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Original Research

Desiccation-Tolerant Rhizobacteria from Cholistan Desert, Pakistan, and Their Impact on *Zea mays* L.

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Abstract

Five desiccation-tolerant rhizobacteria (*Brevibacterium frigoritolerans*-LPS1B, *Bacillus subtilis*-CHFT15, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *Pseudomonas stutzeri*-CHP413A) isolated from Pakistan's Cholistan desert were characterized on the basis of morphological, biochemical, and 16S rDNA ribotyping. The desiccation tolerance was checked at various relative humidity levels (5, 23, and 100%) for a period of 1-40 days. Heavy metal and antibiotic resistance, auxin, cytokine, siderophore, hydrogen cyanide production, and phosphate solubilization of select bacterial isolates was also investigated. Pot experiments with corn in sandy and pure soil were also carried out to check the plant growth-promoting potential of select strains after 90 days of growth. After harvest, various growth parameters like seed germination, root and shoot length, number of leaves, dry weight per gram fresh weight, and chlorophyll contents were determined. The inoculation of *P. stutzeri*-CHP413A resulted in 3, 33,12, and 37% increases in seed germination, number of leaves, shoot and root length, and dry weight·g^{-1} fresh weight, respectively, in sandy soil ($p<0.05$).

Keywords: desiccation, bacteria, exopolysaccharide, phytohormones, *Zea mays*

Introduction

Abiotic stresses affect the microbial community in soil, resulting in the loss in productivity of agricultural crops [1]. The environmental factors include scarcity of food, drought, salinity, flooding, temperatures, and pH [2, 3]. Rhizospheric microbial communities possess physiological flexibility to a particular environmental factor by changing the composition and biomass of community [4].

Plant growth-promoting rhizobacteria (PGPR) live in the rhizospheric soil and have an association with roots of various plants either directly or indirectly for enhancing the growth and development of plants [5]. PGPR strains possess various properties, like exopolysaccharide production [6] and enzyme ACC deaminase [7], to cope with a variety of abiotic and biotic stresses [8] such as salt stress [9], flooding stress [10], drought stress [11], heavy metal stress [12], and pathogen attack [13]. Desiccation is one of the major problems in the agriculture sector causing limited crop yield in most of the arid and semiarid zones worldwide [14]. This form of abiotic stress causes specific and non-specific reactions, and damage at sub-cellular, cellular, and whole plant level with respect to plant water relation [15].

Generally, microorganisms live within the envelope of the exopolysaccharide (EPS) matrix in many environments like soils, lungs, etc. However, the most frequently discussed possibility is that an EPS envelope matrix protects bacteria from drought by enhancing water retention and from fluctuations in water potential by regulating the diffusion of organic carbon sources [16]. EPS also helps the bac-

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teria in colonization with the plant roots due to fibrous network material that connects the bacteria to the root surface [17]. Inoculation of maize plants (*Zea mays* L.) with desiccation-tolerant indole acetic acid (IAA) producing beneficial bacteria increased drought tolerance of plants growing in arid as well as semiarid areas [18, 19].

Therefore, the present study was an attempt to isolate and characterize exopolysaccharide (EPS) and indole acetic acid (IAA)-producing desiccation-tolerant bacteria from Cholistan desert, a natural habitat that provided the best benefit for desiccation-stressed plants.

Materials and Methods

Sample Collection and Bacterial Isolation

Different soil and plant samples were collected from the Cholistan desert and Multan Bypass, Pakistan. Samples were collected in tightly packed plastic bags and transferred to the laboratory, where they were kept at room temperature. The pH and moisture content was determined for each sample. For isolation of single bacterial colonies, tenfold dilution was prepared for each 1% sample, and 100 µl from each dilution was spread on modified Trypticase glucose yeast (mTGY) agar plates containing 5 g casein peptone, 1 g glucose, 2.5 g yeast extract, and 15 g agar in 1000 ml distilled water. The pH was adjusted between 7.2 to 7.5. After 24h of incubation at 37ºC, the growth of the different colonies was obtained. Single colonies were selected and streaked on new mTGY agar plates. The plates were incubated in the same conditions as before to get pure single colonies. Single pure colonies were preserved as 30% glycerol stocks at -80ºC for further use in experiments.

Characterization of Bacterial Isolates

Select bacterial isolates were morphologically and biochemically characterized by Gram stain, spore stain, capsule stain, catalase, oxidase, citrate utilization, urease, starch hydrolysis, nitrate reduction, denitrification, sulfide-indole-motility (SIM), gelatin liquefaction, Voges-Proskauer, H_2S production, and methyl red. The select bacterial isolates showing promising results (LPS1B, CHFT15, CHFT12, CH13, and CHP413A) were grown in LB broth at 37ºC overnight and were processed for DNA extraction using the modified CTAB method as described by Shahzadi et al. [20]. Amplification of part of 16S rDNA was performed using universal primers 16S-F (5'AAACTCAAATGAATTGACGG3') and 16S-R (5'TCCTCCGCTTATTGATATGC3'). Amplification conditions using a BIOER XP-Thermal Cycler were: 95ºC (5 min), 35 cycles of 95ºC (1 min), 55ºC (1 min), and 72ºC (2 min), with a final 5 min chain elongation at 72ºC.

Screening of Desiccation-Tolerant Bacteria

Select bacterial isolates were grown in 50 ml LB broth at 37ºC while shaking at 150×g. After 24h of incubation, the culture was centrifuged at 10,000×g for 5 min. Cell pellet was suspended in phosphate-buffered saline (PBS) and optical density (OD) of each culture was adjusted to 0.5 at 600 nm. Aliquot of 25 µl culture was transferred to a pre-washed, sterile glass slide (25×37.5 mm) and evenly distributed using the tip of another glass slide. Glass slides were left to dry at room temperature (R.H. 33%) for 6 h. A total of 27 slides for each isolate were prepared and placed vertically on the perforated plates (made up of thermo pore packing material) in three different glass desiccators.

The relative humidity of the desiccators was controlled by filling their bases with silica gel (R.H. 5%) or sterile saturated solution of potassium acetate (R.H. 23%) or sterile distilled water (R.H. 100%). Desiccators were placed in a sterile incubator at 37ºC. The survival rate of each isolate, under desiccated state, was calculated by growing bacteria on LB agar up to one month. After every five days, cells deposited over glass slides were re-suspended by adding 100 µl of sterile saline water (0.9%) and rubbing it with a micropipette tip. This suspension was transferred to 10 ml saline water and mixed well. The process was repeated four times to confirm that all cells on the slide were re-suspended. Suspension was further serial. The number of colony-forming units (CFUs) was determined by incubating plates at 37ºC for 24 h.

Cross-Metal Resistance and Antibiotic Susceptibility

Heavy metal tolerance in these isolates was checked by preparing stock solutions of different metal salts such as arsenic pentoxide (500 $\mu g \cdot ml^{-1}$), arsenic trioxide (200 $\mu g \cdot ml^{-1}$), cadmium chloride (100 $\mu g \cdot ml^{-1}$), copper sulphate (100 $\mu g \cdot ml^{-1}$), and potassium chromate (500 $\mu g \cdot ml^{-1}$). The metal tolerance profile was recorded by gradually increasing the concentration of various heavy metals in a stepwise manner with 50 $\mu g \cdot ml^{-1}$ of metal increased each time. The plates supplemented with various heavy metals were incubated at 37ºC overnight. The antibiotic susceptibility of select bacterial isolates was checked by measuring the zone of inhibition against antibiotics, chloramphenicol (30 $\mu g \cdot ml^{-1}$), streptomycin (10 $\mu g \cdot ml^{-1}$), kanamycin (30 $\mu g \cdot ml^{-1}$), oxytetracyclin (30 $\mu g \cdot ml^{-1}$), ampicillin (10 $\mu g \cdot ml^{-1}$), and erythromycin (15 $\mu g \cdot ml^{-1}$).

Screening for Plant Growth-Promoting Traits

Cytokinin Production

To check cytokinin production by the select axenic bacterial isolates, a modified cucumber cotyledon greening bioassay was used. Bacterial cultures were streaked on half sides of the M9 medium containing 20% glucose, 0.2% casamino acid, 2 pg/l biotin, and 0.7% agar for 72 h, and then the etiolated cotyledons from five-day-old cucumber seedlings were placed on the other half by avoiding direct contact [21]. After 24 h of incubation at 25ºC in the dark, the plates were exposed to light (55 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for three h at room temperature. Chlorophyll content of the cotyledons

relative to negative control (bacterial culture's lack of ability to produce cytokinin) was measured by a UV 4000 spectrophotometer, O.R.I. (Germany). Benzyl amino purine (BAP) of 10 µM concentration was taken as positive control. Increased chlorophyll content provides evidence for positive cytokinin activity. The experiment was done thrice with each bacterial culture.

Auxin Synthesis

Auxin estimation was carried out by following the method of Tang and Borner [22]. Briefly, 5 ml tryptone water was inoculated with bacterial isolates and incubated at 37ºC for 24 h. After incubation, 2-3 drops of orthophosphoric acid and 2 ml of Salkowski reagent (4.5 g of $FeCl_3$ per liter in 10.8 M H_2SO_4) were added to 1 ml supernatant, and after 30 min of incubation at room temperature, the color development from yellow to pinkish was monitored at 600 nm by a UV 4000 spectrophotometer.

Phosphate Solubilization

Bacterial isolates were stabbed by sterile toothpick on Pikovskaya medium ($g \cdot ml^{-1}$) containing glucose 10, yeast extract 0.5, $(NH_4)_2SO_4$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.1, $Ca_3(PO_4)_2$ 5, NaCl 0.2, KCl 0.2, $MnSO_4 \cdot 2H_2O$ 0.002, $FeSO_4 \cdot 7H_2O$ 0.002, and agar 1.5, and plates were incubated at 25ºC for 4-5 days. Transparent zone of clearing around the colonies of bacterial cultures indicated that phosphate has been solubilized in the clearing zone.

Hydrogen Cyanide Production

Bacterial isolates were screened for the production of hydrogen cyanide (HCN) by adapting the method of Lorck [23] described by Ahmed et al. [24]. Bacteria were streaked on N-agar plate, and a filter paper soaked in 0.5% picric acid solution was placed on the top of the agar surface. Plates were incubated at 30ºC for 4 days. Development of orange to red color indicated HCN production.

Siderophore Production

Siderophore production was checked by the method of Louden et al. [25]. Select bacterial strains were streaked on the plates of Chrome Azurol-S (CAS) medium. Yellow colored colonies of bacterial strains indicated siderophore production.

Selection of EPS Producers

EPS-producing isolates were selected on the basis of their highly mucoid growth on the solid medium and positive string test. Microscopic examination confirmed the production of extracellular polysaccharide. For microscopy, bacterial cells were fixed on a glass slide with 3% acetic acid for 30 min and then stained with Alcian Blue 8GX in 3% acetic acid. Finally, extra stain was removed by washing with distilled water and slides were viewed under a compound microscope.

Quantitative Estimation of EPS

Select bacterial isolates were grown in N-broth at 37ºC for 72 h with constant stirring at 150×g. After 72 h of incubation, cell-free extract of each isolate was prepared by centrifuging at 16,000×g for 15 min at 4ºC and then filtered through 0.2 µm filter paper using a filter assembly attached to a vacuum pump. Polysaccharides were precipitated out by mixing 1:3 volumes of extract and cold acetone and kept for 24 h at -20ºC. Precipitate was separated by centrifuging at 16,000 g for 15min at 4ºC and re-suspended in water. The suspension was lyophilized and stored at -20ºC. Quantitative estimation EPS was determined by the Phenol/Sulfuric acid essay described by DuBois et al. [26]. According to this assay, 0.5 ml sugar solution was mixed with 0.25 ml of 5% aqueous solution of phenol, and 1.5 ml H_2SO_4 (conc.) was added immediately to the mixture. The samples were allowed to stand for 10 min, vortexes for 1 min and kept for 10 to 20 min in water bath at 25ºC to 30ºC. Absorbance of each sample was measured at 490 nm by UV spectrophotometer. Blank control was prepared by substituting sugar aliquot with DDI. The concentration of extracellular polysaccharide was determined with reference to standard curve of glucose solution.

Fermentation Optimization for EPS

Media Optimization

To investigate the effects of various media on EPS production by select bacterial isolates, four different growth media, including Luria Bertani (LB) broth, tryptic soya broth (TSB), EPS selective medium [27], and Pharma media were used. Bacterial cultures were inoculated in TSB medium for making 500 µL as a starter culture and incubated at 37ºC for 24 h at 150×g. Each bacterial culture was introduced into four different growth media having cell number approximately to 10^7 $cells \cdot ml^{-1}$ and re-incubated at 37ºC for 48 h with shaking at 150×g. The growth was monitored spectrophotometrically at 600 nm, while EPS production was estimated after acetone precipitation.

Temperature Optimization

For the preparation of inoculum, bacterial isolates were grown for 24 h in TSB at 37ºC while shaking at 150×g. The number of bacterial cells was adjusted approximately to 10^7 $cells \cdot ml^{-1}$ and 500 µl was introduced into EPS medium. After the inoculation of starter culture, flasks were incubated in the range of 25ºC to 42ºC for 48 h with shaking at 150×g. The growth of each bacterial isolate was monitored at 600 nm and EPS production was estimated after acetone precipitation.

pH Optimization

To check the increase in bacterial cell number and EPS production, 500 µl starter culture of each bacterial isolate having 10^7 $cells \cdot ml^{-1}$ after 24 h of incubation at 37ºC was

inoculated into EPS medium. All the flasks were incubated at pH 6 to 10 for 48 h with shaking at 150×g and growth and EPS production were determined.

Plant Growth Experiments in the Field

Plant growth-promoting ability of selected bacterial isolates was checked with maize (*Zea mays*). Maize seeds were surface sterilized in 0.2% mercuric chloride ($HgCl_2$) for 10 min and then washed thrice with sterile distilled water to remove any traces of $HgCl_2$ from the seeds. The surface-sterilized seeds were transferred to bacterial cell suspensions having bacterial cell density of approximately 10^7 cells·ml^{-1} for 1 h, while control seeds were soaked in sterile distilled water for the same time period. After seed bacterization, 12 seeds were sown into each pot containing 3 kg of mixture of sand and soil (1:1) or field soil alone. Two replicates were also included for each bacterially treated and untreated seed groups. Pots were watered equally on a daily basis up to one month. Afterward, pots were watered twice a day up to maturation of plants. Germination of seeds was recorded after two weeks and percentage germination was calculated from the data. Thinning of the plants was carried out after one month and only seven plants were left to grow in each pot. Plants were harvested after maturation and they were monitored for root length, shoot length, fresh mass, dry mass, and chlorophyll content in each type of treated and control plant.

Statistical Analysis

All the data were tested statistically by analysis of variance (ANOVA) using SPSS + Version 20. Each treatment in pot experiment with maize was analyzed with five replicates and a standard deviation (SD) was calculated. The data expressed are mean±SD of each replicate, then means were compared by Duncan's multiple range test.

Results

Isolation and Purification of Bacterial Isolates

Samples collected from Cholistan desert and Multan Bypass were spread on LB plates and incubated at 37ºC. After 24 h, single colonies were picked and streaked on the same medium plates. A total of 200 bacterial isolates were isolated and five were selected for their ability to produce highly mucoid colonies on agar medium and form a long string when picked up with inoculating loop. Blue mucosubstance due to Alcian blue staining of the selected isolates revealed their potential to produce EPS.

Strains Characterization

All the selected isolates were morphologically and biochemically identified using a compound microscope and different biochemical tests. The morphological features of bacterial isolates were circular, entire, convex, smooth, viscous, and opaque. All the isolates showed Gram-positive chains of rods as a result of Gram staining, capsule forming, and motile under compound microscope, and gave biochemically positive results of catalase, oxidation, fermentation, denitrification, and starch hydrolysis (Table 1). Then those bacterial isolates showed high tolerance against desiccation by producing EPS and giving promising results with respect to plant-promoting activities (LPS1B, CHFT15, CHFT12, CH13, and CHP413A) characterized through 16S rDNA sequencing. The sequences of strains LPS1B, CHFT15, CHFT12, CH13, and CHP413A were blasted on NCBI and obtained closest homology with *Brevibacterium frigoritolerans*, *Bacillus subtilis*, *Bacillus subtilis*, *Bacillus subtilis*, and *Pseudomonas stutzeri*, respectively.

Table 1. Morphological and biochemical tests for the selected isolates.

Bacterial isolate	*B. frigoritolerans*-LPSIB	*B. subtilis*-CHFT15	*B. subtilis*-CHFT12	*B. subtilis*-CH13	*P. stutzeri*-CHP413A
Shape	Rods	Rods	Rods	Rods	Rods
Motility	+	+	+	+	+
Gram reaction	+	+	+	+	+
Spore formation	-	+	+	+	-
Capsule formation	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	-
Urease	-	-	-	-	-
Indole	-	-	-	-	-
Oxidation	+	+	+	+	+
Fermentation	+	+	+	+	+
MRVP	-	-	-	-	-
Nitrate reduction	-	+	+	+	+
Denitrification	+	+	+	+	+
Citrate utilization	-	-	+	+	-
Starch hydrolysis	+	+	+	+	+
Gelatin liquefaction	-	+	+	+	+
H_2S production	-	-	-	-	-

Survival Rate of Bacteria Desiccated in PBS

The survival rate of select bacterial strains *B. frigoritolerans*-LPS1B, *B. subtilis*-CHFT15, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *P. stutzeri*-CHP413A in response to

desiccation at 5, 23, and 100% relative humidity under constant pressure was monitored by plate count method following incubation at different time intervals ranging 1-40 days. *B. frigoritolerans*-LPS1B showed a higher survival rate at the beginning of the desiccation period at higher humidity levels (R.H. 23% and 100%) as compared to a drastic decrease in cell numbers at R.H. 5%. In contrast to all other isolates, *P. stutzeri*-CHP413A showed a varying trend by surviving at all humidity levels (5, 23 and 100%) except at the end of the incubation period. It showed high endurance at lower R.H. (R.H. 5%) in contrast to higher humidity levels (R.H. 23% and 100%) (Fig. 1).

Cross Metal and Antibiotic Resistance

All the isolates were sensitive to given concentrations of cadmium (Cd^{+2}) and copper (Co^{+2}). *B. subtilis*-CHFT15, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *P. stutzeri*-CHP413A were found resistant to arsenate As^{+5} at 500 µg/ml, while *P. stutzeri*-CHP413A was also resistant to arsenite (As^{+3}). Moreover, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *P. stutzeri*-CHP413A were able to tolerate Cr^{+6} at 500 $\mu g \cdot ml^{-1}$. All the selected bacterial strains were sensitive to the given concentration of oxy-tetracycline (Tc), kanamycin (Kn), streptomycin (Sm), and erythromycin (E) while resistant to chloramphenicol (Cm). *P. stutzeri*-CHP413A was found to be resistant to chloramphenicol (Cm) as well as ampicillin (Ap) (Table 2).

Table 2. Heavy metal and antibiotic resistance in select isolates.

Bacterial isolate		*B. frigoritolerans*-LPSIB	*B. subtilis*-CHFT15	*B. subtilis*-CHFT12	*B. subtilis*-CH13	*P. stutzeri*-CHP413A
Heavy metals	As^{+5} (500 µg/ml)	-	+	+	+	+
	As^{-3} (200 µg/ml)	-	-	-	-	+
	Cd^{+2} (100 µg/ml)	-	-	-	-	-
	Co^{+2} (100 µg/ml)	-	-	-	-	-
	Cr^{+6} (500 µg/ml)	-	-	+	+	+
Antibiotic Sensitivity	Chloramphenicol (30 µg/ml)	+	+	+	+	+
	Oxy-tetracycline (30 µg/ml)	-	-	-	-	-
	Kanamycin (30 µg/ml)	-	-	-	-	-
	Streptomycin (10 µg/ml)	-	-	-	-	-
	Ampicillin (10 µg/ml)	-	-	-	-	+
	Erythromycin (15 µg/ml)	-	-	-	-	-

Plant Growth Promoting Activities of Bacteria

All the selected bacterial isolates were screened for various plant growth-promoting traits (cytokinin, siderophores, HCN, phosphate solubilization) before the field trial experiments. The results of the present study showed that among phytohormones, the strains of *B. frigoritolerans*-LPS1B, *B. subtilis*-CHFT15, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *P. stutzeri*-CHP413A were able to produce cytokinin, while in the case of indole acetic acid production they showed negative results. Like IAA production, all the strains were also unable to produce siderophores and failed to solubilize inorganic phosphates. Moreover, *P. stutzeri*-CHP413A showed maximum potential to produce HCN, while *B. subtilis*-CH13 gave negative results in the case of HCN production (Table 3).

Optimization of Fermentation Conditions for EPS Production

Growth Media, pH, and Temperature

Microorganisms need nutrients as their energy source; optimum pH and temperature in order to grow and reproduce were optimized. Five different growth media – LB

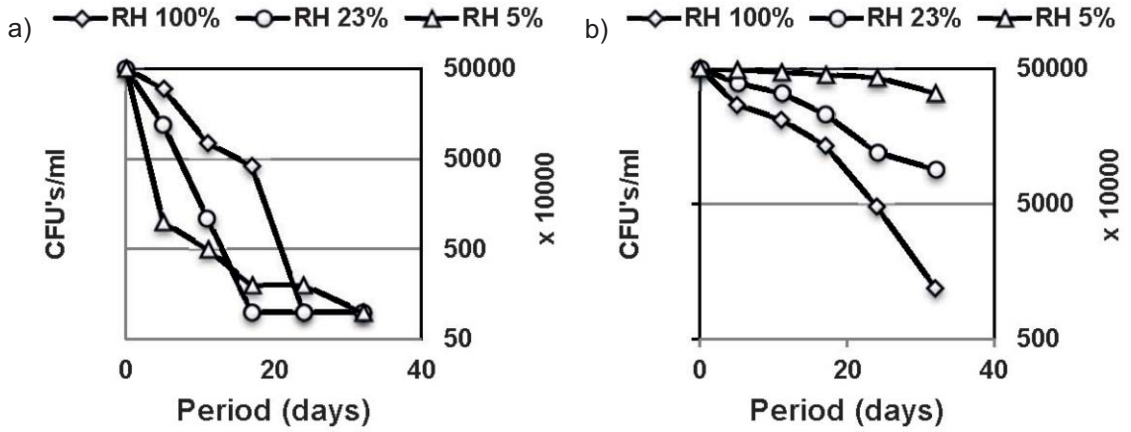

Fig. 1. Survival rates of *B. frigoritolerans*-LPS1B (a) and *P. stutzeri*-CHP413A (b) at various humanities (5, 23, and 100%) for a period of 1-40 days.

Table 3. Screening for multiple plant growth-promoting activities of select bacteria.

Bacterial isolate	Phytohormones		Phosphate solubilization	Hydrogen Cyanide	Siderophore production
	IAA	Cytokinin			
B. frigoritolerans-LPS1B	-	+	-	++	-
B. subtilis-CHFT15	-	+	-	++	-
B. subtilis-CHFT12	-	+	-	++	-
B. subtilis-CH13	-	+	-	-	-
P. stutzeri-CHP413A	-	+	-	+++	+

broth, TSB, EPS medium, and Pharmamedia at various pH (6, 7, 8, 9, and 10) and temperatures (25ºC, 30ºC, 37ºC, 42ºC, and 46ºC) – were tested for their ability to enhance EPS production by the select isolates. After 72 h of incubation, the maximum growth of all isolates was observed in Pharmamedia and maximum EPS yield was observed in TSB medium at optimum temperature of 37ºC and neutral pH of 7 (Fig. 2).

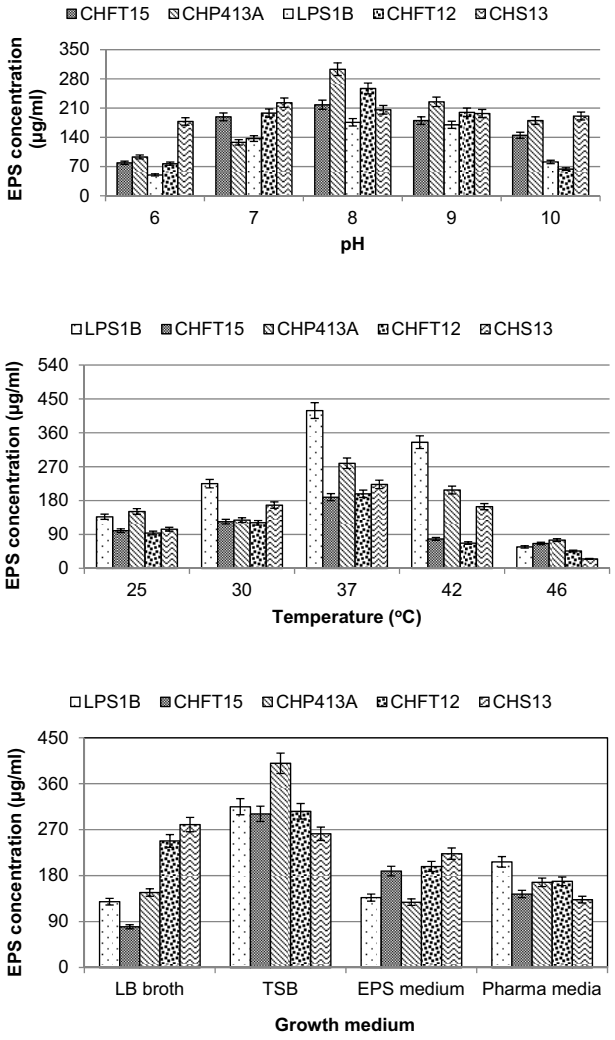

Fig. 2. Impact of pH, temperature, and various growth media on exopolysaccharide production by bacterial strains incubated at 37°C for 72 h at 150×g.

EPS Extraction

Isolation and purification of EPS was accomplished by precipitation with acetone followed by lyophilization of all select bacterial isolates. Maximum dry weight of EPS yielded 2.5 g and protein concentration was estimated 0.888 mg/10 mg of EPS in the case of *P. stutzeri*-CHP413A, while 0.5 g dry weight of EPS and 0.83 mg/10 mg of EPS protein concentration for *B. frigoritolerans*-LPS1B, 0.7 g dry weight of EPS, and 0.8 mg/10 mg of EPS protein concentration for *B. subtilis*-CHFT15 and *B. subtilis*-CHFT12, and 0.4 g dry weight of EPS and 0.7 mg/10 mg of EPS protein concentration were estimated for *B. subtilis*-CH13 (Fig. 3).

Plant Growth Promotion by Desiccation-Tolerant Bacteria

The plant growth-promoting ability of desiccation-tolerant bacterial isolates was checked with *Zea mays*; both in pure soil as well as in mixed soil (Table 4, Fig. 4). Bacterial inoculation had seemed to have significant impact on a number of leaves in mixed soil as compared to uninoculated seeds. The same growth-promoting effect was observed for shoot length and root length as compared to control plants as given in Table 3. The inoculation of *P. stutzeri*-CHP413A resulted in 6, 18, 23, 37, and 100% increases in germination, number of leaves, shoot and root length, and dry weight·g^{-1} fresh weight in pure soil, and 3, 33,12, 37, and 0% increases in above-mentioned plant growth parameters for sandy soil ($p<0.05$). In contrast, numbers of leaves in pure soil were increased as compared to mixed soil and control plants.

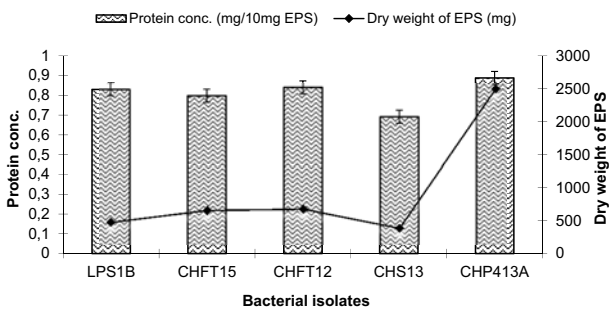

Fig. 3. Dry weight of EPS and protein concentrations in EPS of bacterial strains.

Table 4. Effect of five bacterial inoculations on seed germination, number of leaves, shoot length, root length, and dry weight per gram fresh weight of *Zea mays* grown in pure and mixed soil.

Soil type	Treatment	Germination (%)	Number of leaves	Shoot length (cm)	Root length (cm)	Dry weight·g^{-1} fresh weight (g)
Pure soil	Control	94.4+0.3 (a)	11.0+1.0(a)	59.6+3.2 (a)	33.6+3.6 (a)	0.1+0.1 (a)
	LPS1B	94.4+0.3 (a)	13.0+0.8 (c)	68.6+3.7 (b)	46.4+2.9 (e)	0.2+0.1 (b)
	CHFT15	94.4+0.3 (a)	13.0+0.7 (c)	72.0+3.4 (d)	41.9+3.4 (c)	0.2+0.1 (b)
	CHFT12	100+0.1 (c)	12.0+1.3(b)	70.9+3.7 (c)	40.7+3.2 (b)	0.2+0.08 (b)
	CH13	97.2+0.3 (b)	13.0+1.0 (c)	72.6+3.2 (d)	43.1+3.7 (d)	0.2+0.2 (b)
	CHP413A	100+0.3 (c)	13+1.1 (c)	73.0+3.5 (e)	46.1+3.5 (e)	0.2+0.2 (b)
Mixed soil	Control	100+0.2 (d)	9.0+1.4 (a)	56.0+3.5 (a)	35.3+3.7 (a)	0.2+0.1 (b)
	LPS1B	94.4+0.3 (b)	11.0+0.7 (b)	68.9+3.4 (d)	51.4+2.6 (f)	0.2+0.1 (b)
	CHFT15	97.2+0.3 (c)	12.0+0.8 (c)	69.0+3.1 (e)	49.6+3.9 (e)	0.18+0.09 (a)
	CHFT12	91.6±0.2 (a)	13.0+0.8 (d)	62.9+3.0 (b)	42.3+3.5 (b)	0.18+0.09 (a)
	CH13	94.4+0.3 (b)	13.0+1.0 (d)	66.6+3.0 (c)	43.3+2.9 (c)	0.2+0.1 (b)
	CHP413A	97.2+0.3 (c)	12.0+1.1 (c)	62.9+3.1 (b)	48.6+2.6 (d)	0.2+0.1 (b)

The results shown are means of three independent experiments±standard error.
The $p<0.05$ was calculated by ANOVA, and different letters indicate significant differences between means of each treatments calculated by Duncan's multiple range test (P=0.05).

Discussion

Microorganisms, especially bacteria, help crop plants to increase their tolerance and adaptation to abiotic stresses. In the present investigation, the isolated strains belong to different genera like *Brevibacterium*, *Bacillus*, and *Pseudomonas*; the first two are desiccation-tolerant gram-positive aerobic bacteria, while the third is a desiccation-tolerant gram-negative aerobic rod-shaped bacterium.

Results of desiccation tolerance showed that strain *B. frigoritolerans*-LPS1B showed higher survival rate at the beginning of the desiccation period at higher humidity levels (R.H. 23% and 100%) as compared to a drastic decrease in cell numbers at R.H. 5%. Strains *B. subtilis*-CHFT15, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *P. stutzeri*-CHP413A were found resistant to arsenate As^{+5} at 500 µg·ml^{-1} while *P. stutzeri*-CHP413A was also resistant to arsenite (As^{+3}). Microorganisms bind soluble heavy metals in three ways (biosorption, bioaccumulation, and the binding by metabolic products), which indirectly reduce the negative impact of heavy metals on plants [28, 29]. Strain *P. stutzeri*-CHP413A was found to be resistant to chloramphenicol (Cm) as well as ampicillin (Ap). All strains showed sensitivity against oxy-tetracycline (Tc), kanamycin (Kn), streptomycin (Sm), and erythromycin (E) but were resistant to chloramphenicol (Cm). Strains *B. frigoritolerans*-LPS1B, *B. subtilis*-CHFT15, *B. subtilis*-CHFT12, *B. subtilis*-CH13, and *P. stutzeri*-CHP413A were able to produce cytokinin but unable to produce indole acetic acid. The phytohormone cytokinin controls apical dominance and regulates root and shoot growth, leaf senescence, and chloroplast development [30]. *P. stutzeri*-CHP413A showed maximum potential to produce HCN, while *B. subtilis*-CH13 gave negative results in the case of HCN production. Recently, the beneficial role of HCN in the control of plant root-damaging subterranean termite has been studied [31]. Several investigators have shown that numerous soil bacteria – especially plant growth-promoting bacteria – can produce either cytokinins or gibberellins or both [32-34].

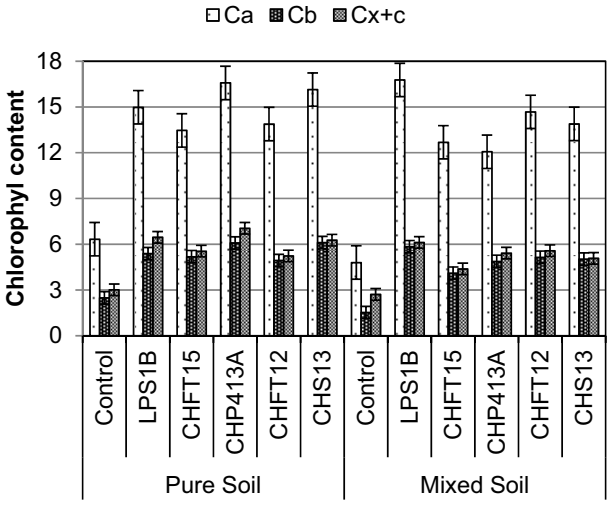

Fig. 4. Effect of bacterial inoculation on chlorophyll *a*, *b*, and carotenoid content of 30-day-old *Zea mays* plants. Ca – chlorophyll *a*, Cb – chlorophyll *b*, and Cx+c – carotenoids. Bars represent mean±SE.

Maximum dry weight of EPS was 2.5 g and protein concentration was estimated 0.888 mg/10 mg of EPS in the case of *P. stutzeri*-CHP413A. Several bacterial strains, especially *Pseudomonas*, survives under abiotic stress by

producing exopolysaccharide [35]. Exopolysaccharide production has a great interest with respect to desiccation because rhizobacteria undergo adaptations. *S. meliloti* has shown adaption in the presence of exopolysaccharide under osmotic stress and also affects the survival rate during dry conditions [36, 37]. Mary et al. [38] observed a decrease in survival rate of rhizobia upon the addition of polysaccharides when dried at an RH of >3%, but survival rate increased to 3% RH. However, specific properties of polysaccharides have different effects on survival rate of microorganism in a desiccated environment [38]. Short exposure to intermediate water activity when the water content is detrimental for the cells leads to higher survival [39, 40].

The inoculation of *P. stutzeri*-CHP413A resulted in 6, 18, 23, 37, and 100% increase in germination, number of leaves, shoot and root length, and dry weight g-1fresh weight in pure soil. The above-described several broad spectrum plant growth promoting attributes of selected rhizo-bacterial isolates, especially the strain *P. stutzeri*-CHP413A, is likely to be the potential candidate for the enhancement of maize growth under drought and a desiccated environment.

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