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Evaluation of Plant Growth Promotory Activities of Rhizobacterial Isolates from Two Plants of Thar Arid Regions

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Abstract : More than thirty rhizobacterial isolates from two ecologically important plant species of Thar Desert were evaluated for their plant growth promontory effects in terms of seed germination, shoot and root length elongation and total biomass production. Isolates T-1 from *Calligonum polygonoides* and TS-2 from *Lasiurus sindicus* respectively were observed as effective seed germination inducers. Up to 37.5% increase over control was detected in shoot length with isolates T-4 and TS-10, whereas maximum root length inducing effect was found with isolates T-4 and TS-1. The total biomass production was highest with isolates T-4 (23.8 mg) in case of *C. polygonoides* and TS-12 (22.1 mg) in case of *L. sindicus*, respectively.

Key words : Rhizobacteria, plant growth promontory activities, North Western hot arid region, *Calligonum polygonoides, Lasiurus sindicus*.

Introduction

The Thar Desert covers western parts of Rajasthan and about 61% of the geographical area of the state. The average rainfall is less than 200 mm with a high diurnal variation in temperature, intense solar radiation and a high rate of evaporation. Low available water capacity, low fertility, vulnerability to wind erosion are major constraints along with high salinity, calcareousness and gypsiferous nature of the soils (Faroda *et al.,* 1999). This area harbors a limited diversity of life forms due to extreme conditions and therefore, it is an area of great ecological importance.

The rhizobacteria that are beneficial to plant are called plant growth promoting rhizobacteria (PGPR). However, the term PGPR has been used for all the rhizosphere bacteria that contribute direct or indirect beneficial effects on plant growth. The direct effects are by biological nitrogen fixation, phyto-hormones (auxins, gibberellins and cytokinin etc.) secretion and solubilization of minerals e.g. phosphate solubilization. In arid zones, the plant rhizosphere becomes an important site where maximum microbial activity could be seen since it provides carbon substrates in nutritionally poor soils (Bhatnagar and Bhatnagar 2005). In the rhizospheric zone, plant-microbial interactions play a crucial role in maintaining soil biological environment. These interactions may be beneficial or harmful to the plant depending on the specific microorganisms and plants involved (Brimecombe *et al.,* 2001; Lugtenberg *et al.,* 1991; Okon 1985).

Over-exploitation of fodder and fuel wood which are two basic necessities to support human life in Thar Desert, created ecological destruction of the desert ecosystem and enhanced desertification (Sinha *et al.,* 1997). Various trees, shrubs and grass species of economical importance (medicinal, food,

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fodder, fuel etc.) have been found to control desertification through sand dune stabilization (Venkateswarlu, 1993). *Calligonum polygonoides* and *Lasiurus sindicus* are one of these plants which have been used for such purposes. It is also increasingly realized that shrubs and grasses are better sand binders than the trees and the microbial communities associated with the plant rhizosphere play a crucial role in maintaining the plant-soil ecological cycle (Rao, 2000).

The effects of PGPR have not been investigated in desert regions. Therefore, the present investigation was undertaken to isolate plant growth promoting bacterial strains from *C. polygonoides* and *L. sindicus* of Thar origin and to study their effect on improving plant production. In our earlier communication, we have studied the phosphate solubilization efficiencies of different rhizobacterial isolates from above plants using tri-calcium phosphate as a sole source of phosphorus in the medium (Gothwal *et al.*, 2006).

Material and Methods

Study site and soil sampling

Rhizosphere soil samples from *C. polygonoides* and *L. sindicus* plants respectively were collected from three different zones of Western sandy plain sub-region covering parts of Bikaner, Jodhpur, Jaisalmer and Barmer districts of Rajasthan, as reported earlier to evaluate the PCR-DGGE based rhizosphere associated bacterial community analysis (Gothwal *et al.*, 2007). These plants are known for their involvement in maintaining ecological balance by soil conservation and sand dune stabilization besides some economical values e. g. wood, fuel, fodder etc.

Bacterial isolates

More than 30 bacterial morphotypes were isolated from rhizosphere soil samples on King's B media (King *et al.*, 1954) at ambient (37 ºC) and high (50 ºC) temperatures, respectively. The higher temperature was chosen because of the temperature variation in the arid region. All the above isolated strains were evaluated for various plant growth promontory effects under *in vitro* conditions.

Inoculum preparation

The isolates were inoculated in 50 ml of sterilized King's B media in 250 ml Erlenmeyer flasks and incubated in an orbital shaker at 180 rpm for 24 h at 37 ºC and 50 ºC (respective to the isolated temperatures). The cell OD of each grown isolate was adjusted to 0.5 at 600 nm and used to study their effects on the seeds of *C. polygonoides* and *L. sindicus*.

Seed sterilization and plating

Seeds of *C. polygonoides* and *L. sindicus* were procured from Central Arid Zone Research Institute, Jodhpur, Rajasthan. These were surface sterilized by keeping them in 0.1 % solution of mercuric chloride for 1-2 min and then in 70 % ethyl alcohol for 5 min and finally washed several times with sterile distilled water. All the surface sterilized seeds were then pre soaked in sterilized distilled water over night prior to plating. Five seeds were transferred aseptically on to 0.7 % water-agar (autoclaved) plates followed by addition of 10 ìl of above prepared bacterial inoculum on each seed so as to coat the surface of seed (Berg *et al.*, 2001). The plates were finally incubated up to 2 weeks at 27 ± 2 ºC in a temperature and photoperiod controlled growth chamber with a 12 h light and 12 h dark periods respectively. After completion of incubation period the seedlings were carefully taken out from the plates. The adhered agar with the seedling was removed by washing under running tap water. The seedlings were then kept on the blotting paper sheets to remove any excess of water or water droplets and observations regarding shoot and root length and total biomass measurements were made. The seed germination count was taken after 6 days. The relative germination was calculated for all the isolates with respect to control considering 100 % germination in control. All the experiments were performed in triplicate along with a control where seeds were coated

with King's B broth only and the mean values were recorded.

Results

More than 30 isolated cultures were used for the observation of their PGPR activity using *C. polygonoides* and *L. sindicus* plant seeds on the basis of seed germination, shoot and root length elongation and wet weight etc.

Effect of rhizobacterial isolates on seed germination

The effect of rhizobacterial isolates on the seed germination of *C. polygonoides* plant has been represented in Table 1. More than 50% isolates showed positive response towards germination. The highest response was observed with isolate T-1 with more than 90% germination, whereas isolate T-5 showed inhibitory effect towards germination. With isolate T-1, the relative germination was found to be increased to about 140% when tested under water agar plate testing conditions. When the effect of rhizobacterial isolates of *L. sindicus* was studied on the seed germination of the same plant, 86.7% germination was recorded with isolate TS-2 (Table 2). This was the highest response recorded among all the tested rhizobacterial strains of *L. sindicus*. This isolate has shown about 145% increase in relative germination compared to the control.

Effect of rhizobacterial isolates on shoot length

Among different rhizobacterial isolates from *C. polygonoides*, T-4 was found most effective on the elongation of shoot length (Table 1). This isolate showed a 37.5% increase in the shoot length over control, followed by isolate T-17 and T-19 (28.1%, each). In case of different rhizobacterial isolates of *L. sindicus* the highest shoot promoting efficiency was detected in isolate TS-10 (Table 2). The average shoot length recorded with this isolate was 3.9 cm which showed about 35% increase over control followed by isolate TS-12 and TS-18 (27.6% and 20.7% increase over control, respectively).

Effect of rhizobacterial isolates on root length

The effect of different rhizobacterial isolates on root length elongation was also studied and it was found that isolate T-4 showed maximum root length promoting effect in *C. polygonoides* seedlings (Table 1). A 36.2 % increase was observed with this isolate over control, followed by T-14 and T-17 (27.6 % and 22.4% increase over control), respectively. In case of *L. sindicus*, it was found that the isolate TS-1 had maximum root length promoting effect (Table 2). A 23.5% increase over control was noted with this isolate followed by TS-12 and TS-10 (17.6% each), respectively.

Effect of rhizobacterial isolates on biomass production

The effect of rhizobacterial isolates on the plant wet weight (overall biomass) in case of *C. polygonoides* was determined after completion of incubation period and it showed that isolate T-4 produced highest wet weight of 23.8 mg which was 67% more over control (Table 1). The next positive response was determined with isolate T-17 and T-14 repectively with an increase of 49.3% and 35.2% over control. The maximum plant wet weight of 22.1 mg was recorded with isolate TS-12 (Table 2) which was highest among all the tested rhizobacterial strains of *L. sindicus*. With this isolate, about 60% increase over control was recorded followed by isolate TS-10 and TS-18 (50% and 30%), respectively.

Overall, looking to all these parameters, the above result suggests that the isolate T-4 (*C. polygonoides*) is the highest cumulative response producing bacterial isolate in seed germination, root length elongation and shoot length elongation respectively (Figure 1 a, b and c) while isolate TS-12 of *L. sindicus* showed maximum plant growth promontory activities (Figure 2 a and b). Therefore, we predict that these isolates could be used for the development of bio-inoculum to improve plant productivity after some more studies.

Table 1 : Plant growth promontory activities of rhizobacterial isolates of *C. polygonoides*

Isolate No.	Shoot / Root	Average length of seedling (cm)	Increase or decrease over control %	Mean wet biomass (mg)	Germination (%)	Relative germination %
T-1	S	3.6	12.5	16.7	93.3	139.8
	R	6.2	6.9			
T-2	S	3.9	21.9	17.9	86.7	130
	R	6.2	6.9			
T-3	S	3	-6.3	8.7	80	119.9
	R	3.7	-36.2			
T-4	S	4.4	37.5	23.8	86.7	130
	R	7.9	36.2			
T-5	S	3.4	6.3	15.8	46.7	70
	R	6.2	6.9			
T-8	S	2.9	-9.4	11.3	73.3	109.9
	R	5.2	-10.3			
T-10	S	3.3	3.1	16.1	66.7	100
	R	6.5	12.1			
T-11	S	4	25	16.9	53.3	79.9
	R	5.7	-1.7			
T-12	S	3.1	-3.1	13.4	66.7	100
	R	3.9	-32.8			
T-13	S	3	-6.3	9.1	53.3	79.9
	R	4	-31			
T-14	S	3.5	9.4	19.2	53.3	79.9
	R	7.4	27.6			
T-15	S	3.9	21.9	18.8	66.7	100
	R	6.5	12.1			
T-17	S	4.1	28.1	21.2	80	119.9
	R	7.1	22.4			
T-19	S	4.1	28.1	15.4	80	119.9
	R	3.7	-36.2			
T-21	S	3.3	3.1	16.1	73.3	109.9
	R	6.5	12.1			
T-22	S	3.1	-3.1	14.6	66.7	100
	R	6.6	3.5			
T-23	S	3.8	18.8	16.8	86.7	130
	R	5.9	1.7			
Control	S	3.2	--	14.2	66.7	100
	R	5.8	--			

Table 2 : Plant growth promontory activities of rhizobacterial isolates of *L. sindicus*

Isolate No.	Shoot / Root	Average length of seedling (cm)	Increase or decrease over control %	Mean wet biomass (mg)	Germination (%)	Relative germination %
TS-1	S	3	3.5	17.2	73.3	122.2
	R	4.2	23.5			
TS-2	S	3.3	13.8	16.9	86.7	144.5
	R	3.8	11.7			
TS-4	S	3	3.5	14.1	60	100
	R	3.2	-5.9			
TS-5	S	3.1	6.9	15.2	40	66.7
	R	3.5	2.9			
TS-7	S	2.6	-10.3	11.7	60	100
	R	3.3	-2.9			
TS-8	S	2.8	-3.5	13.9	60	100
	R	3.6	5.9			
TS-9	S	3.3	13.8	16.9	66.7	111.2
	R	3.8	11.8			
TS-10	S	3.9	34.5	20.7	73.3	122.2
	R	3.9	14.7			
TS-11	S	2.8	-3.4	13.6	46.7	77.8
	R	3.5	2.9			
TS-12	S	3.7	27.6	22.1	80	133.3
	R	4	17.6			
TS-13	S	2.6	-10.3	14	53.3	88.8
	R	3.8	11.8			
TS-15	S	2.2	-24.1	10.1	46.7	77.8
	R	2.9	-14.7			
TS-16	S	2.8	-3.5	13.7	60	100
	R	3.5	2.9			
TS-17	S	3	3.5	15.8	66.7	111.2
	R	3.8	11.8			
TS-18	S	3.5	20.7	17.9	73.3	122.2
	R	3.8	11.8			
TS-20	S	3.2	10.3	16.6	66.7	111.2
	R	3.9	14.7			
Control	S	2.9	--	13.8	60	100

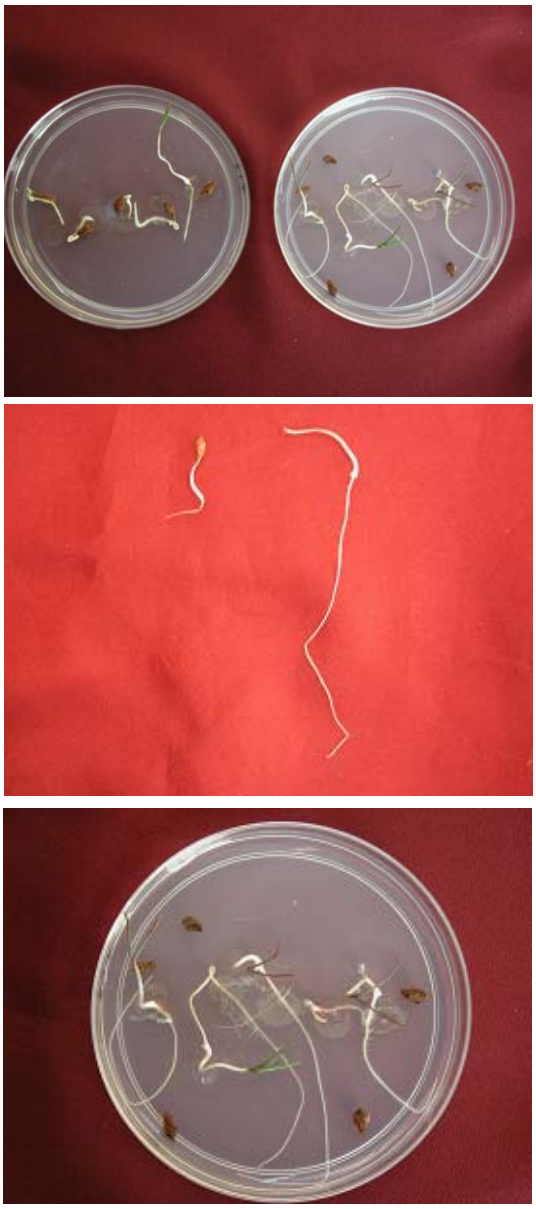

Fig. 1 : Plant growth promotory activities of T-4 isolate from *C. polygonoides*

Fig. 2 : Plant growth promotory activities of TS-12 isolate from *L. sindicus*

Discussion

The beneficial microbes are often used as inoculum for plant growth promotion (Bloemberg and Lugtenberg, 2001). Successful use of microbial inoculants in the field testing conditions requires effective colonization of the microorganisms with the root system of the plant, side by side, expression of a high level of competence of the introduced microbe for the desired biological property e. g. production of antibiotics or plant growth promoting hormones or biosynthesis of catabolic enzymes for degradation of xenobiotic compounds (Ramos *et al.*, 2000). Besides temperature, pH, soil type and plant genotypes the competence of indigenous microbes are among the prime factors that can affect the colonization process (Ikeda *et al.*, 1998; Jjemba and Alexander, 1999). Therefore, ideally the candidate organism should be screened on the plants rather than *in vitro* (Weller, 1988).

We have attempted an initial study on the plant growth promontory activities of rhizobacterial isolates in two different plant species. In this regard, the green house or field trials for screening the PGPR were not conducted rather an *in vitro* approach was used as it was easier as far as time, plant material and growth facilities were concerned (Berg *et al.,* 2001). A varied effect of rhizobacterial isolates on various plant productivity parameters was found in both the plant species. Out of the 17 rhizobacterial isolates of the *C. polygonoides*, T-4 was found to produce the highest biomass which was more than 67% over control. This isolate has also shown the best growth promoting parameters e.g. seed germination, root and shoot length elongation. During our investigation some of the isolates were observed with negative response towards one or other growth promontory activities. These effects might be due to some of the known detrimental interactions inhibiting shoot or root growth by the production of phytotoxins such as cyanide (Alstrom and Burns, 1989).

Inoculation of the PGPRs in forest tree nurseries has been proved to be crucial in enhancing survival of young seedlings when transplanted to the fields (Probanza *et al.*, 2001). Inoculated seedlings with a more developed root system achieve better nutrition and survival after transplanting (Ramos *et al.*, 2006). The strawberry, potato and oilseed rape rhizobacterial isolates have shown stem and root length enhancement under phytochamber conditions and their effectiveness have further evaluated under green house conditions (Berg *et al.*, 2001). In another study, maize rhizobacterial isolates were found to increase grain yield up to 50% (Tilak and Reddy, 2006). In green house conditions, *B. lichaniformis* had shown considerable colonization and competitive ability, which was observed to be used as biofertilizer or biocontrol agent in pepper and tomato (Lucas *et al.,* 2004).

Conclusion

This study was an initiative towards maintaining the ecological balance in the Thar Desert areas where the plant productivity is low because of various hindrances in the plant growth stages. Further development in testing the efficiencies of these isolates under field conditions could open the way for a prominent bio-inoculant development that could find application in maintaining the soil-plant biological environment in arid desert regions. Two of the isolates T-4 and TS-12, respectively, were observed with maximum plant growth promontory activities in this investigation.

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