fibrous nature of browse resources, as observed in most of the villages under study.

Differences in neutral detergent fibre and acid detergent fibre (ADF) contents and cellulose contents in the browse materials were not observed due to similar nature and hardiness of the collected grasses. However, higher content of ADF revealed that the plant materials were fibrous and hence less digestible for livestock. Grasses were hard to cut, reflecting their fibrous nature and thereby expected to have lower palatability for various livestock species. Differences in hemicellulose and lignin content were observed, which can be attributed to differences in the maturity of plant species in the grasslands. Domesticated species, especially yak, pashmina goat, pony and horse were found to sustain on these grasslands, reflecting their better fibre digesting ability. Under harsh climatic conditions, only those grasses survive which can cope with aridity, extreme diurnal temperature fluctuations, strong winds and abrasion, solifluction at higher altitudes and salinity at lower altitudes, nutrient-poor soils and a short growing season. Under such conditions, productivity is generally low and the vegetation is sparse[9]. Growing season is confined to a short period from June to August in summer, and alpine steppe communities with medium to sparse cover (20%) dominate the vegetation. Dominant grass species like *Carex* and *Stipa* have high fibre content. Cold deserts of the Himalaya have short-lived species, which provide cover for 2–3 months. Grazing resources comprise 14.2% of land in Leh and 16.8% in Kargil district, Ladakh. Lower productivity of grasslands could also be attributed to high grazing pressure during sample collection, as this study was conducted during early winter when the crop residues available in households or fields are minimal.

On the basis of the present study, it can be concluded that grasses in the Ladakh region have high fibre content with poor nutritional value for livestock. Productivity of grasslands differs from one area to another, perhaps due to variation in the natural terrain, soil texture and salinization of the land that also affect the nutritional composition of the grasses.

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Manoeuvring prospective rhizosphere-competent bacteria for invigorating growth in chickpea

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The exploration for beneficial rhizosphere-competent bacteria commenced with screening isolated strains for plant growth-promoting attributes, including secretion of indole-3-acetic acid, gibberellins, 1-aminocyclopropane-1-carboxylic acid deaminase, solubilization of phosphate and zinc. The secretion of flavonoid-like compounds revealed quantitative as well qualitative variability among the isolates as their culture supernatant exhibited several fluorescent compounds on TLC plates with different mobilities. Inoculation of seeds with effective isolates under axenic condition enhanced plant growth and induced flavanoids secretion from roots, although the effect was only quantitative. The prospective bioinoculants exhibited competence in lieu of intrinsic antibiotic resistance, amylase production, biofilm formation, root infectivity, salinity tolerance and exopolysaccharide production. Seed bacterization with potential isolates alone and in consortium with rhizobia stimulated growth of chickpea plants under controlled condition.

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THE rhizo-microbiome supports a diverse population of microbes which can benefit plants[1]. Among the microbial communities inhabiting the soil, root-associated beneficial bacteria, known as plant-growth promoting rhizobacteria (PGPR)[2], have been documented as the most predominant group that promotes plant health through nitrogen-fixation, nutrient acquisition and assimilation, secretion of phytohormones, suppression of plant diseases and host induction for the production of signal substances to other symbionts, i.e. flavonoids[3]. Nonetheless, successful application of bioinoculants entails an intimate association between PGPR and the host plant[3], which depends on the competence and colonization pattern of the bacteria. The uneven relationship between associated PGPR and plants has been attributed to climatic conditions, soil properties or the composition or activity of native microbial soil flora that may affect rhizobacterial growth and hence exert their effect on the plant[1]. The best growth-promoting interaction between PGPR and plants under field conditions can be attained by evaluating the rhizobacterial effects on plants and factors affecting their relationship, including the presence of other microorganisms[4]. Therefore, it is necessary to develop efficient strains that are competent as well as able to survive and colonize the rhizospheric soil under field conditions. One possible approach is to explore soil microbial diversity having plant growth-promoting traits and well adapted to a particular environment. Keeping this in view, the present study aimed to identify rhizosphere competent bacterial isolates for their multiple plant growth-promoting activities and to study their effect on growth parameters of chickpea.

Rhizobacteria were isolated from chickpea fields in the vicinity of Punjab, Uttar Pradesh, Chhattisgarh, Himachal Pradesh and West Bengal, India. The rhizosphere samples were collected by carefully removing the plants using a shovel and the soil loosely adhered to the root was taken as rhizosphere soil. This collected soil was serially diluted in sterile water and spread on nutrient agar and King's B media. The plates were incubated at 30°C for 24 h. The morphologically different colonies were selected, purified by subculturing and maintained on the respective slants at 4°–5°C. Chickpea nodulating *Mesorhizobium ciceri* was procured from the Department of Microbiology, Punjab Agricultural University and maintained on yeast extract mannitol agar.

Screening of all the isolates for phosphate solubilization was done by inoculating overnight-grown bacterial cultures on plates containing NBRIP (National Botanical Research Institute's phosphate growth medium) medium[5], followed by incubation at 28°C for 5 days. The colonies were observed for the formation of yellow coloration around them, and P-solubilization index was calculated as the sum of colony diameter and halo zone diameter/ colony diameter. The zinc-solubilization ability was tested using modified Pikovskaya medium containing 0.1% insoluble ZnO and halo around inoculated bacteria was recorded. Zinc solubilization index was then calculated.

Quantitative estimation of indole-3-acetic acid (IAA) production by rhizobacteria was done by inoculating bacterial cultures in Luria broth supplemented without and with (3 mM) tryptophan (Trp) followed by incubation for 72 h at 30°C. Cultures were then centrifuged and 2 ml supernatant was mixed with two drops of orthophosphoric acid followed by 4 ml of the Salkowski reagent. After incubation for 20 min at room temperature, the pink colour developed was read at 530 nm (ref. 6).

For gibberellic acid (GA_3) production[7], bacterial cultures inoculated in nutrient broth and incubated at 37°C for seven days were centrifuged at 8000 rpm for 10 min. Next, 15 ml of the supernatant was mixed with 2 ml of zinc acetate solution (21.9 g of zinc acetate was mixed with 80 ml of distilled water, and 1 ml of glacial acetic acid was added and the volume made up to 100 ml with distilled water). After 2 min, 2 ml of potassium ferrocyanide solution (10.6 g of potassium ferrocyanide in 100 ml of distilled water) was added and centrifuged at 8000 rpm for 10 min. Next, 5 ml of supernatant was added to 5 ml of 30% HCL and the mixture was incubated at 27°C for 75 min. The blank was prepared with 5% HCL. Absorbance was read at 254 nm in a UV–VIS spectrophotometer. Standard curve was prepared using GA_3 solutions of known quantities and the amount of GA_3 produced by the culture was calculated and expressed as μg 25 ml^{-1} broth[8]. For the estimation of 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) activity, bacterial cultures were grown overnight in 10 ml of nutrient broth and the pellet harvested by centrifugation was washed with normal saline and suspended in 7.5 ml of DF medium containing 5 mM of ACC deaminase. After incubation for 24 h at 28°C with shaking, the cells were centrifuged at 8000 rpm for 10 min at 4°C. The pellet was suspended in 1 ml of 0.1 M *Tris*-HCl (pH 7.6) and again harvested by centrifugation at 15,000 rpm for 15 min to be resuspended in 600 μl of 0.1 M *Tris*-HCl (pH 8.5). Next, 30 μl of toluene was added and tube was kept at 4°C for 1 h and then centrifuged at 1200 rpm for 10 min at 4°C. To 200 μl of toluenized cells, 20 μl of 0.5 M ACC deaminase was added, vortexed and incubated at 30°C for 15 min. Next, 1.0 ml of 0.56 M HCl was then added, vortexed and centrifuged for 10 min at 12,000 rpm. To 1 ml of the supernatant, 800 μl of 0.56 M HCl and 300 μl of 2,4-dinitrophenylhydrazine (2% w/v) were added, vortexed and incubated at 30°C for 30 min. Next, 2 μl of 2 M NaOH was added and absorbance was recorded at 540 nm. The ACC deaminase activity was expressed as μmol/mg protein/h using α-ketobutyrate (0.1 and 1.0 μmol) as standard[8].

The extraction of flavanoid-like compounds was done from the supernatant of 100 ml four-day-old broth

culture. The supernatant was mixed with 20 ml ethyl acetate and shaken thoroughly for 10–15 min. Then the upper layer of ethyl acetate was separated from each isolate by discarding the lower layer. The extracts were dried at 40°C and redissolved in 2 ml of 70% ethanol. Total flavonoid content was estimated employing the aluminium chloride method proposed by Zhishen *et al.*[9], using naringen as standard.

The processing of extracted material was done by thin layer chromatography (TLC) using glass plates (20 × 15 cm) coated with silica gel (GF254). The gel slurry was prepared by dissolving 40 g silica gel in 100 ml water, followed by activation at 110°C for 1 h. The plates were charged with a 50 μl aliquot of each sample and developed in solvent-saturated chromatography jars using ethyl acetate : ethanol : water (5 : 1 : 5 v/v/v) solvent mixture. The developed plates were air-dried and visualized under UV light at 365 nm after the addition of liquid ammonia[10].

Surface-sterilized chickpea seeds of GPF-2 variety were imbibed in 10 ml of rhizobacterial isolates, alone and in combination with *Mesorhizobium ciceri* (in 1 : 1 ratio) for 1–2 h. Then they were transferred to sterile petri plates containing double layer of filter paper moistened initially with 4 ml of sterile distilled water, six seeds per plate. The plates were incubated at 22°C for nine days. Roots were cut, weighed and ground in a mortar and pestle with 1 ml of 70% ethanol. Total flavonoid content in the ground material was estimated using the method proposed by Zhishen *et al.*[9] and processed by TLC. Other growth parameters such as root and shoot length, root and shoot fresh weight were recorded.

The experiment was conducted during 2013–14 in the Schools of Biotechnology, PAU, Ludhiana, to study the effect of rhizobacteria as seed inoculants, alone and in consortium with *M. ciceris.* Medium black clayey soil from chickpea fields was autoclaved 3x (1 h, 121°C) at 12 h-intervals and 1 kg sterilized soil was filled per pot. Surface sterilization of seeds was done with 0.1% mercuric chloride for 30 sec followed by washing 5–6 times with sterile distilled water and drying under a stream of sterile air. Bacterization of seeds was carried out by imbibing in 10 ml of bacterial inocula (3×10^8 cfu ml^{-1}) for 3–4 h with gentle shaking followed by drying. For dual inoculation bacterial and *Mesorhizobium* inocula were mixed in 1 : 1 ratio. Seeds imbibed in sterile uninoculated media served as control. Ten seeds were sown per pot. The experiment was arranged in a completely randomized design with seven replications per treatment. Growth parameters, including per cent germination, plant height, dry weight, fresh weight and chlorophyll content were recorded.

Antibiotic resistance spectrum of the isolates was studied using filter paper discs containing standard concentration (μg/disc) of antibiotics, viz. ampicillin (AM^{25}), chloramphenicol (C^{25}), tetracycline (TE^{30}), streptomycin (S^{10} and S^{25}), kanamycin (K^5), amoxicillin (AMx^{10}) and carbenicillin (CB^{100}). Antibiotic discs of different concentrations were placed on the lawns of bacteria and incubated at 28°C for 72 h. The plates were observed for zone of inhibition around the discs[11].

Amylase assay was carried out using a reaction mixture consisting of 0.5 ml enzyme extract and 0.5 ml (1.0%) soluble starch in 50 mM phosphate buffer (pH 7.0), incubated at 30°C for 3 min followed by addition of 1 ml of 3,5-dinitrosalicylic acid to stop the enzymatic reaction. The mixture was then incubated in a boiling water bath for 5 min and after cooling at room temperature 10 ml distilled water was added and reading of the colour was recorded at 540 nm. One unit (U) of amylase is defined as the amount of enzyme that liberates 1 μg of reducing sugars, measured as glucose/min (ref. 12).

Freshly grown bacterial cultures in nutrient broth were diluted to a final optical density of 0.02 (at 600 nm) and 150 μl of culture was transferred to polystyrene 96-well microtitre plates. After maintaining for 24 h 30°C, the cultures were removed and microtitre plate wells were gently washed three times with 150 μl of sterile water to remove any loosely allied bacteria followed by drying at 30°C for 30 min. Samples were stained by adding 1% crystal violet solution to each well and incubated for 20 min. The vessels were washed and intensity of crystal violet staining was measured by addition of dimethyl sulphoxide to each dry well. The samples were incubated for 20 min and OD_{590} values were recorded on a plate reader. All samples were tested in seven independent-wells[13].

Root colonization capability of different strains was studied by inoculating pre-germinated, surface-sterilized chickpea seeds with broth of the respective strains for 30 min. The seeds were then placed in sterilized petri plates containing double layers of moistened filter paper, incubated at 22 ± 1°C. After seven days, 0.5 g root tips were removed, surface sterilized in 0.1% mercuric chloride and washed with sterile water thrice. It was then shaken vigorously in 9.5 ml of sterile water in an orbital shaking incubator at 100 rpm. The bacterial suspension was diluted from 10^{-2} to 10^{-5}, poured into petri plates and incubated at 28° ± 1°C. The colonies were counted and cfu ml^{-1} was calculated.

The ability of rhizobacterial isolates to tolerate different salinity levels was examined as described by Zahir *et al.*[14]. Bacterial isolates were inoculated in the respective broths at different salinity levels (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 M NaCl), incubated for 48 h at 28° ± 1°C with shaking at 200 rpm, and growth was measured at 600 nm.

Exopolysaccharide (EPS) production was evaluated by the method of De Vuyst *et al.*[15]. For this, 24 h old bacterial cultures (OD_{600} 0.3) were inoculated in 100 ml of medium described by Verhoef *et al.*[16] supplemented with varying NaCl concentration (0, 0.2, 0.4, 0.8, 1.2 M),

incubated in a 160 rpm shaker for 48 h at 30°C, centrifuged under optimized conditions (1000 rpm for 15 min at 4°C), and the supernatant was precipitated using three volumes of pre-chilled acetone. Weight of freshly precipitated EPS was recorded.

Experimental data were analysed using standard analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range test (DMRT) to determine the significant difference between groups of data. The results were considered significant at $P \leq 0.05$ level. Standard error was calculated for all mean values.

The rhizo-microbiome discoveries could fuel advances in sustainable agriculture by developing microbial inoculants as biofertilizers, biocontrol or stress protection products[17]. A total of 63 soil-inhabiting bacteria differing in morphological and cultural characteristics were isolated from different chickpea rhizospheres; 35 were picked from King's B medium while 28 from nutrient agar medium.

All the isolates were tested for their plant growth-promoting traits to select the candidates having potential to emerge as bioinoculant. The tri-calcium phosphate solubilizing ability was revealed by 15 isolates on NBRIP medium, which ranged from 1.14 to 2.3 (Table 1). Although the isolate Ps19d did not exhibit P-solubilization activity on NBRIP medium, it solubilized phosphate on Pikovskaya medium in another set of experiments conducted by Kumari *et al.*[18]. This indicates that the visible halo/zone formation on agar plates due to the production of organic acids as the criterion for isolation of phosphate solubilizers is a less reliable approach, and more efficient results could be drawn from broth-based assay. A similar experiment conducted for selecting Zn-solubilizing bacteria revealed that 20 isolates were able to solubilize ZnO, including Ps14d and Ps14c which were efficient P-solubilizers. In the soil, both macro- and micronutrients undergo a complex dynamic equilibrium of solubilization and insolubilization that is greatly influenced by soil pH and microflora, ultimately affecting their accessibility to plants[19]. A search for microorganisms with dual ability to solubilize micro- and macronutrients could proficiently enhance plant growth by reducing the load of chemical fertilizers. The importance of insoluble P-solubilizing microbial strains *in vitro* for managing nutrient requirements of plants has been demonstrated by Suseelendra *et al.*[20].

Evaluating the rhizobacterial isolates for their ability to produce IAA, it was observed that all isolates tested positive for IAA synthesis. Nevertheless, presence of Trp greatly affected IAA production; although it is considered to be strain-dependent[1]. In the absence of Trp, IAA production ranged from 1.78 to 39.02 μg/ml which increased to 9.96–45.7 μg/ml in the presence of 3 mM Trp (Table 1). This is likely to be associated with biosynthesis of IAA using Trp as precursor as the most common pathway. The correlation of IAA biosynthesis by rhizobacteria with stimulation of root proliferation is well documented[21], as it increases the ability of young seedlings to anchor themselves to the soil, and obtain water and nutrients from their environment, thus enhancing their chances for survival[22]. Apart from IAA production, the beneficial effect of PGPR on growth and yield of many crop plants can likely be highlighted, at least in part, by gibberellin production[23]. The gibberellin production by 29 rhizobacterial isolates ranged from 11.90 to 30.70 μg/ml, the highest being observed with isolates Ps14c and B-I (25.30 and 24.89 μg/ml respectively; Table 1). Probanza *et al.*[24] reported that inoculation with *Bacillus licheniformis* and *Bacillus pumilus* improved growth of *Pinus pinea* plants, apparently through production of bacterial gibberellins.

The rhizobacteria also have immense potential to enhance plant growth under stress conditions by altering the synthesis of stress-induced ethylene through the production of enzyme ACC deaminase. The search for ACC-deaminase producing bacteria revealed that the isolates were able to use ACC deaminase as the sole source of nitrogen. Biochemical assay for ACC deaminase showed that isolates Ps14c, B20b and B-I (4.61, 2.56 and 1.41 μM α-ketobutyrate/mg protein/h respectively) were the strongest producers (Table 1). In corroboration with the present findings, Ali *et al.*[25] reported ACC deaminase production by nine isolates of drought-tolerant *Pseudomonas fluorescens*, maximum being observed with strain SogrP4 (3.71 μM α-ketobutyrate/mg protein/h). ACC deaminase-containing bacteria reduce the need of plants to actively protect themselves against various environmental stresses by upregulating the genes involved in plant growth and protein production while downregulating those involved with ethylene stress and defence signalling pathways[26].

The role of flavonoids in the nitrogen-fixing symbiosis between legumes and rhizobia as plant chemotactic signals to rhizobia and as activators of *nod* gene expression is well documented[27]. Flavonoids exuded through the plant roots interact with the product of rhizobial *nodD* gene, which then colonizes the soil in the vicinity of the root hair[28]. In the present study, evaluation of PGPR for the production of flavonoid-like compounds revealed a great variation in the range, viz. 25.55–85.77 μg/100 ml (Table 1). Reportedly, bacteria, especially *Pseudomonas* and *Bacillus* associated with the rhizosphere of various leguminous crops, may assist rhizobia in root colonization by producing compounds similar to that of flavonoids[10]. To ascertain compounds in the culture media, ethyl acetate extracts of broth culture supernatant fluids of different isolates were processed by TLC using naringin (flavanone glycoside) as a standard. Under UV transillumination, the TLC plates showed a single fluorescent pigment in culture supernatants of isolates B-20d and B-I having similar mobility as that of naringin, while culture supernatant of isolate Ps14c showed two fluorescent

Table 1. Plant growth-promoting determinants of selected rhizobacteria

Isolates	P-solubilization index	Zn-solubilization index	IAA production (μg/ml)		Gibberellic acid production (μg/25 ml)	ACC-d activity (μM α-ketobutyrate/ mg/h)	Flavonoids production (μg/100 ml)
			No tryptophan	3 mM tryptophan			
Ps13b	–	–	13.07 ± 1.04	12.84 ± 0.25	14.38 ± 1.28	0.76 ± 0.02	–
Ps14c	1.71 ± 0.06	1.52 ± 0.12	7.61 ± 0.28	24.86 ± 2.15	25.30 ± 2.21	4.61 ± 0.12	85.77 ± 6.10
Ps14d	2.33 ± 0.05	1.62 ± 0.09	18.83 ± 1.28	16.33 ± 2.19	12.20 ± 1.17	–	–
Ps15a	1.14 ± 0.08	1.27 ± 0.07	21.18 ± 0.10	25.31 ± 1.17	18.80 ± 1.11	–	–
Ps19d	–	1.77 ± 0.15	1.78 ± 0.18	20.42 ± 0.24	20.60 ± 1.17	–	–
B17b	–	–	39.02 ± 1.11	42.77 ± 3.29	15.14 ± 0.28	–	–
B20b	1.20 ± 0.11	1.14 ± 0.08	3.94 ± 0.08	45.70 ± 3.28	15.80 ± 1.08	2.56 ± 0.08	25.55 ± 2.14
B20d	1.14 ± 0.02	–	2.16 ± 0.09	29.93 ± 2.07	21.32 ± 2.11	1.36 ± 0.11	52.92 ± 4.07
B28c	–	1.23 ± 0.03	8.18 ± 0.10	19.62 ± 1.07	24.85 ± 1.18	1.04 ± 0.09	34.67 ± 3.09
B-I	1.55 ± 0.02	–	14.58 ± 0.28	30.50 ± 1.28	24.89 ± 2.17	2.42 ± 0.04	54.75 ± 3.20

Values represent mean ± SE of three replications. IAA, Indole-3-acetic acid; ACC-d, 1-aminocyclo propane-1-carboxylic acid-deaminase.

Table 2. Effect of seed bacterization on induction of flavonoids from roots and growth parameters of chickpea under axenic condition

Treatment	Flavanoids (mg/gfw)	Fresh weight (mg/plant)		Length (cm/plant)	
		Root	Shoot	Root	Shoot
Ps14c	54.64^{b}	61.17^{bc}	10.01^{c}	5.18	1.00^{fg}
Ps14c + R	76.78^{a}	71.13^{a}	16.90^{a}	5.37	3.12^{a}
B20d	51.07^{cd}	55.00^{d}	8.50^{c}	6.67	1.00^{f}
B20d + R	62.14^{b}	68.75^{ab}	8.84^{c}	6.79	1.25^{ef}
B20b	55.35^{b}	63.33^{abc}	9.45^{c}	4.24	1.00^{eg}
B20b + R	58.90^{bc}	65.83^{abc}	13.66^{ab}	4.79	1.94^{d}
B-I	53.92^{bcd}	60.41^{c}	9.10^{c}	5.15	1.95^{d}
B - I + R	73.57^{a}	68.16^{ac}	16.94^{a}	6.13	2.30^{cd}
B28c	60.35^{b}	62.91^{bc}	13.00^{b}	4.33	1.50^{e}
B28c + R	58.21^{bc}	64.16^{abc}	16.45^{a}	4.62	2.50^{b}
Control	45.71^{d}	50.83^{d}	7.90^{c}	4.06	0.80^{g}
CD@5%	8.35	8.13	3.42	NS	0.37

In the same column, significant differences at $P < 0.05$ levels are indicated by different letters. Data followed by the same letter in the same column are not significantly different from each other according to analysis of variance (ANOVA).

compounds, one with higher mobility and other similar to naringin. However, isolate B-20b exhibited one fluorescent spot of lower mobility, whereas none was observed with B28c. Similar findings have been reported by Parmar and Dadarwal[10], where the TLC plates showed no fluorescent pigment in culture supernatant of *Bacillus* CRS70, whereas culture supernatant of *Pseudomonas* sp. CRP55b showed two fluorescent compounds with relative mobility in the solvent system used. The production of flavonoid-like compounds by rhizobacterial isolates confirms their role in enhanced nodulation through better rhizobial colonization and plant growth promotion assisted by phytohormones production and improved nutrient uptake. Andrade *et al.*[29] reported that an increase in nodulation in pea caused by inoculation with *P. fluorescens* was due to an increase in flavonoid exudation by the host plant.

The effect of seed bacterization on flavonoid exudation from plant roots under axenic condition showed higher accumulation of flavonoids in the roots in response to bacterial inoculation compared to uninoculated control (Table 2). Co-inoculation of isolates Ps-14c and B-I with *Mesorhizobium* recorded the highest flavonoid content in the roots (76.78 and 73.57 mg/g fresh wt respectively). It has been reported that rhizobacteria induce phytoalexins (a class of fluorescent compounds, closely related to flavonoids and isoflavonoids) in the roots of several crop plants[30] which results in antibiosis in the rhizosphere for pathogenic organisms and thus increases nodulation by providing better environment for rhizobial colonization. Processing of root extracts by TLC revealed a single fluorescent compound with higher mobility compared to fluorescent pigments observed with the supernatant extract of bacterial isolates. In contrast, Parmar and Dadarwal[10] reported three different compounds in the root extract of plants inoculated with *Pseudomonas*, whereas two spots in *Bacillus*-inoculated extracts. Moreover, seed bacterization resulted in higher root fresh weight, shoot fresh weight, root and shoot length compared to uninoculated control (Table 2).

Table 3. Effect of seed bacterization on growth parameters of chickpea under glasshouse condition

Treatment	% Germination	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Chlorophyll content (mg/g)
B-I + R	95	23.30[a]	9.3[a]	2.06	0.94[a]
Ps14c + R	95	22.62[ab]	8.7[ab]	1.80	0.85[a]
B-I	94	22.55[ab]	9.1[a]	1.91	0.96[a]
Ps14c	93	21.35[ab]	8.1[ab]	1.60	0.75[ab]
Control	90	19.11[b]	6.6[b]	1.20	0.61[b]
CD@5%		4.10	2.12	NS	0.21

In the same column, significant differences at $P < 0.05$ levels are indicated by different letters. Data followed by the same letter in the same column are not significantly different from each other according to ANOVA.

Table 4. Intrinsic antibiotic resistance spectra of rhizobacterial isolates

Isolates	Ampicillin (25 mcg)	Tetracycline (30 mcg)	Streptomycin (10 mcg)	Chloramphenicol (25 mcg)	Kanamycin (5 mcg)	Amoxicillin (10 mcg)	Carbenicillin (100 mcg)
Ps14c	R	S(+)	S(+++)	S(++)	S(+++)	S(+)	S(+)
B20d	R	S(+)	S(+++)	S(++)	S(++)	S(+)	S(+)
B20b	R	S(+)	S(++)	S(++)	S(++)	S(+)	S(+)
B28c	R	R	S(++)	S(++)	S(++)	S(+)	S(+)
B-I	R	R	S(+)	R	S(+++)	S(+)	S(+)

R, Resistant; S, Sensitive; +, Least sensitive; ++, Moderately sensitive and +++, Highly sensitive.

Based on *in vitro* assayed plant growth-promoting traits, isolates Ps-14c and B-I were used as single as well as co-inoculants with chickpea-nodulating *M. ciceri* to determine their plant growth-promoting effect under glasshouse condition. The isolates significantly enhanced plant biomass, plant height and chlorophyll content in inoculated plants compared to uninoculated control (Table 3). However, isolate B–I was the most effective in terms of shoot weight and chlorophyll content compared to Ps–14c. In the co-inoculation experiment, further increase in plant height, plant weight and chlorophyll content in plants was observed (Table 3), which signifies secretion of some plant growth-promoting hormones such as IAA, gibberellins, etc. by *Rhizobium* leading to synergism between the two isolates. There are reports on the production of plant growth regulators like auxins, cytokinins and gibberellins by rhizobia that stimulate and enhance plant growth[31]. Moreover, synergism could also be attributed to the flavonoid-like compounds produced by rhizobacteria, which assist in better colonization of rhizobia.

The replication of successful soil microcosm results of PGPR under field conditions has been reported to be limited by the environment that does not support growth and survival of the introduced microorganisms in the plant rhizosphere[32]. Manipulating soil environment to make it better for bacterial survival is not feasible, but understanding the mechanisms that assist in their subsistence under stress condition would be the key to improving the level and reliability of plant growth stimulation by PGPR. Therefore, we focused on characterizing potent PGPR (Ps14c, B20d, B-20b, B-I and B-28c) for determinants related to their survival and colonization in the rhizo-microbiome that would possibly aid in developing efficient biofertilizer inoculants under field condition.

The intrinsic antibiotic resistance spectra of rhizobacteria offer an ecological advantage of survival in the rhizosphere when they are introduced as incoulum[33]. Most of the isolates were resistant to ampicillin and sensitive to chloramphenicol, streptomycin and erythromycin (Table 4). However, isolate B–I showed intrinsic resistance to ampicillin, tetracycline and chloramphenicol and exhibited less sensitivity to streptomycin, kanamycin, amoxicillin and carbenicillin, which is a desirable feature for developing efficient microbial inoculants.

The survival of organisms in a new environment depends on their ability to utilize the source of nutrients available at a particular site. In this regard, amylase has importance as starch is widely distributed in nature and the enzyme can assist in the easy survival of microorganisms by mediating hydrolysis of starch or any other readily available related α-glucan elsewhere[34]. In the present study, all the five potent isolates produced amylase, the maximum by B20d (38.7 U/ml/min) followed by Ps14c (35.7 U/ml/min; Table 5). Amylase has also been reported to affect the cell wall of some phytopathogenic fungi where glycogen, a polysaccharide closely related to starch, is present. It is unlikely that this enzyme plays a key role in the early phase of antagonistic association[35].

Biofilms are bacterial communities in which the cells are embedded in a matrix of extracellular polymeric compounds attached to the surface, which protects them from deleterious conditions[36]. Biofilm formation assayed by crystal violet staining was evident for rhizobacterial isolate B-20d followed by Ps-14c and B20b (ref. 37; Table 5). The dense biofilm matrix limits diffusion of

Table 5. Competence-related determinants of potent rhizobacterial isolates

Isolates	Amylase activity (U/ml/min)	Biofilm formation (OD_{590})	Growth under varying NaCl concentration (OD_{600})					Exopolysaccharide production at different salinity levels (fresh weight in g)					
			0.5 M	1.0 M	1.5 M	2.0 M	2.5 M	0 M	0.2 M	0.4 M	0.8 M	1.0 M	1.2 M
Ps 14c	35.70 ± 3.11	0.111 ± 0.009	0.115 ± 0.02	0.085 ± 0.004	0.015 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–
B20d	38.70 ± 4.29	0.353 ± 0.016	0.892 ± 0.09	0.784 ± 0.09	0.539 ± 0.05	0.192 ± 0.01	0.085 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.17 ± 0.03	0.22 ± 0.06	0.13 ± 0.02	0.12 ± 0.02
B20b	17.50 ± 2.96	0.078 ± 0.001	0.780 ± 0.07	0.669 ± 0.08	0.493 ± 0.04	0.115 ± 0.01	0.082 ± 0.01	0.16 ± 0.01	0.18 ± 0.02	0.19 ± 0.04	0.18 ± 0.04	0.31 ± 0.08	0.10 ± 0.01
B28c	19.00 ± 2.87	0.101 ± 0.008	0.65 ± 0.07	0.480 ± 0.01	0.296 ± 0.02	0.061 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–
B-I	19.70 ± 3.01	0.073 ± 0.003	0.770 ± 0.08	0.568 ± 0.02	0.346 ± 0.03	0.105 ± 0.01	0.024 ± 0.02	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.03	0.21 ± 0.06	0.35 ± 0.10	0.28 ± 0.07

Values represent mean ± SE of three replications.

bioactive substances and nutritional elements secreted by root bacteria which remain concentrated at the root surface and directly affect plant growth. It has been reported that plant roots are not passive targets for bacteria and inability of PGPR to act as efficient bioinoculants under field condition has often been related to their lack of capability to colonize plant roots. However, biofilm formation could faithfully reflect bacterial colonization. Likewise, the results of viable counts of bacteria from infected chickpea roots indicated rapid colonization by B20b (190×10^6 cfu/g) followed by B20d (119×10^6cfu/g), B–I (37×10^6 cfu/g), Ps14 c (90×10^5 cfu/g) and B28c (67×10^5 cfu/g). Efficient colonizers can be developed after inoculating seedlings with a combination of different bacteria, followed by growth of the seedlings in a gnotobiotics system[38].

Screening for osmoadaptation revealed that isolates B20d, B20b and B-I were able to tolerate up to 2.5 M salinity level, whereas B28c was relatively less tolerant (Table 5). Isolate Ps-14c which surpassed in all growth-promoting traits was quite sensitive to salinity even at 0.5 M level (OD_{600} 0.155). Salt-tolerant characteristics of PGPR reflect their ability to adapt and persist under stressed environment[39]. Paul and Nair[39] reported that the root colonization ability of the salt-tolerant *Pseudomonas* strain was not hindered even at higher salinity levels in the soil.

Bacterial EPS plays a crucial role in auto-aggregation, biofilm development and protection against salinity stress. The study of EPS production at different salinity levels by osmotolerant isolates revealed increasing trend in fresh weight of EPS (Table 5), thus implicating the possible role of biofilm and EPS production for effective colonization under adverse conditions. The EPS improved soil aggregation, water stability and microbial biomass stimulated root exudation under stress[40].

The screening strategies used in the present study could provide valuable strains with multiple plant growth-promoting traits that are rhizospheric competent and can establish successfully in the introduced environment. This would possibly help in combating the differences observed in the performance of efficient strains under field conditions.

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First fossil dragonfly from India

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In the Indian wetland palaeoecosystem, no dragonfly has been reported from the Cenozoic sediments until now. Here, we report a well-preserved fossil dragonfly (Odonata: Anisoptera) recovered from the late Neogene sediments of the Chotanagpur plateau, Jharkhand, eastern India. It is characterized by

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