

## COLONIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON TWO DIFFERENT ROOT SYSTEMS

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### Abstract

Phytohormones producing bacteria enhance the plants growth by positively affecting growth of the root. Plant growth promoting bacteria (PGPR) must colonize the plant roots to contribute to the plant's endogenous pool of phytohormones. Colonization of these plant growth promoting rhizobacteria isolated from rhizosphere and soil of different crops was evaluated on different root types to establish if the mechanism of host specificity exist. The bacteria were isolated from maize, wheat, rice, canola and cotton and phytohormone production was detected and quantified by HPLC. Bacteria were inoculated on surface sterilized seeds of different crops and seeds were germinated. After 7 days the bacteria were re-isolated from the roots and the effect of these bacteria was observed by measuring increase in root length. Bacteria isolated from one plant family (monocots) having fibrous root performed well on similar root system and failed to give significant results on other roots (tap root) of dicots. Some aggressive strains were able to colonize both root systems. The plant growth promoting activities of the bacteria were optimum on the same plant from whom roots they were isolated. The results suggest that bacteria adapt to the root they naturally inhabit and colonize the same plant root systems preferably. Although the observe trend indicate host specificity but some bacteria were aggressive colonizers which grew on all the plants used in experiment.

**Keywords:** PGPR, Phytohormones, Host specificity, HPLC

### Introduction

The highest concentrations of microbes are present in the immediate surroundings of the plant roots, forming associations ranging from symbiotic to pathogenic nature (Lynch *et al.*, 1990). The roots are constantly sloughing off and releasing exudates in their surroundings, where complex biological and ecological interactions occur between the plants and the microbes (Bais *et al.*, 2006). The niche around the roots where these interactions occur is called as rhizosphere(Lynch *et al.*, 1990; Bais *et al.*, 2006). Rhizosphere includes the rhizoplane (the root surface) and the soil in the surroundings of the root, mostly ranging few millimeters from the root surface (Lynch *et al.*, 1990).

Among microbes inhabiting rhizosphere, those able to impart beneficial effects to plants are called as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1980; Glick, 1995). PGPR enhance the growth of plant in either a direct or an indirect manner (Mia *et al.*, 2010; Zahir *et al.*, 2012). Among many other mechanisms, one growth promoting mechanism is to contribute to the endogenous pool of hormones present in plant, such as indole-3-acetic acid (Patten & Glick, 2002). The production of indole-3-acetic acid (IAA) (Brown, 1972; Tien *et al.*, 1979; Patten & Glick, 2002), gibberellins (Brown, 1972) and cytokinins(Hussain *et al.*, 2010) by root associated bacteria is well documented. Auxins, cytokinins and gibberellins have been discussed in detail by researchers (Frankenberger & Arshad, 1995) which, when applied to plants, help in increasing plant yield and growth. PGPR producing these phytohormones positively affect the growth of plants by increasing the growth rate and increasing the yield (Brown, 1972; Tien *et al.*, 1979;

Patten & Glick, 2002). These phytohormones promote root and shoot growth and also induce lateral roots. Elongation of primary root or the induced lateral roots help the seedlings in anchoring them in soil and also increase nutrient uptake due to increased surface area (Patten & Glick, 2002).

Induction of plant growth promoting effect require successful colonization of the rhizoplane(Patten & Glick, 2002). The existence of plant-microbe specificity is reported (Dazzo & Hubbell, 1975; Anollés & Favelukes, 1986; Chanway *et al.*, 1991). Host variation for a given beneficial microbe asserts that the host plant genes play important role in the output of these interactions (Smith & Goodman, 1999). In the present study the host specificity in plant-microbe interaction was studied on different root systems. Bacteria were isolated from rhizosphere of different crops and then they were experimentally colonized on roots of different crops to observe the microbe-plant interaction and colonization of the bacteria.

### Materails and Methods

**Isolation and Identification of bacteria:** Roots and rhizospheric soil of 5 different crops from Auriga Research Farms in the vicinity of Lahore city, Punjab, Pakistan were taken at different intervals of 2013-2014. 10 samples from each crop of wheat (*Triticumaestivum*), maize (*Zea mays*),rice (*Oryza sativa*),cotton(*Gossypiumhirsutum*)andcanola(*Brassica napusL.*) were taken. The extracts of the roots and soil were serially diluted and cultured on LauriaBertani (LB) agar (Difco<sup>®</sup>) plates with spread plate method. The strains were isolated and named in a series with first letter indicating the source of isolation as C, K, M, R and W

represents canola, cotton, maize, rice and wheat. Biochemical profile of the isolated strains was determined with conventional biochemical testing. Final identification of isolates was made with the help of Remel RapID identification kits (Oxoid<sup>®</sup>) and ERIC Electronic RapID software.

**Growth conditions for phytohormone detection:** All the isolated strains were tested for two phytohormones production, gibberellic acid GA3 and indole-3-acetic acid. The purified bacteria were first grown in DF salt minimal (Dworkin & Foster, 1958) medium at 32°C for 24 hours. After 24 hours growth the absorbance of culture was taken at 600 nm and the inoculum was diluted with 0.9% sodium chloride (normal saline) until it reached McFarland-1 standard (approximately  $3 \times 10^8$  CFU/ml). 1ml of these standardized inocula were added in 100 ml LB broth (Difco<sup>®</sup>) supplemented with 2 mg/ml L-tryptophan (filter sterilized) and without L-tryptophan. The media flasks were incubated in orbital shaking incubator at 32°C with 130 rpm shaking for 48-72 hours.

**Qualitative detection of IAA:** After 72 hours incubation on previously described conditions the growth was centrifuged at  $5500 \times g$  for 10 minutes. The calorimetric detection of the indole-3-acetic acid was made by mixing 1 ml culture supernatant and 2 ml Salkowski's reagent (1.0 ml 0.5 M FeCl<sub>3</sub> in 50 ml 35% HClO<sub>4</sub>) and strength of red color was observed after 25 min with naked eye (Gordon & Weber, 1951).

**Quantification of Phytohormones with HPLC:** For High performance liquid chromatography (HPLC) analysis, the sample was prepared by carefully separating the supernatant after centrifugation at  $5500 \times g$  for 10 minutes. Then the supernatant was diluted in HPLC mobile phase whose composition is described later. It was then filtered with 0.2 µm nylon filter (Millipore<sup>®</sup>) for detection and quantification of phytohormones.

Filtered supernatants were analyzed with Hitachi LaChrom Elite system<sup>®</sup> equipped with L-2550 column oven, L-2130 pump and L-2455 diode array detector. The column used was Discovery (Supelco<sup>®</sup>) C<sub>18</sub>, 25 cm x 4.6 mm ID with 5 µm particle size. The Method used to quantify the phytohormones with HPLC was that of Kelen *et al.*, with slight modifications (Kelen *et al.*, 2004). The composition of mobile phase was acetonitrile : water (35 : 65; v/v) with 30 mM phosphoric acid at pH 4. The column temperature was  $25^\circ\text{C} \pm 0.1^\circ\text{C}$  with isocratic elution at flow rate of 1 ml/minute. For each sample analysis 10 µl injection was used and elutes were detected at 210 nm by diode array detector. Standards used were of gibberellic acid (GA3) (Sigma<sup>®</sup>) and indole-3-acetic acid (IAA) (Acros Organics<sup>®</sup>) made in HPLC mobile phase.

**Colonization and root elongating assay:** To observe if the bacterial strains can successfully colonize the roots of the different crops, the seeds were coated with bacteria and allowed to germinate. Strains producing indole-3-acetic acid in excess of 10 µg/ml in the laboratory media were selected (with exception of CTIB strain) to observe the colonization and effect of phytohormones. Seeds of 5

crops, wheat (*Triticumaestivum*cv. Sehar 2006), maize (*Zea mays*), rice (*Oryza sativa* cv. Sadaf 777), cotton (*Gossypiumhirsutum*) and canola (*Brassica napus*Lcv. Omega 2) were provided by Auriga Seed Corporation. The seeds were surface sterilized by soaking first in 70% (v/v) ethyl alcohol for one minute and later in 1% sodium hypochlorite for 10 minutes. Then sterile water was used to wash seeds 3-5 times. Now the seeds were incubated in bacterial solution of the isolated strains standardized at one McFarland-1 standard ( $3 \times 10^8$  CFU/ml) with the method described earlier. The seeds were incubated in the bacterial culture for 1 hour. Controls were incubated in 0.1 M MgSO<sub>4</sub> for same amount of time. These seeds were placed in sterile sand pots in growth chamber. The sand was sterilized twice in autoclave with 15 psi pressure for 20 minutes. The pots used had capacity of 1 Kg sand with a diameter of 7 cm and height was 11.5 cm. The sand was poured (900 gram per pot) in the pots and then soaked with 10 ml sterile quarter strength hoagland solution aseptically in laminar flow cabinet. 3 seeds per pot were implanted in the sand aseptically and three replicates for each bacteria and root combination were made. The pots were placed in growth chambers at 20°C for canola and wheat, whereas for maize, rice and cotton it was 32°C. Pots were incubated in darkness for first day and then given cycles of 12 hour light followed by 12 hours of darkness. The pots were daily watered with 10 ml quarter strength sterile hoagland solution. The seeds which were unable to germinate after two days were marked and later on discarded.

The colonization of bacteria was tested by observing successful re-isolation of bacteria and phytohormone effect (root lengthening) after 7 days. 3 replicates for root elongation assay were performed and root showing highest activity (longest root) in a pot was selected to measure root length. For re-isolation of bacteria from root, roots were cut with sterile surgical blade into one cm parts and shaken in 0.9% sodium chloride at 150 rpm in orbital shaker for one hour (normal saline) for performing bacterial count with spread plate count method using LB agar (Difco<sup>®</sup>). The tip of the roots was used for re-isolating the bacteria. The bacterial strains were confirmed by observing colony morphology and gram staining. Biochemical tests were performed where necessary.

## Results and Discussion

**Isolation and identification of bacteria:** In total 102 strains of bacteria were isolated. After screening of the isolates, 22 strains were confirmed as phytohormone producer. The strains were identified as *Pseudomonas* spp. (8 isolates), *Serratia*spp. (9 isolates), *Azotobacter*spp. (2 isolates), *Bacillus* spp. (2 isolates) and one *Klebsiella pneumonia*. These bacterial species have been reported to produce phytohormones by other researchers (Srinivasan *et al.*, 1996; Patten & Glick, 2002; Ahmad *et al.*, 2005; Koo & Cho, 2009; Sachdev *et al.*, 2009). *Azotobacter* and *Bacillus* were identified on the basis of biochemical tests as described by Bergy's Manual of Determinative

Bacteriology as the available identification kits did not cover these organisms.

**Quantification of phytohormones:** IAA and GA3 were detected and quantified by calculating the peak area and comparing with standards (Fig. 1). The rate of IAA production of the strains was variable, as low as 0 µg/ml to as high as 74.02 µg/ml in LB medium (Difco) without

tryptophan. The IAA production was significantly increased in the LB medium supplemented with tryptophan as evident from the results. Gibberellic acid GA3 was detected in only three isolates which are *Pseudomonas stutzeri* strain CT1B, *Pseudomonas aeruginosa* strain MD3B and *Serratia liquefaciens* strain KD5B. Detailed data of phytohormone production is summarized in Table 1.

**Table 1. Production of GA3 and IAA with and without supplemented 2 mg/ml tryptophan in LB broth, after 72 hours of growth of isolated bacteria.**

Bacterium	Strain	IAA (µg/ml)		GA3 (µg/ml)
		Without Tryptophan	With 2 mg/ml Tryptophan	
<i>Pseudomonas stutzeri</i>	CT1B	8.12 ± 0.02*	12.50 ± 0.10	25.31 ± 0.31
<i>Pseudomonas fluorescens</i>	WT1C	4.45 ± 0.06	6.84 ± 0.04	
<i>Pseudomonas fluorescens</i>	WT3A	56.31 ± 0.14	125.10 ± 0.53	
<i>Pseudomonas fluorescens</i>	WT3B	8.74 ± 0.03	12.78 ± 0.06	
<i>Serratia liquefaciens</i>	WT3C	0.00 ± 0.0	16.21 ± 0.14	
<i>Serratiamarcescens</i>	WT4A	18.52 ± 0.04	50.60 ± 0.19	
<i>Serratia liquefaciens</i>	WT5A	7.54 ± 0.02	25.30 ± 0.25	
<i>Azotobacterspp.</i>	WD1B	1.3 ± 0.01	4.59 ± 0.08	
<i>Serratia liquefaciens</i>	WD2B	0.00 ± 0.0	26.83 ± 0.23	
<i>Bacillus</i> spp.	WD3A	0.00 ± 0.0	12.52 ± 0.26	
<i>Serratia liquefaciens</i>	WD3B	68.72 ± 0.16	147.06 ± 0.47	
<i>Azotobacterspp.</i>	WD3C	4.62 ± 0.05	16.52 ± 0.07	
<i>Pseudomonas putida</i>	ST1B	56.29 ± 0.21	70.52 ± 0.36	
<i>Pseudomonas fluorescens</i>	RD1A	47.93 ± 0.27	120.53 ± 0.27	
<i>Serratia liquefaciens</i>	RD1B	63.48 ± 0.31	88.69 ± 0.52	
<i>Serratia liquefaciens</i>	MT2B	39.97 ± 0.07	53.37 ± 0.18	
<i>Bacillus</i> spp.	MT2C	61.74 ± 0.37	83.24 ± 0.35	
<i>Pseudomonas aeruginosa</i>	MD3B	40.76 ± 0.26	72.49 ± 0.38	16.58 ± 0.26
<i>Serratiamarcescens</i>	MD3C	61.60 ± 0.15	83.91 ± 0.13	
<i>Klebsiella pneumonia</i>	KT2C	74.02 ± 0.17	139.24 ± 0.57	
<i>Serratia liquefaciens</i>	KD5B	65.97 ± 0.41	93.50 ± 0.37	34.05 ± 0.04
<i>Pseudomonas putida</i>	KD5D	69.55 ± 0.25	90.61 ± 0.16	

\*Mean with ± standard error for 3 replicates

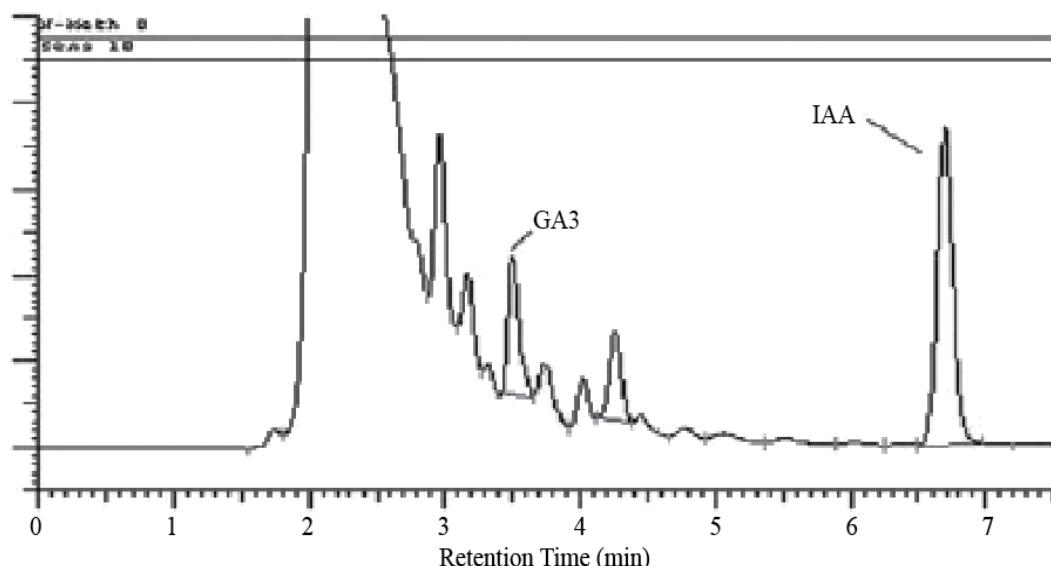


Fig. 1. HPLC chromatogram of *Serratia liquefaciens* strain KD5B showing GA3 at 3.5 RT and IAA at 6.7 RT.

**Analysis of root elongation and colonization:** The phytohormones produced by *Pseudomonas aeruginosa* strain MD3B significantly enhanced the growth of the roots on the maize. This strain was isolated from the maize, a monocotyledon plant having tap root. It enhanced the root growth of the maize by 112% than the control and lateral roots were also induced. This particular strain stimulated root growth on all the inoculated seeds, but the effect was more vibrant on the roots of maize and wheat. In wheat it increased the root length by 110% as compared to the control. The most pronounced effect (growth promotion) was observed on maize and the effect on the fibrous root plants having monocotyledon seed (maize, wheat and rice) was significantly higher than that on the plants having tap root (canola and cotton).

As there was no medium provided to the bacteria in pots, so they would only grow if they can colonize the root and live on the exudates released by the root. MD3B colonized the roots of all 5 plants but the recovery from the maize, wheat and rice (all members of *Poaceae* family) was more pronounced and the highest count was recovered from the roots of maize (Fig. 3). The *Poaceae* family has fibrous root system and the activity of this isolate was highest on the members of this family sharing same root system. Bacterium

colonized the roots of canola and cotton (both having tap root) but the recovery was low as compared to others plant (monocots) and the growth promoting effect was insignificant. Keeping in view that this strain was isolated from the rhizosphere of same family member that is maize, it is suggestive that it had adapted to the root system it inhabited as observed by other researchers that microbes adapt themselves to best suit the niche they live in (Ryan *et al.*, 2009). The exudates from the roots effect the colonization and in turn effect the ability of the bacteria to produce the growth promoting effects (Bais *et al.*, 2006). The rate of recovery for this strain (MD3B) was highest from maize that is  $10^6$  CFU/cm of root and in wheat and rice it was  $10^5$  CFU/cm of root, but it was low in cotton and canola (Fig. 3). As the members of *Poaceae* family share same root system and exudates, the effect of exudates in colonization was evident. Both canola and cotton share the tap root system but this colonization of these two plant roots was low. It suggests that this bacterium is aggressive in colonization so that it colonized both root systems but it is more adapted to the fibrous root system. This trend can be observed in Fig. 2, where the log values of CFU of bacteria recovered from the roots of different plants are plotted.

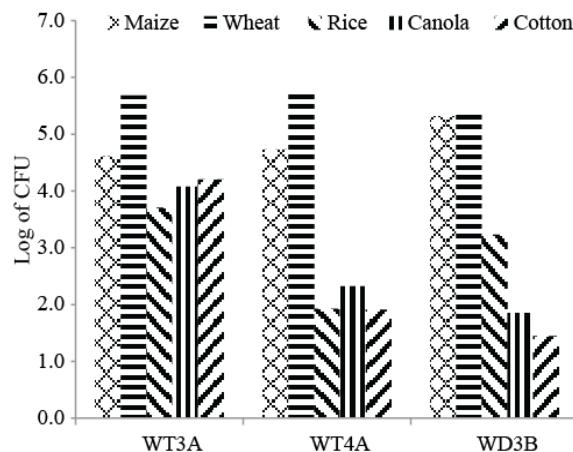


Fig. 2. Colonization of 3 wheat isolates WT3A, WT4A and WD3B on maize, wheat, rice, canola and cotton roots.

The growth promotion and colonization data from the roots of maize indicate that inoculation of bacterial strains isolated from the fibrous root system resulted in growth promotion of maize whereas isolates of canola and cotton did not produce any substantial growth. The recovery of tap root isolates was low; indicating that they didn't colonized the roots of the maize. This host variation response to the PGPRs signifies the role of plant genes in supporting the microbes colonization on the root (Smith & Goodman, 1999).

Wheat and rice displayed same trend in the root growth promotion by the inoculated PGPR (Table 2). The length of the root was increased by the strains isolated from the fibrous roots of the monocotyledon plants. The wheat root colonization of the strains isolated from wheat roots was quite high and second to only *Pseudomonas*

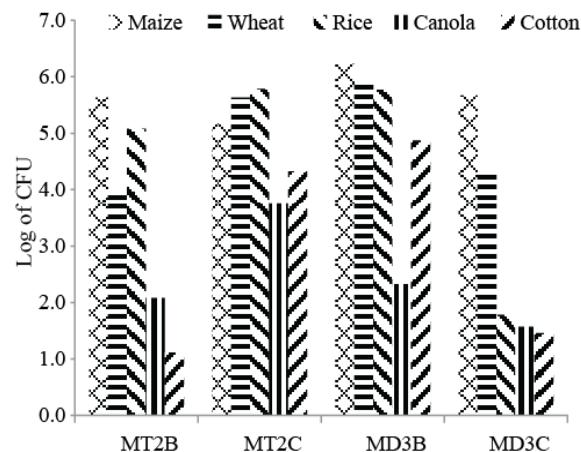


Fig. 3. Colonization of 4 maize isolates MT2B, MT2C, MD3B and MD3C on maize, wheat, rice, canola and cotton roots.

*aeruginosa* strain MD3B. Some strains isolated from the tap roots of the cotton and canola also colonized and increased the root length of wheat and rice (Figs. 4 & 5).

In canola and cotton only the strain isolated from the tap roots (canola and cotton) grew well and produced plant growth promoting effect (Figs. 4 & 5). Only selective strains isolated from fibrous root belonging to *Pseudomonas* genus increased the root length. One special case was that of *Bacillus* strain MT2C isolated from the maize, where the colonization on the cotton roots was moderate but root growth was not enhanced as expected. One possible explanation might be that the strain has the metabolic machinery to grow on the root but it does not possess the required enzymes to convert the available precursor to synthesize IAA, as IAA is synthesized by a number of pathways (Spaepen *et al.*, 2007).

**Table 2.** Length of roots of different plants treated with bacterial inoculation observed after 7 days of seed germination.

Bacterial Treatment	Root length (mm)				
	Maize	Wheat	Rice	Canola	Cotton
Control (0.1% MgSO <sub>4</sub> )	136.4 ± 1.3*	69.3 ± 1.5	62.1 ± 2.1	31.4 ± 1.8	84.4 ± 2.3
CT1B	74.8 ± 4.7	71.5 ± 3.1	104.9 ± 3.7	45.3 ± 2.7	93.2 ± 1.7
WT3A	196.4 ± 3.7	101.2 ± 1.9	74.2 ± 1.8	49.4 ± 2.1	107.1 ± 5.3
WT4A	151.7 ± 2.9	86.9 ± 3.4	63.6 ± 2.4	29.1 ± 1.3	85.7 ± 2.3
WD3B	264.3 ± 3.8	114.3 ± 2.7	69.1 ± 4.6	34.7 ± 1.9	78.5 ± 4.1
ST1B	189.2 ± 2.3	95.2 ± 3.6	75.7 ± 3.3	39.4 ± 3.2	98.9 ± 2.4
SD1A	231.6 ± 3.6	98.2 ± 4.3	103.3 ± 1.8	39.9 ± 1.3	91.3 ± 2.9
MT2B	213.4 ± 4.1	85.8 ± 3.2	93.7 ± 3.7	33.1 ± 2.4	75.1 ± 1.5
MT2C	183.8 ± 2.0	115.4 ± 1.5	125.3 ± 2.5	41.6 ± 1.6	87.8 ± 3.1
MD3B	290.1 ± 1.4	145.7 ± 1.7	110.4 ± 2.8	44.5 ± 4.1	113.2 ± 3.6
MD3C	154.2 ± 1.9	74.2 ± 3.9	59.5 ± 1.4	33.7 ± 3.2	88.5 ± 1.3
KT2C	143.4 ± 2.6	81.6 ± 3.7	66.8 ± 3.1	56.2 ± 1.7	157.1 ± 3.4
KD5B	139.1 ± 3.4	70.9 ± 2.5	72.7 ± 3.5	43.6 ± 3.4	125.4 ± 2.2
KD5D	127.6 ± 3.3	125.3 ± 1.3	68.0 ± 1.4	52.4 ± 2.8	142.4 ± 2.9

\*Mean with ± standard error for 3 replicates

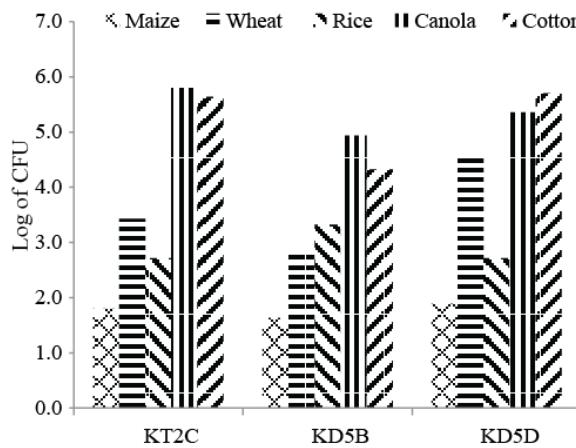


Fig. 4. Colonization of 3 cotton isolates KT2C, KD5B and KD5D on maize, wheat, rice, canola and cotton roots.

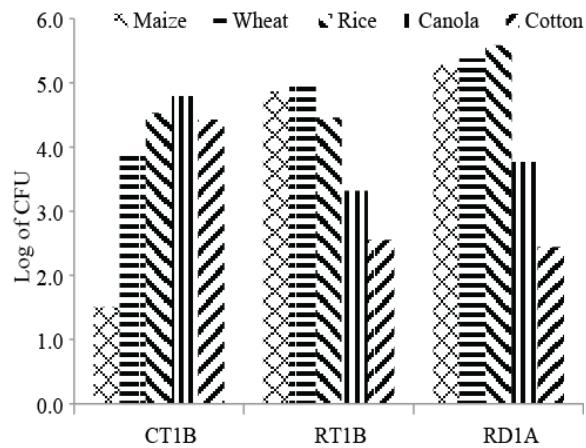


Fig. 5. Colonization of 2 rice isolates RT1B, RD1A and one canola isolate CT1b on maize, wheat, rice, canola and cotton roots.

**Table 3.** The population of inoculated bacteria ( $3 \times 10^8$  CFU/ml) recovered from the roots after 7 days.

Bacterial Treatment	Recovered population from roots (CFU/cm of root)				
	Maize	Wheat	Rice	Canola	Cotton
Control (0.1% MgSO <sub>4</sub> )	0 ± 0.00*	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
CT1B	$3.2 \times 10^1 \pm 0.71$	$7.3 \times 10^3 \pm 0.41$	$3.4 \times 10^4 \pm 0.47$	$6.2 \times 10^4 \pm 0.29$	$2.7 \times 10^4 \pm 0.25$
WT3A	$4.1 \times 10^4 \pm 0.36$	$4.8 \times 10^5 \pm 0.09$	$5.1 \times 10^3 \pm 0.36$	$1.3 \times 10^4 \pm 0.24$	$1.6 \times 10^4 \pm 0.39$
WT4A	$5.4 \times 10^4 \pm 0.25$	$5.3 \times 10^5 \pm 0.47$	$8.5 \times 10^1 \pm 2.51$	$2.1 \times 10^2 \pm 0.32$	$8.2 \times 10^1 \pm 3.71$
WD3B	$2.1 \times 10^5 \pm 0.21$	$2.7 \times 10^5 \pm 0.26$	$1.7 \times 10^3 \pm 0.62$	$7.1 \times 10^1 \pm 0.02$	$3.8 \times 10^1 \pm 0.52$
RT1B	$7.4 \times 10^4 \pm 0.27$	$9.3 \times 10^4 \pm 0.35$	$2.9 \times 10^4 \pm 0.24$	$2.1 \times 10^3 \pm 0.37$	$3.6 \times 10^2 \pm 0.36$
RD1A	$1.9 \times 10^5 \pm 0.21$	$2.4 \times 10^5 \pm 1.17$	$3.9 \times 10^5 \pm 0.41$	$5.9 \times 10^3 \pm 0.11$	$2.8 \times 10^2 \pm 0.25$
MT2B	$4.3 \times 10^5 \pm 0.19$	$8.3 \times 10^3 \pm 0.44$	$1.2 \times 10^5 \pm 0.28$	$1.2 \times 10^2 \pm 0.48$	$1.3 \times 10^1 \pm 4.21$
MT2C	$1.5 \times 10^5 \pm 0.39$	$4.3 \times 10^5 \pm 0.04$	$6.1 \times 10^5 \pm 0.62$	$5.7 \times 10^3 \pm 0.42$	$2.1 \times 10^4 \pm 0.62$
MD3B	$1.7 \times 10^6 \pm 0.04$	$7.3 \times 10^5 \pm 0.31$	$5.9 \times 10^5 \pm 0.16$	$2.1 \times 10^2 \pm 0.53$	$7.4 \times 10^4 \pm 5.39$
MD3C	$4.7 \times 10^5 \pm 0.25$	$2.1 \times 10^4 \pm 0.58$	$6.1 \times 10^1 \pm 0.03$	$3.7 \times 10^1 \pm 0.22$	$2.9 \times 10^1 \pm 0.71$
KT2C	$6.3 \times 10^1 \pm 1.04$	$2.7 \times 10^3 \pm 0.28$	$5.2 \times 10^2 \pm 0.47$	$6.3 \times 10^5 \pm 0.07$	$4.4 \times 10^5 \pm 0.36$
KD5B	$4.3 \times 10^1 \pm 0.17$	$6.9 \times 10^2 \pm 0.06$	$2.1 \times 10^3 \pm 0.03$	$8.7 \times 10^4 \pm 0.46$	$2.1 \times 10^4 \pm 0.51$
KD5D	$7.8 \times 10^1 \pm 0.51$	$3.5 \times 10^4 \pm 0.42$	$5.2 \times 10^2 \pm 0.45$	$2.3 \times 10^5 \pm 0.75$	$5.1 \times 10^5 \pm 0.15$

\*Mean with ± standard error for 3 replicates

It was observed in the study that bacteria adapt to their niche and modify their enzymes systems for assured survival. General fashion observed was that the bacteria isolated from the fibrous root (monocots) colonized the same type of the root and promoted growth by producing phytohormones. The type of root and root exudates affects the colonization of microbe on the roots (Chanway *et al.*, 1991; Brimecombe *et al.*, 2001). It is safe to conclude that the plant root type plays important role in the supporting the PGPR. In some cases, PGPR also play their role as observed that some bacteria grow on all the roots used in study (Table 3). These bacteria are aggressive in nature having diverse gene pool that supports growth and colonization on multiple roots. But the general trend observed was that PGPR isolated from the one root type (fibrous) produce pronounced effects on the same type of the root. Microbes themselves also effect the amount and type of exudates (Hale & Moore, 1980). Thus both plant and microbe have their role in plant microbe interaction and only compatible match would result in positive interaction and growth enhancement of the plants.

From a commercial point of view, knowing which plants your bacterial strain successfully colonize and increase its growth and yield is necessary for planning right biofertilizer for the crop at hand. In local market there are multiple biofertilizers available but the host specificity mechanism is not well studied and in number of cases these products fail to perform in the field. Further studies in this respect that which genes of the plant and microbe are involved in successful colonization of the host plant are required to elaborate the mechanism.

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