Differential modulation of phytoelemental composition by selected *Pseudomonas* spp.

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Abstract

Plant growth promoting rhizobacteria (PGPR) are bioresources with potential application in ecofriendly agricultural practices. The beneficial effects of PGPR have been attributed to their ability to produce phytohormone, organic acid, siderophore, and also due to nitrogen fixation among others. In the present study, previously isolated plant growth promoting rhizospheric *Pseudomonas* spp. were evaluated for growth enhancement effect in *Vigna unguiculata* seedlings. Elemental profiling of treated plant was further carried out by inductively coupled plasma-mass spectroscopy. Results of the study showed significant increase in growth parameters such as shoot length, root length and root numbers for treated plants when compared to control. Most of the macro and micro elements were also found to get modulated by interaction with applied *Pseudomonas* spp. However, a differential modulation was observed for plants when treated with each of the *Pseudomonas* spp., which could be due to their variable interaction with the selected plant. The results of the study indicate the role of each of the associated microbial partner to specifically influence the plant nutrient mobilization along the soil plant axis. The cumulative effect of the plant microbiome hence may decide the global nutritional status of plants as per the available environmental conditions.

Keywords Rhizobacteria · ICP-MS · *V. unguiculata* · Plant growth promotion

Introduction

Plant–rhizobacterial interactions have been studied extensively for various agrological as well as environmental aspects (Chandra and Kumari 2017). Plant growth promoting rhizobacteria (PGPR) can be extracellular (*Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia*) or intracellular (*Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, endophytes and *Frankia*) (Gupta et al. 2015). The diverse mechanisms employed by these organisms include the production of phytohormones, ACC deaminase, siderophore, nitrogen fixation, enhanced mineral uptake and biocontrol against numerous phytopathogens (Vacheron et al. 2013). By IAA production, they have been demonstrated to modulate the cell elongation, division and differentiation in plants. Microbial population also secrete

organic acids which convert the insoluble phosphates into soluble monobasic and dibasic ions and thereby making it available to plants. Siderophore producing bacteria restrict the growth of plant pathogens due to their strong affinity towards Fe(III). The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase of microbial origin facilitates plant growth and development by decreasing the ethylene level, inducing salt tolerance and reducing drought stress in plants (Zahir et al. 2008). These beneficial features of rhizobacteria have significant impact on growth and yield of plants. Among the various rhizobacteria, *Pseudomonas* spp. have ubiquitous distribution and have diverse plant growth promoting as well as biocontrol mechanisms. However, the global changes introduced in plants due to rhizobacterial interaction are not fully known. The wide range of antifungal compounds produced by plant growth promoting rhizobacteria includes amphisin, 2,4-diacetylphloroglucinol (DAPG), oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides (Loper et al. 2007). Among these, phenazine derivatives are one of the important antifungal products of *Pseudomonas* spp.

Due to the easiness with culture handling and large-scale production, many *Pseudomonas* spp. based formulations

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have already been introduced. Hence the Genus *Pseudomonas* forms an important candidate to study the global impact of plant beneficial mechanisms of rhizobacteria on plant system. Such studies are very important to explore the opportunities to design tailor made rhizobacterial formulations to specifically modulate the nutritional composition of plants. As they have been isolated from rhizosphere of numerous plants like cotton, rice, banana, rape, sugar cane, wheat and barley, they have immense promises to explore (Loaces et al. 2010; Ravindra Naik et al. 2008; Patten and Glick 2002; Rameshkumar 2012; Mavrodi et al. 2001; Parejko et al. 2012). So, in this study selected *Pseudomonas* spp. have been investigated in detail to identify their impact on elemental composition of the model plant *Vigna unguiculata*.

Materials and methods

For the study, previously isolated *Pseudomonas* spp. from Western Ghat rhizosphere were selected. These include *P. putida* (KY823010), *P. monteilii* (KY823008), *P. rhodesiae* (KY823009), *P. fluorescens* (KY823007) and *P. taiwanensis* (KY823006). For the plant growth promotion studies, seeds of *V. unguiculata* were collected and surface sterilized using sodium hypochlorite for 10 min (Jasim et al. 2013). The seeds were further washed several times with sterile distilled water and were allowed to germinate. The germinated seedlings were then treated with overnight grown cultures (10^7 CFU/mL) of *P. putida*, *P. monteilii*, *P. rhodesiae*, *P. fluorescens* and *P. taiwanensis* for 30 min. Treated seedlings along with control (distilled water and nutrient broth) were then observed for enhanced plant growth by planting it in double-sterilized soil. In each set of growth promotion study, ten seedlings were used per set in triplicate. Both control and treated seedlings were watered daily and observed periodically. The plants were harvested after 1 week and growth parameters such as root length, shoot length and root numbers were analysed (Jimtha John et al. 2017).

Pseudomonas spp. with enhanced plant growth enhancement effects were selected further to identify its potential to modulate the elemental composition of *V. unguiculata*. So, the plant samples treated with *P. monteilii*, *P. rhodesiae* and *P. fluorescens* were selected for ICP-MS analysis. These were oven dried for half an hour at 50 °C and powdered using mortar and pestle. From this, aliquotes of 0.1 g were digested at 85 °C with 8 mL HNO_3 (Supra pure) in a microwave digestion system. The digested samples were further made up to 25 mL with ultrapure water and then stored in sterile vials. The samples for ICP-MS (THERMO FISCHER iCAP, Q) analysis were diluted with 10% acid and from that 1 mL was again diluted to 25 mL. After ICP-MS analysis,

the elemental concentration in ppb was converted to $\mu\text{g}/\text{mg}$ using the formula $(\text{ICP-MS result} \times 25 \times 0.025) \div 0.1$. Triplicates were maintained for each plant samples. The results were analysed using statistical programme Origin Pro 7. One-way analysis of variance was used for comparison among the groups. Post hoc multiple comparison tests were used to determine the significant difference among groups, $P < 0.05$ was considered as significant.

Results and discussion

Among the selected *Pseudomonas* spp., three of them enhanced the growth of *V. unguiculata* when compared to control. *P. monteilii* treatment showed the highest enhancement of mean shoot length, root length and root numbers when compared to control. The shoot length of *P. monteilii*-treated seedlings was 20.73 ± 0.69 cm and was high when compared to that of the distilled water and nutrient broth control ($\text{DW}-14.48 \pm 0.79$ cm, $\text{NB}-13.11 \pm 0.27$ cm). The root length of the same (5.49 ± 0.27 cm) was also found to get enhanced when compared to the control. The root numbers were more in the case of *P. fluorescens* (9.85 ± 0.99) treatment and the same for control were 6.38 ± 0.55 and 6.38 ± 0.75 for DW and NB, respectively. The shoot length (18.73 ± 0.28 cm), root length (3.92 ± 0.22 cm) and root numbers (9 ± 0.50) were also higher in *P. rhodesiae*-treated seedlings than that of DW and NB control. *P. putida* treatment showed a shoot length of 16.25 ± 0.42 cm, root length of 3.74 ± 0.06 cm and root numbers of 9.33 ± 0.60 ; whereas *P. taiwanensis* mediated shoot length to 16.14 ± 0.23 cm, root length to 2.9 ± 0.12 cm and root numbers to 9.19 ± 0.37 (Fig. 1). The bacterial isolates used in the study have been demonstrated to have efficient growth enhancement effect in various plant systems (Jimtha John et al. 2017). Hence, the result can be due to the colonization of selected bacteria on *V. unguiculata*. Seed bacterization with *Pseudomonas* spp. has previously been described to result in increased growth parameters such as root length, shoot length and dry mass of sorghum seedlings (Praveen Kumar 2012). The inoculation of *Pseudomonas* sp. on peanut and maize has also reported to result in increased seed germination, growth and phosphorous content (Anzuay et al. 2017).

By ICP-MS analysis, *P. fluorescens* treated plants were found to have enhancement of phosphorous and magnesium while there was decrease of potassium and calcium contents when compared to control. Micronutrients like aluminium, iron, zinc and sodium of same plants were lower than control. However, nickel, manganese, caesium, strontium, cerium, neodymium, ytterbium, europium, bismuth, beryllium and lanthanum concentrations in *P. fluorescens* treated plants were observed to be higher than in controls. Chromium, cobalt, praseodymium, scandium, lutetium,

Fig. 1 Growth promotion analysis of *Pseudomonas* spp. on *Vigna unguiculata* seedlings by pot experiment along with untreated control. **a** Shoot length and root length. **b** Root number. DW distilled water and NB nutrient broth

rhodium, thallium, indium, rubidium, arsenic, holmium, erbium, thulium, thorium, gadolinium, dysprosium, lithium, barium, lead, boron, vanadium, gallium, selenium and cadmium concentrations were lower in *P. fluorescens* treated plants than in control (Fig. 2).

In *P. rhodesiae* treated plants macro elements like phosphorous and magnesium were high, but potassium and calcium contents were lower. Aluminium, manganese, iron, chromium, gallium, vanadium, neodymium, rubidium, strontium, barium, lanthanum, cerium, lead, lithium, boron, arsenic, cadmium, praseodymium, gadolinium, samarium, beryllium, indium, caesium, europium, scandium, terbium, bismuth, thorium, uranium, holmium, erbium, thulium, ytterbium, lutetium, thallium and dysprosium concentrations in plants treated with *P. rhodesiae* were higher than control. However, zinc, sodium, cobalt, copper, nickel, yttrium, selenium and rhodium of *P. rhodesiae* treated plants showed decreased concentration than control (Fig. 2).

The macro elements like phosphorous, potassium, calcium and magnesium contents in *P. monteilii* treated plants were lower than controls. Manganese, sodium, nickel, copper neodymium, lanthanum, barium and thorium of treated plants were higher than control. Aluminium, iron, chromium, gallium, vanadium, neodymium, rubidium, strontium,

Fig. 2 Elemental composition variation (macronutrients) in *Pseudomonas* spp. (*P. monteilii*, *P. fluorescens* and *P. rhodesiae*) treated seedlings of *Vigna unguiculata* when compared to control obtained from ICP-MS data. DW distilled water, NB nutrient broth control

barium, cerium, lead, lithium, arsenic, cadmium, zinc, praseodymium, gadolinium, samarium, beryllium, indium, caesium, europium, scandium, terbium, bismuth, uranium, holmium, erbium, thulium, ytterbium, lutetium, thallium and dysprosium concentrations in *P. monteilii* treated plants were lower than control (Fig. 2) (Table 1).

While comparing the macronutrient enhancement by *Pseudomonas* spp., both *P. fluorescens* and *P. rhodesiae* treated plants showed an elevated level of phosphorous and magnesium with a decrease in potassium and calcium

Table 1 Elemental composition variation in *Pseudomonas* spp. (*P. monteilii*, *P. fluorescens*, *P. rhodesiae*) treated seedlings of *V. unguiculata* when compared to control (DW distilled water, NB nutrient broth) obtained from ICP-MS data

	Concentration of elements in µg/mg (DW)	Concentration of elements in µg/mg (NB)	Concentration of elements in µg/mg (<i>P. fluorescens</i>)	Concentration of elements in µg/mg (<i>P. monteilii</i>)	Concentration of elements in µg/mg (<i>P. rhodesiae</i>)
Al	2773.419 ± 0.579785	2362.462 ± 0.300888	2644.735 ± 0.519615	2279.399 ± 0.057735	4322.849 ± 0.57735
Fe	1548.341 ± 0.57735	1529.137 ± 0.015275	1437.867 ± 0.608276	1141.111 ± 0.057735	2362.847 ± 0.57735
Zn	1018.497 ± 0.574485	1093.336 ± 0.060828	692.4619 ± 0.635085	395.8311 ± 0.057735	556.2129 ± 0.057735
Na	798.2303 ± 0.635085	731.2288 ± 0.028868	648.3131 ± 0.057735	607.5434 ± 0.057735	539.4947 ± 0.11547
Mn	315.6843 ± 0.611991	569.3644 ± 0.011547	658.2657 ± 0.608276	604.6754 ± 0.057735	618.3861 ± 0.23094
Cu	287.652 ± 0.57735	371.3465 ± 0.110151	137.1203 ± 0.550757	70.57084 ± 0.288675	398.2044 ± 0.635085
Ni	95.74449 ± 0.57735	231.4784 ± 0.110151	274.1513 ± 0.608276	126.2504 ± 0.11547	51.16571 ± 0.608276
Rb	73.07984 ± 0.57735	65.63367 ± 0.060828	64.49175 ± 0.057735	65.13879 ± 0.057735	76.60876 ± 0.173205
Ba	48.95014 ± 0.57735	50.54808 ± 0.090185	40.48197 ± 0.57735	38.16625 ± 0.057735	55.73448 ± 0.057735
Pb	39.20162 ± 0.264575	40.24077 ± 0.058026	28.5152 ± 0.11547	11.7652 ± 0.11547	41.74654 ± 0.057735
Ce	17.31047 ± 0.763763	46.77984 ± 0.191594	47.63744 ± 0.11547	24.23715 ± 0.057735	72.37724 ± 0.057735
Sr	34.12062 ± 0.8544	34.06756 ± 0.066583	36.20203 ± 0.11547	25.50155 ± 0.11547	38.36365 ± 0.057735
La	9.635971 ± 0.52915	22.24709 ± 0.063509	25.37034 ± 0.23094	33.07198 ± 0.011547	36.2054 ± 0.057735
Nd	7.148222 ± 0.519615	20.55475 ± 0.005774	25.33701 ± 0.173205	21.82394 ± 0.57735	31.43724 ± 0.173205
Cr	12.1051 ± 0.46188	16.44843 ± 0.055076	11.19034 ± 0.173205	10.33974 ± 0.057735	23.71692 ± 0.057735
B	9.644166 ± 0.80829	8.268384 ± 0.057166	6.790562 ± 0.057735	12.50646 ± 0.57735	10.5506 ± 0.057735
Co	12.6119 ± 0.51316	13.24436 ± 0.057449	11.22673 ± 0.057735	11.04435 ± 0.005774	12.04435 ± 0.005774
V	7.825573 ± 0.503322	7.136994 ± 0.057166	5.167253 ± 0.57735	6.115242 ± 0.57735	15.33155 ± 0.057735
Y	14.56597 ± 0.503322	11.21149 ± 0.060828	10.89192 ± 0.057735	6.321809 ± 0.57735	16.93436 ± 0.011547
Ga	4.764481 ± 0.623538	9.072364 ± 0.011547	3.195728 ± 0.680686	5.055294 ± 1.154701	14.80622 ± 0.005774
Pr	6.855615 ± 0.011547	5.335955 ± 0.05	5.051339 ± 0.028868	3.046101 ± 0.034641	8.327819 ± 0.057735
Se	4.424484 ± 0.057735	4.375814 ± 0.05837	3.63396 ± 0.34641	2.525315 ± 0.017321	3.321944 ± 0.057735
Gd	5.304772 ± 0.190539	4.122109 ± 0.057735	3.855442 ± 0.57735	2.249681 ± 0.005774	6.251332 ± 0.057735
Sm	4.240168 ± 0.042564	3.91512 ± 0.057822	3.410008 ± 0.173205	2.031552 ± 0.005774	5.6794 ± 0.173205
Li	2.208819 ± 0.000902	2.493184 ± 0.046159	2.011212 ± 0.014565	1.668211 ± 0.57735	3.313335 ± 0.057735
As	4.642374 ± 0.041886	3.339049 ± 0.046194	2.744356 ± 0.34641	1.673618 ± 0.57735	4.752068 ± 0.057735
Dy	1.679462 ± 0.005774	1.92816 ± 0.054271	1.01443 ± 0.005774	1.032609 ± 0.017321	2.933963 ± 0.057735
Sc	0.847444 ± 0.005774	0.809872 ± 0.058014	0.473593 ± 0.1	0.656396 ± 0.057735	1.230956 ± 0.023094
Cd	1.58425 ± 0.112694	0.924124 ± 0.11576	0.405736 ± 0.208167	0.37792 ± 0.23094	1.663265 ± 0.04772
Th	0.432498 ± 0.017559	0.865954 ± 0.003464	0.181 ± 0.057735	0.933131 ± 0.011547	1.452252 ± 0.011547
Er	0.978176 ± 0.026458	0.81665 ± 0.005508	0.633949 ± 0.11547	0.424043 ± 0.011547	1.26346 ± 0.011547
Cs	0.455627 ± 0.028918	0.438053 ± 0.01	0.310439 ± 0.057735	0.408961 ± 0.005774	0.654966 ± 0.005774
Yb	0.171043 ± 0.017321	0.452301 ± 0.034641	0.607498 ± 0.057735	0.120148 ± 0.005774	0.767828 ± 0.005774
Eu	0.174358 ± 0.025981	0.455287 ± 0.021362	0.497044 ± 0.057735	0.023871 ± 0.005774	0.752082 ± 0.028868
Tb	0.436285 ± 0.002887	0.459525 ± 0.058924	0.328601 ± 0.057735	0.235315 ± 0.005774	0.643939 ± 0.002887
Ho	0.426438 ± 0.017321	0.367546 ± 0.058832	0.256826 ± 0.057735	0.235315 ± 0.028868	0.511165 ± 0.009033
U	0.089015 ± 0.01	0.288567 ± 0.055664	0.02649 ± 0.005774	0.035929 ± 0.005774	0.73126 ± 0.005774
Tl	0.096498 ± 0.001	0.119401 ± 0.027555	0.014297 ± 0.005774	0.076135 ± 0.028868	0.120861 ± 0.005774
Tm	0.0399 ± 0.010536	0.096134 ± 0.002517	0.035139 ± 0.005774	0.027294 ± 0.017321	0.157744 ± 0.023094
Be	0.050758 ± 0.002887	0.049947 ± 0.004071	0.051831 ± 0.000577	0.027745 ± 0.005774	0.087557 ± 0.001732
In	0.05772 ± 0.006429	0.076439 ± 0.005292	0.040781 ± 0.005774	0.040492 ± 0.005774	0.116225 ± 0.005774
Lu	0.090961 ± 0.006083	0.080174 ± 0.015319	0.062445 ± 0.005774	0.029883 ± 0.011547	0.111271 ± 0.005774
Re	0.0043 ± 0.005196	0.00513 ± 0.005947	0.002436 ± 0.00052	0.001627 ± 0.000173	0.000466 ± 0.000561
Bi	0.052993 ± 0.000643	0.057524 ± 0.001	0.074106 ± 0.005774	0.045035 ± 0.003464	0.078048 ± 0.001155

Data represented as mean ± standard deviation

concentrations than *P. monteilii* treated plants. In the case of micronutrients, most of the elements (aluminium, manganese, iron, vanadium, boron) in *P. rhodesiae* treated plants were elevated than in *P. fluorescens* and *P. monteilii* treated plants. Other elements like neodymium, rubidium, strontium, barium, lanthanum, cerium, lead, lithium, arsenic, cadmium, praseodymium, gadolinium, samarium, beryllium, indium, caesium, europium, scandium, terbium, bismuth, chromium, gallium, thorium, uranium, holmium, erbium, thulium, ytterbium, lutetium, thallium and dysprosium were lower in *P. monteilii* treated plants than that of *P. fluorescens* treated plants except nickel, caesium, strontium, cerium, neodymium, ytterbium, europium, bismuth, beryllium and lanthanum. In case of *P. rhodesiae* treated plants, other elements except zinc, sodium, cobalt, copper, nickel, yttrium, selenium and rhenium were elevated than in *P. fluorescens* and *P. monteilii*. Even though similar studies have not been conducted previously in detail, current results confirm the role of rhizobacterial interaction in determining the elemental composition of plants. In a previous study, *Pseudomonas* sp. A3R3 have been reported to be effective in promoting the phytoremediation potential of both host (*Alyssum serpyllifolium*) and nonhost (*Brassica juncea*) plants by improving either the Ni accumulation or biomass production (Ma et al. 2011). At the same time, inoculation of plants with phosphate solubilizing organism has also reported to result in increased P contents and associated 10–15% increase in crop yields (Gyaneshwar et al. 2002). Another study has also suggested bacterial role in protecting plants from the inhibitory effects of nickel, lead, and zinc by providing the plants with sufficient iron (Burd et al. 2000). Inoculation of *P. fluorescens* at a concentration of 10^9 cfu/mL was also observed to cause significant increase in availability of Fe by 34.75% in plants (Pratiwi et al. 2016). Macro and micronutrient modulation and associated increase of growth have also been reported in Sorghum due to seed bacterization with *Pseudomonas* spp. (Kumar et al. 2012). In another study, nutrient content (P, Fe, Zn, K and Mg) and plant growth of strawberry have been suggested to be modulated by *Bacillus* and *Pseudomonas* sp. (Esitken et al. 2010). These indicate the remarkable modulatory effect of plant elemental composition by associated microorganisms.

Plant growth promoting rhizobacteria are potential tools for the sustainable agricultural trend for the future. In the study, previously isolated *Pseudomonas* species were found to enhance the growth of *V. unguiculata* seedlings when compared to that of control. From the ICP-MS analysis result, differential modulatory effect of *Pseudomonas* species on the elemental composition of plants could be confirmed. Hence, a detailed insight into the plant growth promoting and element modulatory functions *Pseudomonas* can pave the way to make use of rhizobacteria to engineer plants with desired nutritional composition.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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