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Plant growth promotion of radish by rhizosphere dwelling bacteria

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ABSTRACT

The present study was carried out with the aim to develop a plant growth-promoting rhizobacterial inoculant for improving the growth of radish. A total of 20 rhizobacteria were isolated and then tested for multifarious plant growth-promoting traits. The isolates exhibited tricalcium phosphate and zinc solubilization, production of siderophores, auxins, ammonia, and growth on different nitrogen-free media. Out of 20 isolates, two potential bacterial isolates, RM3 and RL3, identified as *Bacillus* and *Pseudomonas*, respectively, were selected based on PGP traits to evaluate their potential to promote the growth of radish under pot conditions. In greenhouse experiment, these isolates, when used as a consortium, showed a significant improvement in soil enzymatic activities (*viz*. dehydrogenase: 12.99 µg TPF formed h^{-1} g^{-1} of soil, alkaline phosphatase: 11.94 µg PNP formed h^{-1} g^{-1} of soil and urease: 401.65 µg urea hydrolysed formed h^{-1} g^{-1} of soil) and plant growth parameters (shoot length: 38.76 cm, leaf area: 267cm^2, fresh leaf weight:106.05 g, dry leaf weight:1.23 g, number of leaves per plant: 23, fresh root weight:111.69 g, dry root weight: 2.06 g, root length: 34.04 cm and root diameter: 3.33 cm) relative to control as well as other bioinoculants. This study concludes that using these two potential rhizospheric isolates as a consortium would improve the soil health and yield of radish.

Key words: *Raphanus raphanistrum* L.**,** Enzyme activities, Plant growth promotion, Rhizospheric bacteria

INTRODUCTION

Diverse plant growth promoting rhizobacterial (PGPR) communities may be found in the rhizospheric soil, which has favourable impacts on crop yield. These soil microbial consortium can be exploited as low-cost inputs for sustainable and environmentally friendly agricultural practices (Gosal and Kaur, 5). Because of the rise in food-borne illnesses in recent years, the importance and potential of PGPR have gained momentum to improve vegetable cultivation for healthy human nutrition in a sustainable and environmentally friendly way. *Raphanus sativus* L., commonly known as radish belongs to the family Brassicaceae and phylogenetically belongs to the Rapa/Oleacera lineage. These are low in calories and high in calcium, copper, magnesium, potassium, vitamin B6 and vitamin C. Radish is a short-duration crop, so its root growth should be rapid and uninterrupted, which needs optimum fertilization. Thus, the effect of biofertilizers on radish crop needs to be evaluated. Keeping all the aspects in view, the present study aimed to assess the potential of isolated rhizobacteria with multiple functional traits as biofertilizer on radish crop.

MATERIALS AND METHODS

Rhizospheric soil samples were collected from vegetable crops grown around Sangrur, Mansa, Ludhiana, Ahmedgarh Mandi, Jagraon and PAU Organic farming fields in Punjab to isolate the PGPR. Soil samples collected from random spots of the same field were pooled to form one representative sample. The rhizobacteria were isolated from the collected samples using serial dilution spread plating technique and purified using the streak plate method.

The isolated bacteria were tested for their biochemical, microscopic, and cultural characteristics as colony morphology and gram staining (Holt *et al*, 9). Isolated rhizospheric bacteria were tested for their ability to exhibit various functional activities. The PGP traits, which were tested included qualitative and quantitative estimation of phosphate solubilisation, growth on different nitrogen-free media, zinc solubilization, auxin production, Siderophore production, ammonia production and ammonia excretion (Kaur, 12).

Seeds of radish (*Raphanus raphanistrum* subsp. *sativus*) variety “safed mooli-2” were surface sterilized and primed with two selected bacterial isolates (alone and in combination). These were evaluated in comparison to untreated control as well as commercially available biofertilizer consortium biofertilizer (Consortium of *Azotobacter, Bacillus* and *Pseudomonas*) recommended by PAU. Radish was grown from October to December 2020 in pots containing 3kg of soil at the greenhouse of Punjab Agricultural University, Ludhiana. The two bacterial inoculants used in pot experiments were selected

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based on their multiple plant growth promoting abilities. The treatments were replicated thrice in RBD. The experiment had a total of 11 treatments with three replications (Table 1)

Soil and plant samples were collected from all treatments for analysis at different intervals (15 DAS, 45 DAS and 60 DAS). Soil samples were analysed for soil enzyme activities (dehydrogenase, alkaline phosphatase, and urease) by the method of (Kaur, 10). Different plant parameters such as plant height, number of leaves per plant, root length, diameter, leaf area index, and chlorophyll content were analysed (Harleen, 8). The data were statistically analysed using SPSS Software by ANOVA at 5% level of significance.

RESULTS AND DISCUSSION

A total of 20 rhizospheric bacteria with different morphological features were isolated. The pure isolates were maintained by streaking on nutrient agar slants and stored at 4°C. All the bacterial colonies were smooth in texture, yet the colour varied from creamish white to transparent. Some of the colonies were also of light yellow, pinkish, light brown and grey colour. Seventy-five per cent of the isolates were shiny in appearance while 25% of them (RS2, RM5, RL2, RO1 and RJ2) were dull. Eleven isolates were opaque and the rest of the nine (RS1, RS2, RS4, RM1, RM2, RL2, RL3, RO4, RA1) were translucent. Except for four isolates (RM3, RL1, RO1 and RA1) which have irregular margins, the rest of the 16 bacterial isolates have entire colony margins. Six isolates were found to be spherical in shape whereas, 16 were having rod appearance and 70% of isolates were found to gram-negative and non-motile.

Table 1. Treatments used for pot experiment

Treatments	Detail of the experiment
T1	Control
T2	100% RDF
T3	75% RDF
T4	100% RDF+RM3
T5	75% RDF+RM3
T6	100% RDF+RL3
T7	75% RDF+RL3
T8	100%RDF+CBF1
T9	75% RDF+ CBF1
T10	100% RDF+CBF2
T11	75% RDF+CBF2

*RDF- Recommended dose of fertilizer
*CBF1: Consortium of isolated rhizobacteria (RM3 and RL3)
*CBF2- Consortium biofertilizer recommended by PAU for wheat, onion, sugarcane, maize, turmeric and potato

All the isolates exhibited variable reactions for the biochemical tests, most of the isolates were found to be positive for catalase (55%), indole production test (65%), oxidase (70%), MR (65%) and urease (80%). However, in the case of VP, citrate utilization and TSIA test, the majority of the isolates (65% in VP and 60 % in citrate utilization and TSIA test) were found to be negative. Similarly, Shrivastava (15) identified the rhizobacterial isolates on the basis of morphological and biochemical characterization. Results of his study revealed that most of the isolates tested positive for catalase, citrate utilization and urease.

In the rhizosphere, one of the most common modes of action of plant growth promoting rhizobacteria is phosphorous solubilization. In our study, around 50% of isolates showed the solubilization of tricalcium phosphates on Pikovskaya's agar (Table 2). The phosphate solubilization index varied from 0.1 to 1.6. The solubilization index of isolate RL3 was found to be significantly higher (1.6) followed by the isolate RO3 (1.5) (Table 2). Likewise, in the study conducted by Sharma *et al*. (14), rhizobacterial isolates were screened for their phosphate-solubilizing ability. Six isolates showed the P-solubilization ability (4.5 to 10.3 mm) by the formation of a clearance zone around the colonies.

In liquid media, the phosphorous solubilization varied from 64 to 119 µg/ml (Table 2). The significantly higher P-solubilizing activity was shown by isolate RL3 (119 µg/ml) followed by isolate RJ1 (103 µg/ml), whereas the lowest phosphate solubilization ability was found in isolate RS1 (64 µg/ml). Phosphorous is an important nutrient for plant growth (Kaur and Gosal, 13) and various strains of *Agrobacterium, Achromobacter*, *Bacillus*, *Serratia, as well as Pseudomonas,* have been reported for solubilizing varying quantities of phosphates in soil by producing organic acids (citric acid, gluconic acid and oxalic acid) and phosphorus mineralizing enzymes such as Phytases and phosphatases (Goswami and Deka, 6).

Seven isolates were capable of producing solubilization zones on zinc oxide medium. Isolate RL3 (2.1 cm) showed the highest ZnO-solubilization zone formation. In the case of solubilization of $Zn_3(Po_4)_2$, 20% of the isolates were able to solubilize zinc phosphate. Out of all the isolated rhizobacteria, 10% of the isolates were found to have Zn-solubilizing activity in the range of 1.1-1.5 cm whereas 10% of isolates exhibited Zn-solubilizing activity in the range of 0.5-1 cm. Four isolates (RS3, RM3, RL3 and RA1) were able to solubilize $ZnCO_3$ and the highest

Table 2. Screening of rhizospheric isolates for plant growth promoting traits.

Isolate	Tricalcium phosphate solubilization		Zinc solubilization zone (cm)			Production of					Growth on nitrogen free medium		
	Solubilization index	Phosphate solubilization (µg ml^{-1})	ZnO	$Zn_3(PO_4)_2$	$ZnCO_3$	Auxins (µg ml^{-1})		Siderophore (mm)	Ammonia Production	Ammonia excretion (µg/ml)	Noris Glucose Medium	Burk's medium	Jensen's medium
						Without Tryptophan	With Tryptophan						
RS1	0.1^{g}	64^{f} ±0.007	-	-	-	6.04^{i}±0.71	21.9^{ij} ±0.29	-	+	1.12^{h} ±0.03	+	+	+
RS2	-	-	-	-	-	12.2^{c} ±0.02	33.9^{ef}±0.42	-	++	1.48^{gh} ±0.01	+	+	+
RS3	-	-	1.2^{de}	-	1.21^{b}	8.7^{e} ±0.36	32.0^{f} ±0.02	-	++	1.48^{gh} ±0.05	+	+	+
RS4	-	-	-	-	-	10.4^{d} ±0.34	33.1^{f} ±0.89	-	+++	1.96^{d} ±0.01	+	+	+
RM1	-	-	-	1.5^{a}	-	8.9^{e} ±0.92	45.7^{a}±0.29	-	+	2.54^{a} ±0.02	+	+	+
RM2	0.3^{f}	74^{e} ±0.028	-	-	-	7.7^{f} ±0.06	41.5^{b}±0.75	7.0^{b}	+	1.57^{g} ±0.002	+	+	+
RM3	1.1^{d}	90^{c} ±0.032	1.4^{b}	-	1.26^{c}	21.3^{a} ±0.26	46.9^{a}±0.41	8.5^{a}	+	1.96^{d} ±0.06	++	++	++
RM4	1.4^{bc}	99^{b} ±0.014	1.3^{c}	-	-	9.8^{d} ±0.15	37.4^{c} ±0.07	5.1^{d}	++	2.01^{d} ±0.25	+	+	+
RM5	-	-	-	1.03^{b}	-	8.8^{e} ±0.36	32.0^{f} ±0.54	-	+++	2.36^{b} ±0.10	++	++	++
RL1	-	-	1.2^{d}	-	-	12.9^{b} ±0.14	36.9^{c} ±0.69	3.8^{e}	++	2.24^{c} ±0.09	+	+	+
RL2	-	-	-	-	-	6.1^{g} ±0.18	32.2^{f} ±0.11	-	+++	1.08^{h} ±0.08	+	+	+
RL3	1.6^{a}	119^{a} ±0.024	2.1^{a}	1.00^{c}	1.15^{a}	8.3^{ef} ±0.05	28.9^{g} ±0.23	-	+	1.44^{g} ±0.019	+	+	+
RO1	0.6 ^{e}	81^{e} ±0.019	-	0.9^{d}	-	9.9^{d} ±0.37	20.7^{j}±0.44	-	+++	1.89^{de} ±0.057	++	++	++
RO2	0.3^{ef}	74^{e} ±0.013	-	-	-	6.2^{g} ±0.24	22.4^{i}±0.01	-	++	1.73^{f} ±0.066	+	+	+
RO3	1.5^{b}	100^{b} ±0.016	1.0^{e}	-	-	3.6^{hi} ±0.12	35.4^{d} ±0.53	2.5^{f}	+++	1.80^{ef} ±0.019	+	+	+
RO4	-	-	0.8^{f}	-	-	4.0^{h} ±0.17	26.3^{h} ±0.42	6.3^{c}	++	1.03^{h} ±0.029	+	+	+
RO5	-	-	-	-	-	12.0^{c} ±0.29	27.4^{h}±0.81	-	+++	1.90^{de} ±0.005	+	+	+
RA1	1.4^{c}	98^{b} ±0.001	-	-	0.6^{d}	3.9^{h} ±0.13	27.3^{h}±0.68	-	+++	2.33^{bc} ±0.084	++	++	++
RJ1	1.3^{e}	103^{b} ±0.024	-	-	-	3.1^{i} ±0.01	21.0^{ij} ±0.43	-	+	1.12^{h} ±0.003	+	+	+
RJ2	-	-	-	-	-	12.2^{c} ±0.40	33.9^{de}±0.27	-	+++	1.48^{gh} ±0.24	++	++	++

was shown by isolate RM3 (1.26 cm) followed by isolate RS3 (1.21 cm) (Table 2). The findings by Chiranjeevi *et al*. (3) showed the zinc phosphate solubilisation by rhizospheric isolates varied from 2.0 to 6.0 mm. Solubilization of Zinc phosphate by strains of *Pseudomonas fluorescens* has also been investigated. They reported that the zinc solubilization zone in zinc oxide and zinc carbonate amended media was 1.50 cm.

All 20 rhizobacterial isolates showed ammonia production as indicated by the appearance of yellow to brown colour. The ammonia excretion by the radish rhizosphere isolates was found to be in the range of 1.03- 2.54 µg/ml. The significantly higher ammonia excretion (2.54 µg/ml) was observed by RM1 and the isolates RM5 (2.36 µg/ml) and RA1 (2.33 µg/ml) were at par with each other in terms of ammonia excretion (Table 2). Likewise, in a study conducted on screening of rhizosphere-associated PGPR, ammonia excretion has been shown by all the isolated bacteria (Kaur and Gosal, 12). Similarly, Kaur (10), reported that 17 of the 20 isolates were observed to produce ammonia in Jensen's medium.

The isolated bacteria were tested for their ability to fix atmospheric nitrogen indirectly by testing their ability to grow on nitrogen-free media. All the isolates exhibited luxuriant growth on three nitrogen-free medium viz. Burk's medium, Norris Glucose medium and Jensen's agar medium within 48 h of incubation (Table 2). Many workers have also reported earlier the nitrogen-fixing capability of plant growth-promoting bacteria in view of their ability to grow on nitrogen-deficient media. In a study, 14 bacterial isolates out of 34 could grow on different nitrogen-free media (Dhole *et al*., 4).

In the medium without tryptophan supplementation, significantly higher production of IAA was reported in isolate RM3 (21.3µg/ml), followed by isolate RL1 (12.9 µg /ml), whereas, significantly lower IAA production was shown by isolate RS1 (3.2 µg/ml). In tryptophan supplemented medium, isolate RM3 (46.9 µg/ ml) was recorded with significantly higher IAA production and significantly lower IAA production was observed in isolate RO1 (20.7 µg /ml) (Table 2). Ahmad *et al.* (1) reported that the *Pseudomonas* isolates produced IAA ranging from 5.34 to 22.4 g/ml in the absence of tryptophan in the medium.

Out of the 20 rhizobacterial isolates, six isolates indicated siderophore production in the form of the halo zones on the media with sizes ranging from 2.5 to 8.5 mm. The diameter of the zone indicated a significantly higher siderophore production in isolate RM3 (8.5 mm) followed by isolate RM2 (7.0 mm) (Table 2). The results are in accordance with a previous study where 5 out of 21 isolated bacteria from cereals were reported to produce a siderophore with a maximum halo zone formation of 2.3 cm (Kaur and Gosal, 11). In soil, iron is normally present as ferric oxyhydroxides and hydroxides which is unavailable to the plants. The low molecular weight siderophores produced by the rhizobacteria are capable of chelating available iron in the rhizospheric zone and making it available for plant growth promotion (Goyal *et al*., 7).

Based on plant growth-promoting activities, two bacterial isolates with different morphologies and multiple plant growth-promoting traits were selected for further studies under glass house conditions. These bacteria were tentatively identified as *Bacillus* (RM3) and *Pseudomonas* (RL3) based on biochemical characterization.

The activity of soil dehydrogenase (12.99 µg TPF formed /h/g of soil at 15 DAS, 11.98 µg TPF formed /h/g of soil at 45 DAS and 11.45 µg TPF formed /h/g of soil at 60 DAS), alkaline phosphatase (11.94 µg PNP formed /h/g of soil at 15 DAS, 11.61 µg PNP formed /h/g of soil at 45 DAS and 11.04 µg PNP formed /h/g of soil at 60 DAS) and urease enzyme (356.11 µg urea hydrolyzed /h/g of soil at 15 DAS, 392.31 µg urea hydrolyzed /h/g of soil at 45 DAS and 401.65 µg urea hydrolyzed /h/g of soil at 60 DAS) was observed to be significantly higher in treatment T8 (100% RDF+CBF1) relative to other treatments as well as zero day values [dehydrogenase (7.05 µg TPF formed /h/g of soil), alkaline phosphatase (8.17 µg PNP formed /h/g of soil) and urease (200.11 µg urea hydrolyzed /h/g of soil)] (Fig 1). A significant difference was observed in growth parameters of radish with the application of bacterial inoculants relative to uninoculated control (Table 3). Treatment T8 (100% RDF+ CBF1), significantly improved the overall plant growth parameters (shoot length: 38.76 cm, leaf area: 267cm^2, fresh leaf weight:106.05 g, dry leaf weight:1.23 g, number of leaves per plant: 23, fresh root weight:111.69 g, dry root weight: 2.06 g, root length: 34.04 cm and root diameter: 3.33 cm) over the control (shoot length: 24.44 cm, leaf area: 155 cm^2, fresh leaf weight: 101.71 g, dry leaf weight:0.59, number of leaves per plant:10, fresh root weight: 96.01g dry root weight: 1.7 g, root length: 26.50 cm, and root diameter: 2.75 cm) (Table 2). In rhizospheric soils, the PGPRs produce several vitamins and growth promoting hormones which impart positive effects on the physical and chemical composition and overall growth of the plants by overcoming nutrient deficiency. Thus, the inoculation of these potential rhizobacteria as a consortium in the soil resulted in improved soil enzyme activities as well as yield parameters (Bhat *et al.,* 2).

Table 3. Effect of potential bacterial strains on the growth of radish at harvest in pots under green house conditions.

Treatments	Plant height (cm)	Leaf Area (cm^2)	Fresh weight of leaves (g)	Dry weight of leaves (g)	Number of leaves	Fresh Root Weight (g)	Dry root Weight (g)	Root Length (cm)	Root Diameter (cm)
T1 (control)	24.44^{k}±0.854	155^{k}±4.14	40.02^{k}±0.555	0.59^{k}±0.021	10^{k}±0.127	96.01^{k}±3.66	1.7^{k}±0.051	26.50^{k}±0.893	2.75^{k}±0.102
T2 (100% RDF)	34.09^{h}±1.04	165^{h}±6.69	43.68^{i}±1.61	0.61^{i}±0.012	11^{i}±0.416	100.43^{i}±3.44	1.86^{i}±0.035	25.59^{j}±0.853	3.25^{i}±0.082
T3 (75% RDF)	29.00^{i}±0.522	171^{i}±2.00	69.59^{f}±2.88	0.82^{h}±0.023	13^{h}±0.328	98.23^{g}±3.89	1.75^{j}±0.017	24.05^{i}±0.975	2.79^{j}±0.085
T4 (100% RDF+RM3)	37.69^{g}±0.645	215^{f}±5.42	98.38^{g}±2.03	1.20^{b}±0.047	20^{c}±0.558	106.46^{c}±0.86	1.91^{b}±0.071	31.89^{d}±1.034	3.26^{f}±0.121
T5 (75% RDF+RM3)	34.19^{i}±0.739	185^{e}±2.50	81.76^{i}±1.62	1.02^{d}±0.029	16^{i}±0.360	99.76^{j}±3.86	1.67^{e}±0.037	28.05^{f}±0.025	3.12^{h}±0.036
T6 (100% RDF+RL3)	37.08^{e}±0.869	204^{j}±1.65	97.16^{c}±0.087	1.16^{c}±0.039	18^{e}±0.470	103.72^{f}±2.15	1.88^{f}±0.003	30.2^{g}±1.33	3.24^{d}±0.116
T7(75% RDF+RL3)	31.14^{d}±1.09	176^{d}±6.02	75.92^{d}±1.30	1.01^{i}±0.009	15^{f}±0.175	92.01^{h}±1.74	1.62^{g}±0.056	27.93^{c}±0.478	3.10^{g}±0.069
T8 (100% RDF+CBF1)	38.76^{a}±1.11	267^{a}±2.64	106.05^{a}±3.72	1.23^{a}±0.029	23^{a}±0.165	111.69^{a}±3.92	2.06^{a}±0.011	34.04^{a}±0.675	3.33^{a}±0.075
T9 (75% RDF+ CBF1)	37.3^{j}±1.51	194^{c}±2.27	94.08^{h}±3.13	1.19^{e}±0.016	19^{d}±0.325	101.31^{e}±4.29	1.79^{c}±0.033	29.52^{h}±0.372	3.20^{e}±0.126
T10 (100% RDF+CBF2)	38.01^{c}±1.40	241^{b}±7.16	101.71^{b}±2.47	1.21^{g}±0.031	22^{b}±0.555	107.50^{b}±3.58	1.94^{d}±0.059	32.21^{b}±0.116	3.27^{c}±0.082
T11 (75% RDF+CBF2)	36.23^{b}±0.032	189^{g}±7.83	92.45^{e}±1.49	1.07^{f}±0.042	17^{g}±0.459	100.5^{d}±1.08	1.73^{h}±0.027	29.48^{e}±1.27	3.19^{b}±0.077

*All values represent mean of three replicates± standard deviation
**Superscripts- Denote ranking in descending order, based on Tukey-b test, means with same letter are at non-significant difference to each othe

Fig. 1. Rhizospheric soil (A) dehydrogenase (B) alkaline phosphatase (C) urease enzyme activities of radish at different time intervals in response to application of potential bacterial strains

AUTHORS' CONTRIBUTION

Conceptualization of research (JK); Designing of the experiments (JK); Contribution of experimental materials (JK, SSW); Execution of field/lab experiments and data collection (H, SSW); Analysis of data and interpretation (H, RK); Preparation of the manuscript (RK, B).

DECLARATION

The authors declare no conflict of interest.

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