present study PGPR were isolated from rhizosphere of Sesame plant grown in Haridwar region and three bacteria out of a total of 22 isolates were identified to belong to genus *Pseudomonas*, *Bacillus* and *Azotobacter*. All the three bacteria were found to exhibit a growth enhancing effect on Sesame plant reflected by enhanced growth of seedlings, increased shoot length and number of branches in plants treated with PGPR.

Keywords: Rhizosphere, Sesame, *Pseudomonas. Bacillus, Azotobacter.*

Introduction

Sesamum indicum L. an important oil seed crop commonly called as Sesame is known to be 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 (Anila kumar *et al* 2010, Haruna *et al* 2011). Among various species of Sesame, *S. indicum* remains to be one of the most commonly cultivated species of sesame in India. Sesame oil has been assessed to contain large number of phytochemical compounds. Linoleic acid, oleic acid, palmitic acid and steoric acid are the main unsaturated fatty acids present in sesame oil. Sesame seeds are highly rich in protein, sulphur, calcium and phosphorous content (Van Raheen 1973, Panhwar, 2005). Sesamin, sesomolin, sesamol and sesaminol glucosides and tocopherol are other phyto-components of sesame. The plant possesses antihypertensive effect, anti inflammatory, antioxidant effect, antiaging, serum lipid lowering, blood pressure lowering etc (Namiki 2002). Sesamin an important phytoconstituent of sesame oil is found to protect liver from oxidative damage. There is an ever increasing demand of sesame seed for various medicinal and nutritive purposes (Frank 2005, Kapadial *et al* 2002, Chen *et al* 2005, Kaur and Saini 2000). However, routine cultivation often falls short to fulfill demand (Mahrous *et al* 2015). Fluctuation in rainfall, ordinary cultivation practices, limited use of certified seeds, insects and pests all together adversely effect the crop yield (Osman 1985, Khidir 1981,1997). Hence there is need to develop cultivation practices to obtain maximum possible yield one such approach is utilization of PGPR. Hence, the present study was conducted to isolate bacteria from rhizosphere of

Sesame plants grown in Haridwar region and evaluate their effectiveness in improving growth of plants.

Materials and Methodology

The *Sesame* plants selected for the study were gently removed from soil. The plants were shaken (slowly) to remove the soil loosely attached to roots of plants completely to obtain rhizosphere. This rhizosphere was utilized as study material for further studies. Approximately 1 gm of roots were excised from plant and transferred to glass bottle containing 99 ml of phosphate buffer saline (PBS). The bottles were placed on a rotatory shaker for 5-6 hours to dissociate bacteria associated with the roots. This solution was subsequently utilized for preparation of dilutions from 10^{-2} to 10^{-6}. Adequate sample from these dilutions were spread onto KB agar medium and nitrogen free agar medium. Isolated bacteria were restreaked onto NAM. All the cultures were maintained at 37 ℃. Isolated cultures were further subjected to morphological and biochemical analysis. Reactions characteristic of biochemical reactions occurring inside cells can be utilized in identification and classification of microorganism. A series of biochemical tests provides a microbial fingerprint. Each bacterial culture were examined for gram reaction, shape, motility, catalase, gelatin and starch hydrolysis, IMVIC test, oxidase test, urease production, H_2S production, nitrate reduction as per the methodology of K R Aneja (2001).

Similarity among the isolated bacteria was estimated by using the percentage difference in the program NTSYSPC 2.1d. The similarities between bacterial isolates were calculated according to Jaccard's coefficient (Jaccard, 1908) using NTSYS-pc software package ver 2.10d. A Phylogenetic tress was constructed using UPGMA method. Standard strain of *Pseudomonas aeruginosa* (SCO1), *Bacillus subtilis* (SC02) and *Azotobacter vinelandii* (SC03) were utilized as reference strains for identification and comparison with the isolated bacteria.

Selected bacteria were examined for their effect on growth on Sesame plants. The prepared innoculum was added to seed to obtain a uniform coat of PGPR culture around the seeds. Seeds were surface sterilized with 0.1% $HgCl_2$ for one minute followed by rinsing with sterile water to remove all traces of $HgCl_2$. Seeds were inoculated onto petridishes containing sterile whatman paper soaked with sterile water. Also, seeds were sown in pots containing sterile soil. Petridishes and pots were regularly monitored for germination rate and growth parameters in terms of number of branches, shoot length.

Results and Discussion

A total of about 22 bacterial isolates were obtained from rhizosphere of young and healthy plant of *S. indicum.* Isolated bacteria were maintained as pure culture onto NAM slants and were accordingly abbreviated (SI01-SO22).

Bacterial isolates

SI01, SI03, SI04, SI05, SI08, SI12, SI14, SI17, SI18, SI19, SI21 were found to be gram positive whereas rest of isolates were gram negative. Bacterial strains SI01, SI02, SI04, SI05, SI06, SI08, SI10, SI12, SI15, SI16, SI18 and SI22 were found to be motile whereas rest of the isolates were non motile. Most of the isolated bacteria including SI02, SI03, SI06, SI07, SI09, SI10, SI11, SI13, SI14, SI17, SI18, SI21, SI22 were found to be positive for indole test. Cultures SIO1,SI04,SI07,SI09,SI10,SI13,SI14,SI16,SI18,SI12, SI22 were found to produce enzyme urease as depicted by the result obtained in urease test. Phenyl alanine deamination was reported in SI03, SI05, SI09, SI13, SI15, SI18 and SI20. Among the isolated bacteria SI01, SI02, SI04, SI08, SI11, SI12, SI15, SI17, SI18, SI20 and SI22 were found positive for nitrate reduction. Many of the isolated bacteria were found to produce H_2S (SI03, SI06, SI07, SI08, SI11, SI13, SI16, SI17, SI20 and SI22). Cultures SI02, SI04, SI06, SI07, SI08, SI10, SI11, SI16, SI18, SI19 and SI22 were reported to be positive for catalase test (Table 1). As revealed by the phylogenetic tree (Fig.1) bacterial isolate SI10 exhibited with standard culture *Pseudomonas fluroscens* (SC01). Similarly, bacterial isolate SI18 was found to have 93% similarity with standard culture *Bacillus subtilus* (SC02) and isolated bacteria SI06 was similar to reference strain of *Azotobacter* (SC03). Hence, it can be concluded that bacterial isolate SI10, SI18 and SI06 belongs to genus *Pseudomonas, Bacillus* and *Azotobacter* respectively.

Among various parameter analyzed such as number of branches per plant, length of shoot, root, all of these parameter resulted better growth in the plants treated with PGPR as compared to control. A maximum of 4.2 branches were obtained in control plants. The number of branches increased to 5.3 when the plants were treated with *B. subtilis* (T1). About 6.3 branches per plant were obtained in plants treated with *Pseudomonas* spp. By far *Pseudomonas* (T2) was found to be most effective PGPR as compared to

Table 1: Morphological and biochemical characteristics of bacteria isolated from rhizosphere of *S. indicum*

	Bacterial isolates												
Characteristics	**SI01**	**SI02**	**SI03**	**SI04**	**SI05**	**SI06**	**SI07**	**SI08**	**SI09**	**SI10**	**SI11**	**SI12**	**SI13**
Gram Reaction	+	-	+	+	+	-	-	+	-	-	-	+	-
Shape	Rod	Rods	Rod	Rod	Rod	Rod	cocci	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	-	+	+	+	-	+	-	+	-	+	-
Capsule	-		+	-	-	+	-	-	+	-	+	-	+
Endospore	+	+	-	-	-	-	+	+	-	-	-	-	+
Growth on NAM	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	+	+	+	-	+	+	+	-	+	+	-	-
Gelatin Hydrolysis	-	-	-	+	+	-	-	-	+	+	-	+	-
Starch Hydrolysis	-	-	+	-	-	-	-	+	+	+	+	+	-
Urease	+	-	-	+	-		+	-	+	+	-	-	+
Phenylalanine Deamination	-	-	+	-	+	-	-	-	+	-	-	-	+
Nitrate Reduction	+	+	-	+	-	-	-	+	-	-	+	+	-
H_2S Production	-	-	+	-	-	+	+	+	-	-	+	+	+
Citrate Utilization	-	+	+	+	+	+	-	+	-	+	+	-	+
Methyl red	+	+	-	-	+	-	-	+	+	-	+	+	+
Vogues Prospaur	-	-	+	+	-	+	+	-	-	-	-	-	-
Indole	-	+	+	-	-	+	+	-	+	+	+	-	+
Malonate	-	-	-	+	+	+	+	+	-	-	+	+	+
Esculin Hydrolysis	-	-	+	-	+	+	-	+	-	+	-	+	+
Oxidase	+	-	-	+	-	+	+	-	+	+	+	-	+

Characteristics	**SI14**	**SI15**	**SI16**	**SI17**	**SI18**	**SI19**	**SI20**	**SI21**	**SI22**	**SC01**	**SC02**	**SC03**
Gram Reaction	+	-	-	+	+	+	-	+	-	-	+	-
Shape	Rod	Rod	cocci	Rod	Rod	Rod	Rod	Rod	Rod	+	+	+
Motility	-	+	+	-	+	-	-	-	+	+	+	+
Capsule	+	-	-	+	-	+	-	+	-	-	-	+
Endospore	-	-	+	-	+	-	-	-	-	-	+	-
Growth on NAM	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	+	-	+	+	-	-	+	+	+	+
Gelatin Hydrolysis	+	-	-	+	+	-	+	-	+	+	+	-
Starch Hydrolysis	-	-	-	+	-	+	-	+	-	+	-	-
Urease	+	-	+	-	+	-	+	-	+	+	+	+

Phenylalanine Deamination	+	+	-	-	+	-	+	-	-	-	+	-
Nitrate Reduction	-	+	-	+	+	-	+	-	+	+	+	+
H_2S Production	-	-	+	+	-	-	+	-	+	-	-	+
Citrate Utilization	-	+	-	-	-	-	+	+	-	+	-	+
Methyl red	+	-	+	-	-	+	-	-	-	-	-	-
Vogues Prospaur	-	+	-	-	+	-	+	+	+	-	-	+
Indole	+	-	-	+	+	-	-	+	+	+	+	+
Malonate	-	-	-	+	-	+	-	+	-	+	-	+
Esculin Hydrolysis	+	-	+	+	+	-	-	+	-	+	+	+
Oxidase	+	-	-	+	+	-	-	+	+	+	+	+

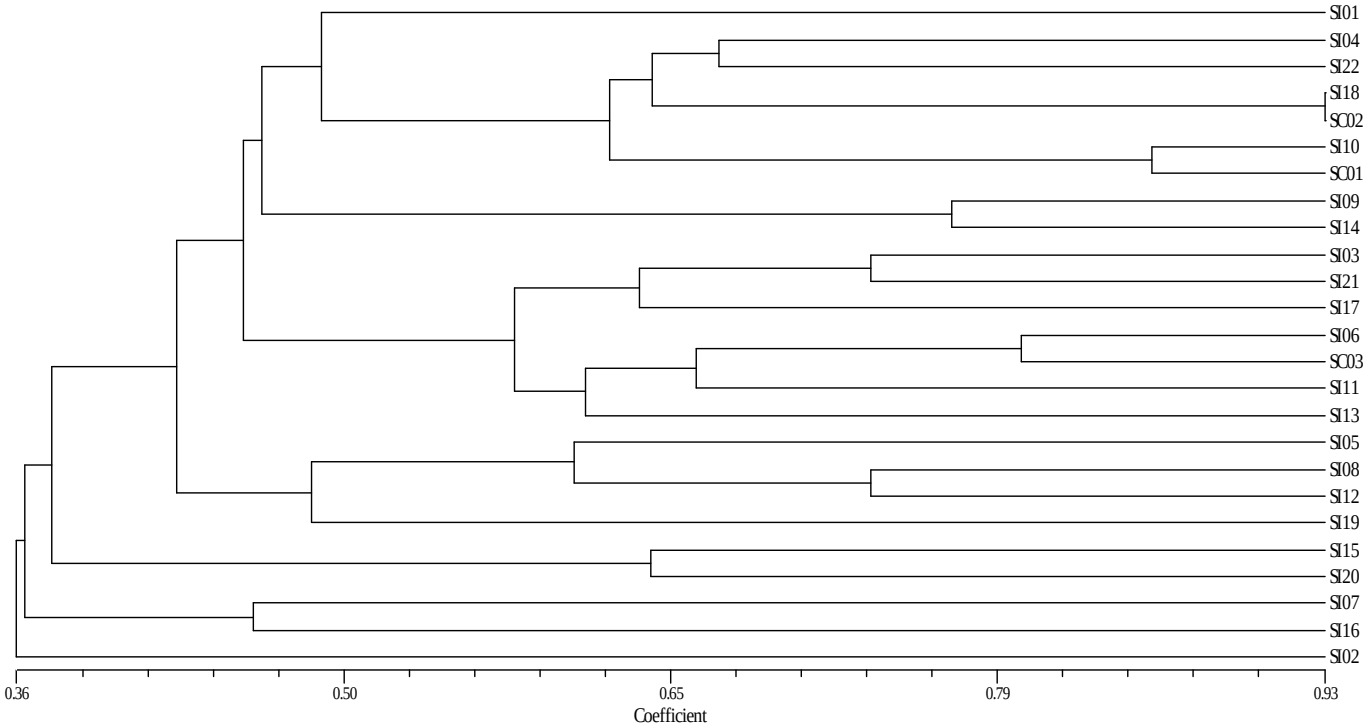

Fig.1 Dendogram based upon morphological and biochemical characters of bacterial isolates

Bacillus +Azotobacter in case when single PGPR were used as innoculum. Plants treated with *Azotobacter* (T3) resulted in 5.6 branches per plant. The number of branches obtained was further enhanced when these bacteria were utilized in combination (Fig.2A).

Plants treated with consortium of *Bacillus* and *Pseudomonas* (T4) resulted in formation of about 6.2 branches per plant. Among different combinations utilized consortium of *Bacillus*, *Pseudomonas* and *Azotobacte*r (T6) was found to give best results with a number of 7.8 branches per plant. Another morphological parameter analysed was shoot length of the plants. In control a maximum of 175.3 cm length was attained by the plants. However, plants treated with innoculum of PGPR either individually or in combination an expected increase in shoot length was obtained. Plants treated with *Bacillus* attained shoot length of about 182.4 cm and plants treated with *Azotobacter* attained a height of 186.4 cm. Similar results were obtained were shoot length of treated and untreated plants were compared. Maximum shoot length of 190.2cm was obtained in plants treated with *Pseudomonas* (when PGPR's were used individually). Beside these increase in shoot length was also achieved when PGPR were used in combination. When plant were treated with *B. subtilis* and *Pseudomonas*, plants attained a height of 190.8 cm. Maximum shoot length of 196.2cm was obtained in the plants treated with *Bacillus* + *Pseudomonas*+ *Azotobacter* (Fig.2B). Several earlier studies have also reported effectiveness of PGPR in enhancing the growth of plant. Farhan *et al*., 2010 reported an increase in chlorophyll content, number of shoots, height of shoots, number of branches, number and area of leaf per plant, number of seeds per plant and percentage of oil in sesame seeds. When sesame crops were cultivated in presence of *P. putida* and *P. fluorescens.* Enhancement in morphological characters such as length of plant, fresh weight of plant and number of pods of sesame plant have also been reported by Zeidan *et al*.,2011. Glick *et al* (1997) found utilization of *P. fluorescens* and *P. putida* resulted in increase in root and shoot length of canola plants. No significant increase was found in terms of percentage of seed germinated between treated and untreated seeds, however seed treated with PGPR exhibited faster germination and enhanced growth of seedlings as compared to untreated seeds (Fig. 3A-C).

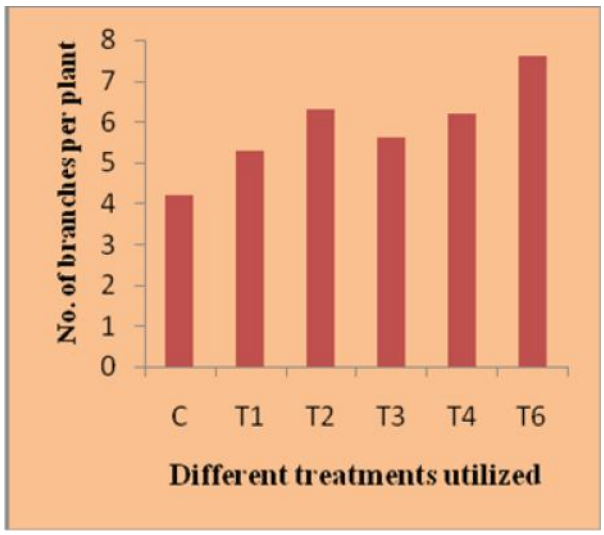

Fig.2A Effect of PGPR on no. of branches

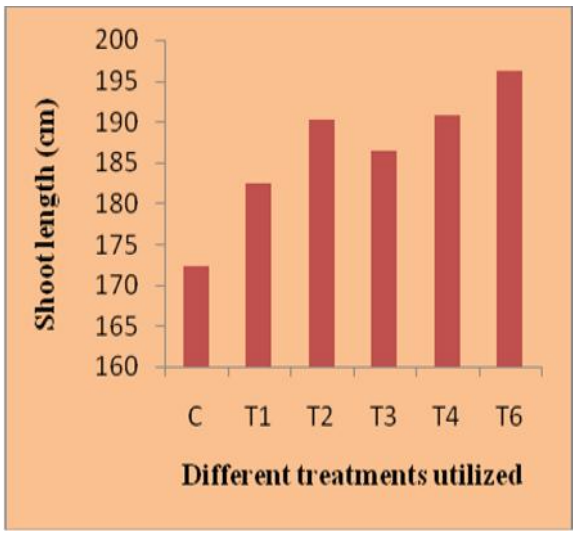

Fig.2B Effect of PGPR on shoot length

C= Control; T1= *Bacillus*; T2= *Pseudomonas*; T3=*Azotobacter*; T4= *Pseudomonas* + *Bacillus*; T6= *Pseudomonas*+*Bacillus*+*Azotobacter*

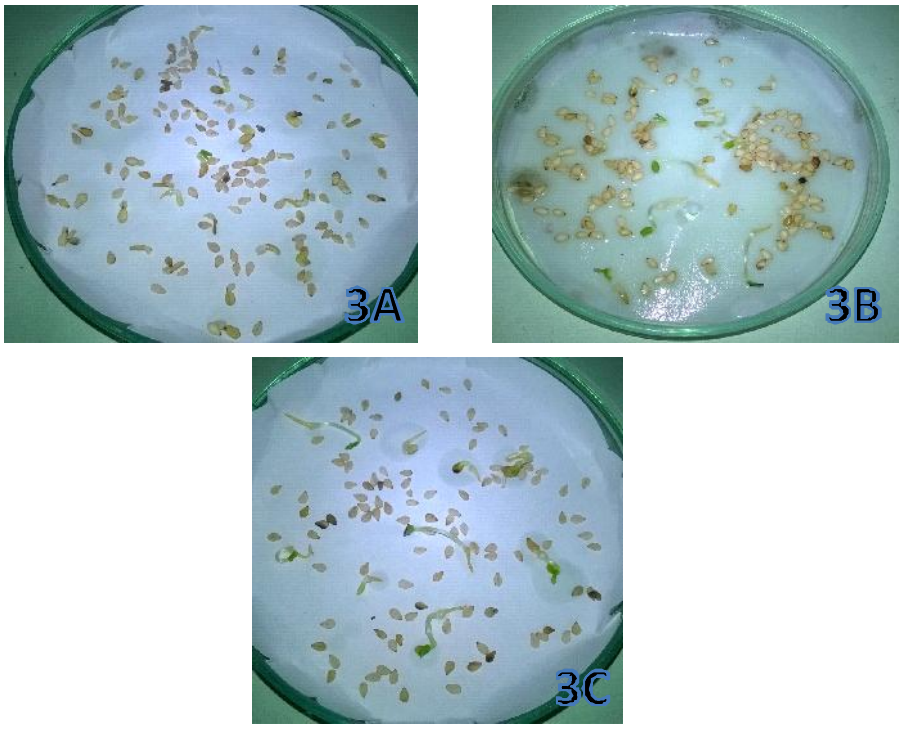

Fig..3A- Control , seeds with slow germination and growth, **Fig.3(B-C)** – Seeds treated with *Psuedomonas, Bacillus* exhibiting faster germination and growth of seedling, after 7-8 days of incubation.

Conclusion

Present study reports presence of *Pseudomonas* spp., *Bacillus*., and *Azotoabacter* spp. to be present in rhizosphere of Sesame plant grown in Haridwar region. Also all these three bacteria were found to exert an overall growth enhancing effect on Sesame plants. Hence, the study supports utilization of bacterial inoculums with cultivated crop to enhance growth and productivity.

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