



ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FROM AGRICULTURAL FIELD AND THEIR POTENTIAL ROLE ON GERMINATION AND GROWTH OF SPINACH (*Spinacia oleracea* L.) PLANTS

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ARTICLE INFO	ABSTRACT
Received 16th, July, 2016, Received in revised form 12 th, August, 2016, Accepted 26th, September, 2016, Published online 28th, October, 2016	Various chemical fertilizers are used in different doses for crop improvement purpose in agriculture. But for several side effects of agrochemicals there is an increased concern on the co-operative activities between plants and the rhizospheric microbes. So, now a days the PGPR (Plant Growth Promoting Rhizobacteria) application opening a new gateway to solve these problems. The PGPRs not only can improve soil fertility but also enhance plant growth. In this article we are going to focus on the potential role of PGPR, isolated from the agricultural land. Here we examined the growth of spinach plant with four isolated PGPR strains in the Kalyani University campus. In all studied cases, we observed that different growth parameters in the uninoculated spinach (<i>Spinacia oleracea</i> L.) plants (i.e-without PGPR) were reduced as compared with treated plants. Absence of PGPR activities were assessed by observing different growth parameters like low germination %, reduced growth and poor rooting and low chlorophyll content. Inoculation of PGPR in spinach plant enhanced the different growth parameters as compared with uninoculated plants. Our present study conducted with 4 out of 20 different bacterial isolates and observed results indicated that SIN 1,SIN 2,SIN 3 ,SIN 4 (named according to the place and priority of isolate such as, Sample Isolate Nadia 1) were very effective to promote plant growth.
Keywords: PGPR, plant pathogen, rhizospheric microbes, soil fertility, soil ecosystem.	

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INTRODUCTION

The rhizospheric zone is the nutrient rich area that promotes the establishment of huge varieties of microorganisms such as, fungi, bacteria and each of them may exhibit neutral, beneficial and hazardous effects on the plants [1]. PGPR can enhance plant growth and development by several mechanisms such as, by producing several compounds (phytohormones, organic acids, siderophores), by fixing atmospheric nitrogen and most of all by producing various biologically active substances or Plant Growth Regulators (PGRs) [2]. It is seen that the rhizobacteria show more versatility in transformation, mobilization and solubilization of nutrients than those from the bulk soils [3]. PGPR are free-living in nature and they have the capacity to invade into the living plants and can create symptomatic infections [4] and when they are applied to seeds or crops they can increase the plant growth and reduce the deleterious effects of soil-borne phytopathogens also [5]. In this study the preliminary objective is to isolate bacteria from the rhizospheric zone of paddy (*Oryza sativa*) and cabbage (*Brassica oleracea* var. *capitata*) plants, then to characterize their morphological, biochemical and PGPR features and

ultimately to observe their effect on the growth and development of Spinach plants in K.U. campus. In addition to this the chlorophyll content of plants were also determined.

MATERIALS AND METHODS

Collection of Soil

The soil samples were collected from paddy and cabbage fields of Chakdaha, Pumlia, Nadia. During collection the upper 7 cm. soils were discarded and the lower soil layer beneath this were collected aseptically in neat and clean polythene packet and then brought to the laboratory and was stored in aseptic condition for further use.

Collection of Seeds

The spinach seeds were bought from Uluberia Seed house. The seeds were of uniform germplasm and the variety was Harita. After purchase the seeds were kept in dried clean place of laboratory for further use.

Isolation of Bacteria

By serial dilution technique the bacterial strains were isolated from the collected soil samples by using Nutrient Agar Media

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(Beef extract- 3g; Peptone-5g; Agar-5g; Distilled water-1000ml., Sodium chloride- 5g, pH-6.8). Plates were incubated at 28°C and after 48-72 hours of incubation the number of bacterial colonies developed was recorded and inoculated separately in Nutrient Agar slants, maintained at 4°C for further work.

Characterisation of bacteria

Characterization of isolated bacteria were performed based on conventional procedures such as, morphological studies, cultural properties, staining nature and biochemical peculiarities.

Morphological characters include colour, elevation and edge of the colony.

Gram nature of each isolates was initially determined by using crystal violet and safranin staining. Among biochemical tests, amylase test, catalase test, gelatin hydrolysis, urease test, citrate utilization were performed based on conventional techniques for bacterial characterization. (followed by Microbiology- A Laboratory Manual, 7th edition by Capuccino Sherman).

Seed germination test

The efficiency of germination of the collected spinach seeds were performed in the Kalyani University research garden by using different combinations of isolated PGPR strains. Atfirst the spinach seeds were surface sterilized with 0.01% HgCl₂ for 2 mins. followed by successive washing with sterile distilled water. Seeds were kept into respective bacterial culture medium containing 10⁶ cells/ml. for 10 mins. After that the seeds were transferred and placed on sterile soil containing pots and incubated for 2-3 days. After 3 days seed germination was recorded in comparison with control. The nature of seed germination was also checked by planting the imbibed seeds into pots using the same technique.

Germination percentage = (Total no. of germinated seeds/Total no. seeds)*100

Exploitation of PGPR on growth of spinach seedlings

Spinach seeds (Harita variety) were sown in (the soil of the pot was sterilized for successive 3 days at 15 lbs pressure for 40 mins.) pot culture after proper imbibitions in the bacterial suspension for 24 hours and the set was kept in Kalyani University campus. The length of root, shoot and chlorophyll content of the test plant samples were measured. For chlorophyll content measurement of green parts of spinach plants the conventional method was followed [6].

RESULTS

In this study PGPR from agricultural field were isolated and enumerated their role for improvement of the growth and yield of spinach in the Kalyani University campus.

Morphological, Biochemical, PGPR characters were determined following different techniques. Colony morphology and biochemical tests of the bacterial isolates were tested by following the standard methods of "Microbiology- A

Laboratory Manual" by Cappuccino Sherman (7th edition). The PGPR tests were done by following standard methods as depicted in table 3

[17],[18],[19,20],[21]. It was noted that the appearance of yellow colouration, bubble production, liquefaction of gelatin, appearance of blue colour and conversion of yellow into pink colour were indicated as presented in table 1. The results of PGPR confirmation tests are denoted in table2.

Table 1 Isolation and characterization of bacteria

Name of the strain	Colony Morphology	Gram nature	Amylase Test	Catalase Test	Gelatin Hydrolysis	Citrate utilization test	Urease test
SIN1	Whitish, smooth	+	++	+	-	-	+
SIN2	Whitish, smooth	+	+	++	-	+	+++
SIN3	Yellowish, elevated	+	+++	+	-	-	-
SIN4	Slightly yellowish, elevated	+	+	+	+	-	+++

**Presence or absence of character is noted by "+" or "-" sign; intensity of character is designated by the no. of "+" sign.

Table 2 PGPR tests of isolated bacterial strains

Name of the strain	Phosphate solubilization test	Ammonia production	IAA production	HCN production
SIN1	+	+++	-	+
SIN2	+	+++	-	-
SIN3	-	++	-	+
SIN4	-	+++	+	-

Table 3 Performed PGPR tests and the methods followed

Name of the PGPR test	Methods followed in this work
Phosphate solubilization test	Pikovskaya's Method (1948)
Ammonia production test	Dye's Method (1962)
IAA production test	Gordon and Weber's Method (1951) modified by Brik <i>et al.</i> (1991)
HCN production test	Bakker and Schipper's Method (1987)

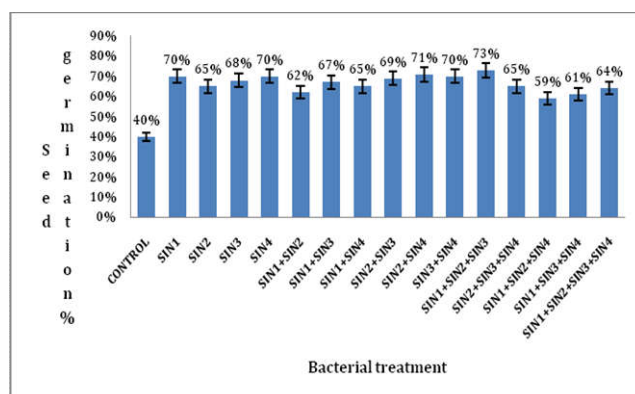


Fig 1 Effect of isolated PGPR on seed germination of spinach in Kalyani University Research Garden.

Effect of isolated PGPR on spinach seed germination in normal environment in Kalyani University research garden was estimated. The outcome of the experiment denoted that the isolated PGPR strains had outstanding effects on the germination of spinach seeds. Atfirst the surface sterilized seeds were dipped into different combinations of isolated PGPR emulsions for 24 hours and then allowed those seeds to germinate in the pots kept in Kalyani University research garden

and after certain time the seed germination percentage was calculated as compared to uninoculated set. The calculation of seed germination percentage, PGPR's effects on root length, shoot length, chlorophyll content are presented in Figure 1, Figure 2, Figure 3, Figure 4 respectively.

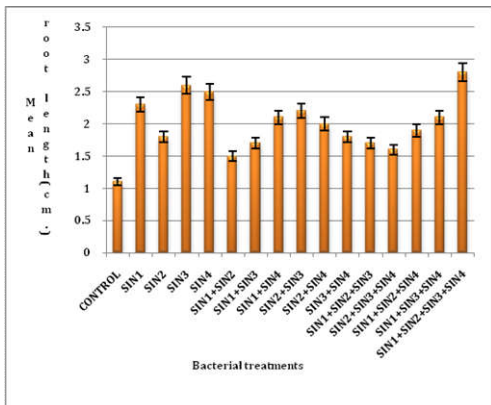


Fig2 Effect of isolated PGPR on root length (cm.) of spinach seedlings at Kalyani University research garden.

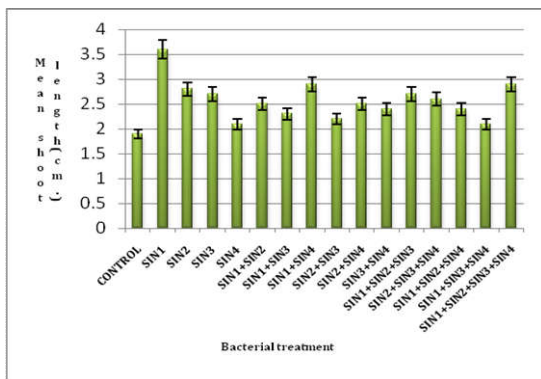


Fig 3 Effect of isolated PGPR on shoot length (cm.) of spinach seedlings at Kalyani University research garden.

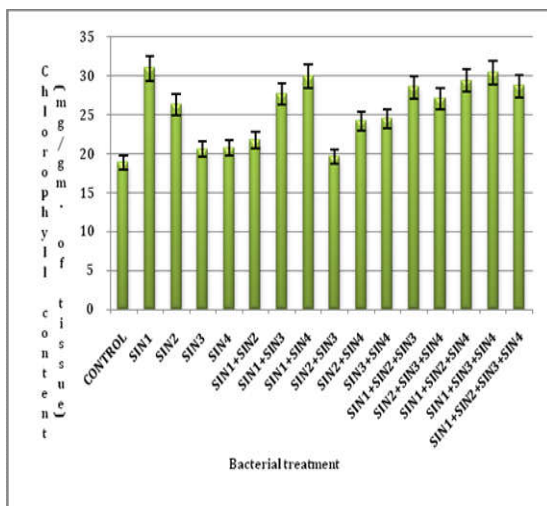


Fig 4 Effect of isolated PGPR on chlorophyll content (mg/gm. of tissue) of spinach seedlings at Kalyani University research garden. (followed by the method Arnon,DI (1949) Plant Physiol 241)

DISCUSSION

The result of this experiment denoted that the isolated PGPR strains showed profound positive effect on enhancing seed germination of spinach. The PGPR isolates exhibited their individuality based on various characterization such as, morphological, staining and biochemical properties that is depicted in table 1. All the tests were performed following the standard methods[7].

In a subsequent study the isolated PGPR strains were applied as inoculants and they showed remarkably improved growth of spinach seedling in respect to root length(cm.),shoot length (cm.) and chlorophyll content (mg/gm of tissue) over the uninoculated plant (i.e. without PGPR treatment).

Reports about PGPR were many and their activities have been proven to enhance growth and yield of many crops such as, rice [8], wheat [9], cucumber [10,22], maize[11,], cotton [12], black pepper[13] and banana [14]. The PGPR is one of the most effective soil microorganism that can enhance plant performance [15]. Therefore, in this experiment also the isolated PGPR strains are found to enhance the seed germination and growth. The result further indicated that the isolated PGPR having plant growth promoting activity can be used as alternative source of biofertilizers in future. Although further study needs for to reach any firm conclusion.

CONCLUSION

From the above study it can be clearly indicated that if the isolated PGPR strains were applied either alone or in combinations they can enhance plant germination, growth and development as well. Without any application of any chemical fertilizers the spinach plant growth can be increased by the using of these PGPR strains. So, there is a scope of using these PGPR strains as good biofertilizers in future.

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