



Plant growth promoting rhizobacteria from heavy metal contaminated soil promote growth attributes of *Pisum sativum* L.

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ABSTRACT

In natural soil environment, microbial communities demonstrate a cooperative relationship with crops yield through the plant growth promoting rhizobacteria (PGPR). In present report, we have explored the PGPRs for the plant growth promoting activities. In our recent report, the isolated strains were isolated from the industrial soil. Out of four rhizobacterial species, two strains (*Bacillus thuringiensis* PS-1 and *Azotobacter chroococcum* PS-2) have shown higher PGPR activity. Both strains were selected for their growth promoting activity in garden pea (*Pisum sativum*) variety P88 which had significantly higher germination percentage. Four different treatments, *P. sativum* without bacterial inoculum, *P. sativum* + *B. thuringiensis* PS-1, *P. sativum* + *A. chroococcum* PS-2 and *P. sativum* + bacterial consortium (control) were considered to make the comparative analysis of parameters like seed germination, plant height, plant relative water content, chlorophyll content, number of pods, number of leaves and root length in *P. sativum*. Among these treatments, seeds treated with bacterial consortium were significantly better in terms of plant height (87.78 ± 4.15 cm; $p < 0.05$); weight (93.45 ± 4.85 g; $p < 0.05$); number of leaves (80 ± 3.54 ; $p < 0.05$) and chlorophyll content (2.56 ± 0.78 ; $p < 0.05$) on day 60 post-inoculation. *P. sativum* + consortium treated group has showed the best result among all the treated and non-treated groups. Meticulous use of these rhizospheric bacteria could aid phytoremediation against the agrochemicals and heavy metals along with plants better growth.

1. Introduction

Industrialization is a significant feature of development and growth. It is incomplete without the applications of metals and chemicals or agrochemicals. Same time, excess release of these metals and chemicals has adversely effected the environment and agricultural land (Singh et al., 2016; Kumar et al., 2015a). During the last few years, heavy metals pollution has attract the attention of the whole world due to its widespread periphery from industry to agricultural lands (Kaur et al., 2017; Kumari et al., 2018a,b; Singh et al., 2017). Inclusion of inorganic contaminants such as heavy metals in the food chain is a new threat and challenge for the scientific community because of their toxicity and persistence in environment (Kumar et al., 2015b, 2016). Accretion of

heavy metals and organic pollutants in landforms is toxic to human beings and other animals, both aquatic and terrestrial (Singh et al., 2015; Kumar et al., 2014a, 2014b). Heavy metals above the recommended levels can negatively affect the beneficial microorganisms through affecting the growth, morphology, biomass and biochemical activities of microorganisms (Kumar et al., 2017).

Plant growth promoting rhizobacteria (PGPR) are the beneficial rhizobacteria which form an alliance with the host plant resulting in the stimulus to the host plant and decreases the occurrence of various plant diseases (Bhattacharyya and Jha, 2012; Georgieva et al., 2018; Gopalakrishnan et al., 2017). PGPR are generally categorized into iPGPR (intracellular) and ePGPR (extracellular) on the basis of their degree of association (Martinez-Viveros et al., 2010; Patel et al., 2017;

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Kizhakedathil and Devi, 2018). iPGPR are usually present inside the nodules of the root cells and ePGPR locates on rhizosphere or in between root cortex. These PGPR usually enhance the production of various metabolites used for plant growth and secretes phytohormones like gibberellic acid, auxin, cytokinins and indole acetic acid by increasing the surface area of roots (Ahemad et al., 2016; Ahemad and Khan, 2012; Meena et al., 2015). Several bacterial species can improve the plant growth by indirect or direct methods and have the potential to survive in contaminated areas (with heavy metals and pesticides) and increases plant growth (Barakat, 2011; Tica et al., 2011). This interaction between PGPRs and microbes invited the attention of researchers in recent years (Dell'Amico et al. 2008; Barakat, 2011). These PGPRs protects plants from heavy metal toxicity in heavy metal contaminated areas and promote plant growth by inducing various phytohormones (Kumari et al., 2018a,b).

Symbiotic association of leguminous plants and rhizobacteria are widely known to detoxify and remove heavy metals contamination from contaminated sites. The nitrogenase activities and nodulation process are very sensitive to heavy metal contamination but recent studies reveal the role of heavy metal tolerant rhizobacterial strains in effective removal of heavy metals and sideways carrying out nitrogen fixation to the leguminous plants (Checcucci et al., 2017). Various plant growth promoting mechanisms such as the production of ACC deaminase, production of siderophores, phytohormones, volatile compounds (such as 2, 3 butanediol and acetoin) makes strains potent for the decontamination and de-toxification of heavy metals through phyto-remediation (Rangel et al., 2017; Hao et al., 2014). However, these PGPR are also recognized to enhance the yield of leguminous plants in heavy metal stress (Arora et al., 2010).

Moreover, *Pisum sativum* is one of the most important legume species worldwide. Due to its high sensitivity to inorganic and organic contaminants, it has been used as an indicator plant in various studies. Most of the previous research has focused on testing abilities of isolated strains nitrogen fixation and very less data is available to test their abilities to enhance plant growth. In our earlier study, we have analyzed heavy metal ions from soil of seven industrial sites and isolated best four plant growth promoting bacteria, those have shown excellent heavy metal resistance (Kumar et al., 2015b). It is well known fact that plant growth promoting activities are variable (Ahemad et al., 2016; Ahemad and Khan, 2012; Meena et al., 2015). The objective of this current study was to check the ability of strains isolated from heavy metal contaminated site in Jalandhar district of Punjab to synthesize plant growth hormones such as indole-3-acetic acid (IAA), siderophores, HCN production, solubilize phosphate and to assess the potential of isolated strains to promote *Pisum sativum* under axenic conditions. The isolated and selected strains which performed better in PGPR production were further selected to check their effect on the enhancement of *Pisum sativum* growth promotion as possible bio-inoculants. Present study deals with the assessment of effects of two isolated rhizobacteria (*Bacillus thuringiensis* PS-1 and *Azotobacter chroococcum* PS-2) on *P. sativum* in terms of efficiency to produce plant growth promoting traits and showing effects on chlorophyll content. Current study could propose the promising application of rhizobacterial strain for the effective growth of crops including legumes like *P. sativum*.

2. Materials and methods

2.1. Selection of rhizobacteria

All the isolated strains used in this study were a native from heavy metal contaminated site (Navyug industries Pvt Ltd) located in the industrial area of Jalandhar (sea level-228 m, Latitude: 31°20'15.4"N and East Longitude: 75°36'37.0"E). This site usually manufactures V belts and the metal analysis of this area crosses hazardous levels. ICP-ES results depicts the average level of heavy metal in that site was

13.1 mg/kg for As, 5.9 mg/kg of Cadmium, 6.5 mg/kg of chromium, 7370 mg/kg of Copper, 1619 mg/kg of Lead and Zinc 10,345 mg/kg were measured (Kumar et al., 2015a,b).

In our previous work, we carried out an extensive identification and characterization of isolated strains through morphological, biochemical and 16S rRNA sequencing. Strains were isolated first by serial dilution method on the specific media and later on healthy and single colonies were further selected for study (Kumar et al., 2015a,b). Partial sequence of isolated strains was obtained using universal primers 1492R (ACCT TGTTACGACTT) and 27F (AGAGTTTGATCMTGGC TCAG). The similarity index between the isolated strains and closely relative strains was almost high (98%).

2.2. Plant growth promoting activities

Plant growth promoting activities including siderophores production, phosphate solubilization, cyanide production (HCN), and Indole acetic acid were performed as per our previous work (Kumar et al., 2015b). Detailed protocols are mentioned under supplementary data S1. Based on PGPR activities, out of four stains, for the present work, we have selected two best bacterial strains. Further, two best strains (*B. thuringiensis* PS-1 and *A. chroococcum* PS-2) were used to check their affect on the growth of *P. sativum*.

2.3. Selection of pea variety and its germination

Seeds of six different varieties of garden Pea (*P. sativum* L.) namely, Arkel, Bonneville, Matar Ageta-6, Mithi Phalo, Punjab 87 and Punjab 88 were procured from Punjab Agricultural University, Punjab, India. Before sowing, seeds were sterilized with 4% solution of sodium hypochlorite for 30 min on a magnetic stirrer and rinsed with sterile distilled water. After seed surface-sterilization, a hundred seeds were sown in an open-field (equal distance from each other). After 5 days, the germination rate (%) was calculated by using an equation given below:

$$\text{Germination rate (\%)} = \left(\frac{\text{No of seeds germinated}}{\text{Total seeds}} \right) \times 100.$$

2.4. Selection of pea variety for field experimentation

Based on high germination percentage, the Punjab 88 variety was selected for further field experimentation work. The seeds were treated with 10% sugar solution and were agitated in a shaker (REMI Orbital Shaking Incubator with LCD, RIS-24 Plus) at 100 rpm for 24 h vigorously until a fine coating of sugar appeared on them. Later, the seeds were equally distributed into 100 ml conical flasks. The first set was kept in distilled water for 24 h and the other was dipped in an individual bacterial suspension of two bacterial cultures *B. thuringiensis* PS-1, *A. chroococcum* PS-2 and in a combination of both the strains, having 10^4 CFU/ml, respectively. The seeds were placed at room temperature overnight. All the experiments were conducted in triplicates and repeated thrice.

2.5. Field study and other parameters

The seeds were sown in agricultural fields of Lovely Professional University (in year 2015 and repetition in year 2016 and 2017) in months of September, October and November, in seven rows in each of the plot (10 seeds in each row) and were irrigated with canal water. The soil is slightly alkaline in nature (pH = 7.8), with low availability of carbon, nitrogen and potassium. Soil is deprived of phosphorous content and the details are provided in the Table 1. The data regarding seed germination, plant height, root length, number of nodes, number of pods, number of flowers etc. were determined after 15, 30 and 60 days of sowing, as per Newman protocol (Goubran and Richards, 1979).

Table 1
Physicochemical properties of the soil of plots 1 to 4.

Particulars	Unit	Soil test value	Status	Soil test Rating		
				Acidic	Neutral	Alkaline
pH		7.8	Alkaline	< 6.5	6.5–7.5	> 7.5
Electric Conductivity	ds/m	0.21	Non-Saline	Non Saline < 1.0	Increasingly Saline 1.0–2.0	> 2.0
Organic Carbon	kg/ha	0.27	Low	Low < 0.50	Medium 0.50–0.75	High > 0.75
Avail. Nitrogen	kg/ha	58.8	Low	< 250	250–500	> 500
Avail. Phosphorous	kg/ha	7.8	Medium	< 10	10–25	> 25
Avail. Potassium	kg/ha	93.2	Low	< 125	125–250	> 250
Exch. Calcium	mg/L	494	Low	< 500	500–1000	> 1000
Exch. Magnesium	mg/L	163.4	High	< 125	125–250	> 250
Avail. Sulphur	mg/L	19.3	Medium	< 10	10–50	> 50
Avail. Zinc	mg/L	1.67	Medium	< 1	1.0–5.0	> 5.0
Avail. Copper	mg/L	0.55	Medium	< 0.5	0.5–2.5	> 2.5
Avail. Iron	mg/L	8.6	High	< 2.5	2.5–10.0	> 10.0
Avail. Manganese	mg/L	13.3	Medium	< 5.0	5.0–20.0	> 20.0
Boron	mg/L	0.34	Low	< 0.5	0.5–1.0	> 1.0

2.6. Measurement of relative water current (RWC)

The quantity of water present in plants was determined by the measurements based on dry weight (DW), fresh weight of the plant material at the time of sampling (FW) and turgid weight (TW). The samples were dried in an oven at 80 °C for 1, 2, and 4 h respectively. The percentage of relative water content of the pea plant at different time intervals were calculated as:

$$\% \text{ RWC} = (\text{FW}-\text{DW}/\text{TW}-\text{DW}) \times 100$$

2.7. Total chlorophyll content

The total chlorophyll content of leaves was estimated using the method of Baghizadeh et al. (2014) where chlorophyll a (at 662 nm) and b (at 645 nm) were checked by UV–visible spectroscopic method. From sowing to maturation no external mineral or nutrients were added. The plants were almost grown until grain maturation. Each treatment group comprises of three replicates containing 10 individual plants. Plants were irrigated regularly throughout the experiment. The chlorophyll content was extracted from 0.1 g of freshly collected pea plant by using 80% acetone and the values of chlorophyll a and b were calculated by using protocol of Lichtenthaler and Wellburn (1983) as follows

$$\text{Chlorophyll a } (\mu\text{g/mL}) = C_a = 12.25A_{663.2}-2.79A_{646.8}$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = C_b = 21.50 A_{646.8}-5.10 A_{663.2}$$

2.8. Statistical analysis

Statistical analyses of the data were performed by using ANOVA (differences among tests) and Tukey's HSD (to compare means).

3. Results

3.1. Isolation and selection of rhizobacteria

The 16s rRNA sequencing analyses have confirmed the isolated strains resemble with the species of *Bacillus thuringiensis* and *Azotobacter chroococcum* and deposited in GenBank under accession numbers: *Bacillus thuringiensis* PS-1 (KJ511861.1), *Azotobacter chroococcum* PS-2 (KJ607246.1) (Kumar et al., 2015b). Further, *B. thuringiensis* PS-1 and *A. chroococcum* PS-2 were selected for the in-vivo study because of their high efficiency against the production of different plant growth

promoting hormones (Details are under supplementary data S2). Both strains were able to pass all the PGPR tests namely Indole acetic acid, phosphate solubilization, siderophore production and HCN production higher. The observed PGPR activities were more than the known PGPR i.e. *Pseudomonas fluorescens* and were significantly different from control (Supplementary Data S2). The IAA amounts produced by selected strains were 0.74 ± 0.005 for PS1 and 0.76 ± 0.005 for PS2, as compared to known PGPR (0.68 ± 0.005). Similarly, the zone of solubilization of phosphate is also greater than known PGPR (i.e. 18 ± 0.005 mm for PS1 and 16 ± 0.004 mm for PS2) as compared to known PGPR (15 ± 0.006 mm). The siderophore production from these two isolates was also higher than the known PGPR (i.e. 0.88 ± 0.007 for PS1 and 0.90 ± 0.005 for PS2 and 0.85 ± 0.006 for known PGPR (*Pseudomonas fluorescens*). As compared to known PGPR, at a significant level ($p \leq 0.05$), the increased siderophore production (6–9%), enhanced acetic acid production (10–13%), increased HCN production (10–12%), and solubilization of phosphate (8–22%) were noticed with the applications of PS2 and PS2.

3.2. Germination and selection of seed variety

The seed variety selected for the current study was Punjab 88 because of its high germination rate 81.66% as shown in Table 2. In the present study, the total number of seeds that grew in Plot 1 (seeds treated with pump water), Plot 2 (seeds treated with *B. thuringiensis* PS-1), Plot 3 (seeds treated with *A. chroococcum* PS-2) and Plot 4 (consortium of *B. thuringiensis* PS-1 and *A. chroococcum* PS-2), were P_1 (77.1 ± 9.5 seeds), P_2 (82.8 ± 4.8 seeds) P_3 (88.5 ± 8.9 seeds) and P_4 (95.7 ± 5.3 seeds) respectively. The details as per single row in each plot are mentioned in Table 3. The bacterial treated group showed increased germination percentage as compared to the non-treated group. Inoculated bacteria showed their importance in the growth of plants, by helping them to respond to environmental stresses, compared to un-inoculated plants.

3.3. Growth parameters

The mean of the net weight of the pea plants was calculated after 15, 30 and 60 days. The plants in Plot 4 (P_4) showed the highest growth followed by plants of P_3 , P_2 and P_1 , respectively. Also, the number of leaves and root length was highest in P_4 plot followed by P_3 , P_2 and P_1 . The stem height was reported highest in P_4 plot compared to P_3 , P_2 and P_1 . All parameters of growth like net weight, number of leaves, root length, stem height and a total height of the plant were found to be highest in Plot 4 (P_4) followed by plants in Plot 3 (P_3) Plot 2 (P_2) and

Table 2
Selection of *Pisum sativum* seeds for experimentation work.

S. No.	Varieties	Seeds Inoculated			Seeds Germinated			Seed Germination (± SD) Mean	Variance (Standard deviation)	Germination Percentage (%)
		First Plot	Second Plot	Third Plot	First Plot	Second Plot	Third Plot			
1.	Arkel	100	100	100	64	61	57	60.66 ± 3.51 ^b	12.33	60.6%
2.	Bonneville	100	100	100	79	74	67	73.33 ± 6.02 ^a	36.3	73.33%
3.	Matar Ageta-6	100	100	100	49	52	54	51.66 ± 2.51 ^b	6.33	51.66%
4.	Mithi Phali	100	100	100	55	58	60	57.66 ± 2.51 ^b	6.33	57.66%
5.	Punjab 87	100	100	100	74	70	78	74 ± 4 ^a	16	74%
6.	Punjab 88	100	100	100	82	84	79	81.66 ± 2.51 ^a	6.33	81.66%

Mean values followed by different letters in same column are significantly different (one-way ANOVA; Tukey's test, $p \leq 0.05$).

Plot 1 (P_1). The details are given in Table 4 and supplementary data S3.

3.4. Relative water current

The relative water current of the whole plant was recorded after 1, 2 and 4 h of desiccation. The plants of Plot 4 (P_4) have showed the highest relative water current followed by Plot 3 (P_3) Plot 2 (P_2) and Plot 1 (P_1) respectively. After 15 days, the seeds inoculated with a combination of both the cultures show good relative water content (0.27 ± 0.01) as compared to singly inoculated (0.18 ± 0.04 for Plot 2 & 0.20 ± 0.05 for plot 3) and untreated samples (0.11 ± 0.01). Similar results were observed in day 30 and day 60 plants, respectively. The relative water current after 1, 2 and 4th h in all the treated groups after 15 days was significantly different than other treated groups (Table 5).

3.5. Chlorophyll content

The chlorophyll content was calculated and compared after 60 days in all the groups. The chlorophyll content (Chl a and Chl b) in the treated groups was higher compared to the non-treated groups. After 60 days, the seeds inoculated with a combination of both the cultures show high chlorophyll a content (0.82 ± 0.04) compared to singly inoculated (0.71 ± 0.09 for Plot 2 & 0.68 ± 0.09 for plot 3) and untreated samples (0.65 ± 0.1). Similar findings were observed for chlorophyll b content and the chlorophyll values of a, b and total chlorophyll in all the groups after 60 days was significantly different than other treated groups (Table 6).

4. Discussion

Various researchers have elucidated that the enhanced growth of plant is directly proportional to the increase in the parameter like siderophore production, nitrate production indole acetic acid production, phosphate solubilization, and HCN production (Ma et al., 2009; Dell'Amico et al. 2008; Barzanti et al., 2007; Idris et al., 2004). Various bacteria have been isolated worldwide showing potential to survive in heavy metal stress environment (Ma et al., 2009; Dell'Amico et al.

2008). Moreover, bacteria were also used to evaluate toxicity from heavy metals in which heavy metal indices the PGPR activities of the strains (Mishra et al., 2016). In current study, as compared to control, porph amplification in the siderophore 9 and 11% for strains PS1 and PS2 was found. Similarly, an enhanced indole acetic acid production for PS1 (6%) and PS2 (9%) were found. Compared to control, enhanced phosphate solubilization for PS1 (17%) and PS2 (14%) was found.

In present report, under the field experiments, the early stage plant growth of *P. sativum* was affected by the bacterial inoculum. As compare to control (un-inoculated), the inoculum of bacterial strains *B. thuringiensis* PS-1 and *A. chroococcum* PS-2 has significantly increased the germination. Increase in net dry weight and biomass content was noticed which was linked with the higher N, K and P content. Both the selected strains, PS-1 and PS-2 were effective in net dry weight increase respectively but less than the consortium treated groups over the control. The chlorophyll content also increases in consortium treated groups as compared to other treated groups respectively. The present observations which were showing increase in various parameters were found to be similar with the work carried out by Belimov et al. (2005). In present work, bacterial isolates enhanced the plant growth and increased the nutrient uptake process which may be good for nutrient deprived soils. The mechanism behind the enhanced nutrient uptake and plant growth was associated with the enhancement in the plant growth promoting traits including siderophore production phosphate solubilization ability and indole-3-acetic acid production, (Ahmad et al., 2008; Ma et al., 2009). With respect to a number of seeds sown, the *P. sativum* (P_1) groups were significantly different from *P. sativum* + *A. chroococcum* PS-2 (P_3) treated groups and *P. sativum* + consortium treated groups. *P. sativum* + *B. thuringiensis* PS-1 (P_2) treated groups showed significantly different results with *P. sativum* + consortium treated groups but similar results as *P. sativum* treated groups and *A. chroococcum* PS-2 (P_3) treated groups. *P. sativum* + *A. chroococcum* PS-2 (P_3) treated groups showed similar results as *P. sativum* + *B. thuringiensis* PS-1 (P_2) treated groups and consortium treated groups. The net weight, number of leaves, total height and root length after 15 days is significantly different in all the treatment groups. The stem height in *P. sativum* + consortium treated group is

Table 3

Number of *Pisum sativum* seeds (out of 10) sown rows in field after exposure with different bacteria (Plot 1 = Control; Plot 2 = *Bacillus thuringiensis* PS-1; Plot 3 = *Azotobacter chroococcum* PS-2 and Plot 4 = Consortium).

Row	<i>Pisum sativum</i> + D. water (P_1)	<i>Pisum sativum</i> + <i>Bacillus thuringiensis</i> PS-1 (P_2)	<i>Pisum sativum</i> + <i>Azotobacter chroococcum</i> PS-2 (P_3)	<i>Pisum sativum</i> + Consortium (P_4)
1	8	8	8	9
2	7	9	9	9
3	8	8	10	10
4	6	8	9	9
5	9	9	8	10
6	8	8	10	10
7	8	8	8	10
Average/SD	7.71 ± 0.95 ^c	8.28 ± 0.48 ^{bc}	8.85 ± 0.89 ^{ab}	9.57 ± 0.53 ^a

Mean values followed by different letters in same row are significantly different (one-way ANOVA; Tukey's test, $p \leq 0.05$).

Table 4

Growth parameters of *Pisum sativum* after 15, 30 and 60 days of exposure with different bacteria (Plot 1 = Control, Plot 2 = *Bacillus thuringiensis* PS-1; Plot 3 = *Azotobacter chroococcum* PS-2 and Plot 4 = Consortium).

S. No.	Growth Parameter(s)	Observation	Plot 1 (P ₁)	Plot 2 (P ₂)	Plot 3 (P ₃)	Plot 4 (P ₄)
1	Net Weight (g)	15th day	1.14 ± 0.34 ^d	1.72 ± 0.25 ^c	1.89 ± 0.13 ^b	2.14 ± 0.44 ^a
		30th day	9.30 ± 0.04 ^c	11.32 ± 0.15 ^c	13.81 ± 0.36 ^b	16.21 ± 0.057 ^b
		60th day	48.02 ± 2.25 ^d	63.80 ± 2.68 ^c	78.20 ± 1.18 ^b	93.45 ± 4.85 ^a
2	No. of leaves	15th day	7.25 ± 0.15 ^d	16.40 ± 1.13 ^c	19.46 ± 0.67 ^b	24.1 ± 0.26 ^a
		30th day	21.02 ± 0.35 ^c	34.14 ± 0.18 ^b	37.24 ± 0.85 ^b	43 ± 0.097 ^a
		60th day	37.02 ± 0.56 ^c	5.41 ± 0.78 ^d	69.26 ± 0.95 ^b	80 ± 3.54 ^a
3	Root length (cm)	15th day	5.80 ± 0.76 ^d	7.30 ± 0.85 ^c	8.40 ± 1.05 ^b	9.2 ± 0.94 ^a
		30th day	7.30 ± 0.75 ^c	9.70 ± 1.25 ^{bc}	11.4 ± 0.94 ^{ab}	13.5 ± 0.1.33 ^a
		60th day	16.00 ± 0.34 ^c	22.40 ± 0.58 ^b	37.20 ± 0.14 ^a	39.38 ± 1.2 ^a
4	Stem height (cm)	15th day	9.40 ± 0.42 ^b	9.20 ± 0.62 ^b	10.2 ± 0.85 ^b	13.5 ± 0.74 ^a
		30th day	15.20 ± 0.78 ^c	18.6 ± 0.54 ^{bc}	24.30 ± 1.12 ^{ab}	28.6 ± 2 ^a
		60th day	27.00 ± 0.15 ^c	43.0 ± 1.08 ^b	43.10 ± 1.86 ^b	48.4 ± 2.24 ^a
5	Total height (cm)	15th day	15.20 ± 1.38 ^d	16.50 ± 0.14 ^c	18.60 ± 0.52 ^b	22.7 ± 0.81 ^a
		30th day	22.50 ± 0.89 ^d	28.30 ± 0.42 ^c	35.70 ± 0.14 ^b	42.1 ± 2.5 ^a
		60th day	43.00 ± 1.87 ^d	65.00 ± 2.16 ^c	80.30 ± 2.76 ^b	87.78 ± 4.15 ^a
6	No. of nodes	60th day	17.02 ± 0.05 ^c	24.32 ± 0.87 ^b	33.42 ± 0.15 ^a	36 ± 0.99 ^a
7	No. of internodes	60th day	48.15 ± 4.3 ^c	58.02 ± 0.95 ^b	67.43 ± 1.35 ^a	72 ± 1.85 ^a
8	No of pods	60th day	5.4 ± 0.44 ^c	6.15 ± 0.62 ^c	8.25 ± 0.06 ^b	11.36 ± 1.2 ^a
9	No. of flowers	60th day	5 ± 0.74 ^b	6.12 ± 0.84 ^b	13.28 ± 0.57 ^a	16.24 ± 1.4 ^a

Mean values followed by different letters in same row are significantly different (one-way ANOVA; Tukey's test, $p \leq 0.05$).

significantly different from *P. sativum* (P₁), *B. thuringiensis* PS-1 (P₂), and *A. chroococcum* PS-2 (P₃) treated groups. The relative water current after 1 h in *P. sativum* (P₁) groups after 15 days was significantly different than other treated groups. The *P. sativum* (P₁) and consortium treated groups were significantly different after 2 h. The net weight in *P. sativum* (P₁) and *B. thuringiensis* PS-1 (P₂) treated groups were significantly similar whereas they both were found to be significantly different from *P. sativum* + *A. chroococcum* PS-2 (P₃) and consortium treated groups after 30 days. The number of leaves in *P. sativum* (P₁) and *P. sativum* + consortium were significantly different from all other treatment groups. *P. sativum* + *B. thuringiensis* PS-1 (P₂) treated groups were significantly different from *P. sativum* + consortium treated groups with respect to root length and stem height results after 30 days. *P. sativum* + *A. chroococcum* PS-2 (P₃) treated groups was also found to be significantly different from *P. sativum* (P₁) groups in terms of root length and stem height after 30 days. The total height after 30 days was found significantly different in all the treatment groups. The relative water current after 1 h in 30 days treatment was found significantly different in all the treatment groups. After 2 h and 4 h, *P. sativum* + *A. chroococcum* PS-2 (P₃) and consortium treated groups were significantly different from others. The net weight, number of leaves, total height after 60 days was found to be significantly different in all the treatment groups. The root length was found to be similar in *P. sativum* + *A. chroococcum* PS-2 (P₃) and consortium treated groups but was statistically different from *P. sativum* (P₁) and *B. thuringiensis* PS-1 treated groups after 60 days. The number of nodes and number of internodes in *P. sativum* + *A. chroococcum* PS-2 (P₃) and consortium treated groups

Table 5

Relative Water Current (RWC) in *Pisum sativum* raised plots after 15, 30 and 60 days of exposure with bacterial Inoculum (Plot 1 = Control, Plot 2 = *Bacillus thuringiensis* PS-1; Plot 3 = *Azotobacter chroococcum* PS-2 and Plot 4 = Consortium).

No. of Days	Time period	Plot 1 (P ₁)	Plot 2 (P ₂)	Plot 3 (P ₃)	Plot 4 (P ₄)
15	After 1 h	0.22 ± 0.04 ^b	0.42 ± 0.05 ^a	0.48 ± 0.15 ^a	0.52 ± 0.13 ^a
	After 2 h	0.11 ± 0.02 ^b	0.21 ± 0.05 ^{ab}	0.23 ± 0.03 ^{ab}	0.3 ± 0.04 ^a
	After 4 h	0.11 ± 0.01 ^b	0.18 ± 0.04 ^{ab}	0.20 ± 0.05 ^{ab}	0.27 ± 0.01 ^b
30	After 1 h	5.40 ± 0.04 ^d	6.80 ± 0.08 ^c	7.90 ± 0.12 ^b	8.34 ± 0.66 ^a
	After 2 h	3.80 ± 0.02 ^c	4.10 ± 0.11 ^c	5.4 ± 0.18 ^b	6.89 ± 0.48 ^a
	After 4 h	3.80 ± 0.07 ^c	4.00 ± 0.04 ^c	5.20 ± 0.08 ^b	6.44 ± 0.83 ^a
60	After 1 h	16.20 ± 0.12 ^d	33.8 ± 1.25 ^c	45.1 ± 0.89 ^b	48.22 ± 0.9 ^a
	After 2 h	15.60 ± 0.04 ^c	30.10 ± 0.87 ^b	41.80 ± 1.25 ^a	44.78 ± 1.2 ^a
	After 4 h	15.40 ± 0.67 ^d	30.76 ± 0.56 ^c	40.24 ± 1.62 ^b	43.2 ± 1.78 ^a

Mean values followed by different letters in same row are significantly different (one-way ANOVA; Tukey's test, $p \leq 0.05$).

Table 6

Chlorophyll a, chlorophyll b and Chl a/b ratio of *Pisum sativum* after 60 days of exposure with different bacteria (Plot 1 = Control, Plot 2 = *Bacillus thuringiensis* PS-1; Plot 3 = *Azotobacter chroococcum* PS-2 and Plot 4 = Consortium).

Parameter	Plot 1 (P ₁)	Plot 2 (P ₂)	Plot 3 (P ₃)	Plot 4 (P ₄)
Chlorophyll a	0.65 ± 0.1 ^a	0.71 ± 0.09 ^a	0.68 ± 0.09 ^a	0.82 ± 0.04 ^a
Chlorophyll b	0.27 ± 0.1 ^a	0.31 ± 0.1 ^{ab}	0.29 ± 0.2 ^{ab}	0.32 ± 0.07 ^a
Chlorophyll a/b	2.4 ± 0.1 ^a	2.29 ± 0.2 ^a	2.34 ± 0.1 ^a	2.56 ± 0.78 ^a

Mean values followed by different letters in same row are significantly different (one-way ANOVA; Tukey's test, $p \leq 0.05$).

were found to be similar but were statistically different from *P. sativum* (P₁) and *B. thuringiensis* PS-1 treated groups after 60 days. The number of pods in *P. sativum* (P₁) and *B. thuringiensis* PS-1 treated groups were found to be similar but were statistically different from *A. chroococcum* PS-2 (P₃) and consortium treated groups after 60 days. The number of flowers in *P. sativum* (P₁) and *B. thuringiensis* PS-1 treated groups were statistically similar to each other and also the result of *P. sativum* + *A. chroococcum* PS-2 (P₃) and consortium treated groups were statistically similar after 60 days of treatment. The Relative water current after 1 and 4 h were statistically different in all the treatment groups whereas in 2 h the *P. sativum* + *A. chroococcum* PS-2 (P₃) and consortium treated groups was found statistically similar to each other but varied statistically with other treatment groups after 60 days. The level of chlorophyll a and chlorophyll a/b was found to be statistically different in all the

treatment groups. The chlorophyll b in *P. sativum* (P₁) and all other groups were found to be statistically similar.

Our consortium of both the isolates *B. thuringiensis* PS-1 and *A. chroococcum* PS-2 have shown significant effect on plant growth (i.e. pea) where soil is nutrient deprived. Same results have been confirmed by the De Freitas and Germida (1992b), where bacterial strains exhibited enhanced plant growth at early stage of plant. According to Lazarovits and Norwak (1997), perfect experimental or climatic conditions can enhance the plant growth and grain yields. Under suitable climatic conditions, the increased production of rhizospheric microorganisms and phytohormones is observed. Along with it, plant growth as well yield also increases (Turyanitsa et al., 1995; Zimmer et al., 1995; Ahmad et al., 2008).

Presently, the new approaches for improving crop production to discover a broad variety of rhizobacteria having characteristic qualities including agrochemical and heavy metal ion tolerance (Checcucci et al., 2017; Ahemad and Khan, 2012; Ma et al., 2009), organic or bioactive agrochemicals with biological controls on pathogens etc. (Poole et al., 2008; Russo et al., 2008) salinity tolerance (Tank and Saraf, 2010; Mayak et al., 2004). In addition to that, they also support PGPR properties such as like phosphate solubilization, HCN production, nitrate, siderophores production indole acetic acid etc. (Ahemad and Khan, 2012; Tian et al., 2009). Hence, various diverse forms of symbiotic and non-symbiotic bacteria are using globally to enhance the growth of plants under stressed conditions including chemicals and heavy metal ions stress (Ma et al., 2009).

5. Conclusion

In present study, we have investigated the plant growth promoting activities of the rhizobacteria *Bacillus thuringiensis* PS-1 and *Azotobacter chroococcum* PS-2 those were isolated from the heavy metal stressed areas. In *in-vivo* experiments, both strains were tested for their plant growth promoting activities against pea (*Pisum sativum*) variety P88. Both strains have shown significant results w. r. t. the plant growth activities and their applications in the growth of *P. sativum*. The parameters like seed germination, plant height, plant relative water content, chlorophyll content, number of pods, number of leaves and root length of *P. sativum* were enhanced significantly. Consortium treated group (i.e. PS2 > PS1 > Control) has showed the best result among all the treated and non treated groups (at $p < 0.05$). In concluding remarks, investigated strains may be used as bio-agents in agriculture to enhance the plant growth.

Conflicts of interest

The author declares that no conflict of interest exists.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.bcab.2019.01.035>.

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