Antonie van Leeuwenhoek (2017) 110:253–270
DOI 10.1007/s10482-016-0796-0

ORIGINAL PAPER

# Characterisation of *Pseudomonas* spp. and *Ochrobactrum* sp. isolated from volcanic soil

**Shashank Kumar Mishra · Mohammad Haneef Khan · Sankalp Misra · Vijay Kant Dixit · Praveen Khare · Suchi Srivastava · Puneet Singh Chauhan**

Received: 18 August 2016 / Accepted: 24 October 2016 / Published online: 16 November 2016


**Abstract** Soil bacteria may have properties of plant growth promotion but not be sufficiently beneficial for plants under stress conditions. This challenge has led researchers to extend their searches into extreme environments for potential soil bacteria with multiple plant beneficial traits as well as abiotic stress tolerance abilities. In the current study, an attempt was made to evaluate soil bacteria from an extreme environment, volcano soils, based on plant growth promoting and abiotic stress mitigating characteristics. The screening led to the isolation of eight (NBRISH4, NBRISH6, NBRISH10, NBRISH11, NBRISH13, NBRISH14, NBRISH16 and NBRISH26) bacterial isolates capable of withstanding stresses, namely temperature (up to 45 °C), salt (up to 2 M NaCl) and drought (up to 60% Poly Ethylene Glycol 6000) in vitro. Further, the selected isolates were notable for their in vitro temporal performance with regards to survival (in terms of colony count), phosphate solubilisation, biofilm formation, auxin, alginate and exo-polysaccharide production abilities under abiotic stresses i.e. 40 °C temperature; 500 mM NaCl salt and drought (PEG) conditions. In vivo seed treatments of individual selected bacteria to maize plants resulted into significant enhancement in root and shoot length, root and shoot fresh and dry weight and number of leaves per plant. Overall, the plant growth promoting and abiotic stress tolerance ability was most evident for bacterial isolate NBRISH6 which was identified as an *Ochrobactrum* sp. using 16S rRNA based phylogenetic analysis.

**Keywords** Abiotic stress · *Ochrobactrum* sp. · Plant growth promotion · *Pseudomonas* spp. · Volcano

## Introduction

The world economic forum has speculated that the effect of abiotic stresses shall increase significantly by 2025, risking 30% of the crop production worldwide (World Economic Forum 2011). These abiotic stresses include the consequences of the constant increase in water deficit, irregular rainfall, rises in global earth temperature and sea level, and frequent flood situations (Singh 2013). In India too, these conditions will drastically affect the huge low-income population practicing traditional agriculture and therefore will influence its economic, employment and nutritional

**Electronic supplementary material** The online version of this article (doi:10.1007/s10482-016-0796-0) contains supplementary material, which is available to authorized users.

S. K. Mishra · M. H. Khan · S. Misra · V. K. Dixit · P. Khare · S. Srivastava · P. S. Chauhan (✉)
Division of Plant Microbe Interactions, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India
e-mail: puneetnbri@gmail.com; puneet@nbri.res.in

safety. To reduce the impact of these factors, researchers are applying several eco-friendly and economical approaches including plant growth promoting rhizobacteria (PGP/PGPR) in agriculture for crop improvement (Gupta et al. 2015; Gopalakrishnan et al. 2015; Barea 2015; Shrivastava and Kumar 2015). PGPR possess multiple plant beneficial traits such as solubilisation of phosphate, production of siderophores and auxin, and nitrogen fixation (Gadhave et al. 2016; Abiala et al. 2015; Oteino et al. 2015). In addition, PGPR protect themselves and plants using biofilm formation, and alginate and exo-polysaccharide (EPS) production, to indirectly antagonise the harmful effects of soil borne root pathogens (Bogino et al. 2013; Qurashi and Sabri 2012; Redmile-Gordon et al. 2014). These agriculturally important microbes are also recognised to modulate morphology of roots and improve the utilisation of nutrients essential for plant growth (Hayat et al. 2010).

Notwithstanding their multiple plant beneficial characteristics, PGPR are typically incapable of improving plant fitness under abiotic stresses such as high salt, temperature and drought conditions in local habitats (Rodriguez et al. 2008). The indigenous microflora also faces similar issues of limited adaptability under varied environmental conditions (Rodriguez et al. 2008; Zhu et al. 2007). With the increasing reach of saline, drought and arid/semi-arid areas in India (Singh 2013), an alternative such as 'exotic' bacteria with both plant beneficial and abiotic stress tolerance characteristics is needed, so that they can perform even under challenging soil conditions (Rodriguez et al. 2008). Similar to the discovery of *Thermus aquaticus Taq* polymerase from an extreme environment (Saiki et al. 1988; Chien et al. 1976), there is high probability that soil bacterial isolates from extreme conditions may present both multiple PGP and abiotic stress tolerance traits. Therefore, we chose to extend our search for bacteria with multiple PGP traits to extreme environments such as volcano sites or hot springs. In India, the soils of the Andaman and Nicobar islands have rarely been explored for such bacterial isolates and such studies are warranted. Characterisation of isolates based on molecular tools such as 16S rRNA gene is essential for identification before their commercial use or in-field application. Consequently, this study was undertaken to isolate, characterise and screen bacteria from an extreme environment (volcanic soils) based on their PGP and abiotic stress tolerance traits in vitro. Furthermore, selected isolates underwent an in vivo test for plant growth promotion in terms of length, dry weight and leaf count.

## Materials and methods

### Sample collection

Samples were collected from seven different locations (Volcano mud, Baratang; Soil, Smith island; Lime stone cave, Baratang; Sand, Uttara; Lichen adhered stone of cave, Baratang; Soil, Sippighat; Sand, Wondoor beach) nearby to a volcano in the Andaman and Nicobar islands, India (Table 1). These areas were selected for as they were previously unexplored regarding bacterial diversity. The collected samples were subjected to serial dilution followed by spreading on Nutrient Agar (NA; HiMedia, India). The inoculated NA Petri plates were kept at 28 °C for 24 h. Different isolates appearing on NA were segregated onto NA plates based on their varying morphological appearance until pure cultures were obtained. Individual bacterial isolates were maintained on NA medium and simultaneously stored in 30% glycerol and kept at −80 °C.

### Screening of bacterial isolates based on EPS and alginate production along with abiotic stress tolerance ability

The selected bacterial isolates were tested and further screened for their ability to produce EPS and alginate using the phenol–sulphuric acid method (Titus et al. 1995) and carbazole–boric sulfuric acid method (Srivastava et al. 2008; Mishra et al. 2012) respectively. The initial inoculum used to determine these abilities of the bacterial isolates was approximately $7.5 \times 10^7$ CFU $ml^{-1}$. After the selection of bacterial isolates based on EPS and alginate production ($\mu g\ ml^{-1}$), these isolates were tested for their capability to tolerate abiotic stresses such as temperature (30, 40 and 45 °C), drought (30, 45 and 60% poly ethylene glycol 6000; PEG) and salt (0.5, 1 and 2 M NaCl) by growing them on nutrient broth (NB;

**Table 1** Sampling sites along with their geographical coordinates, isolate codes with the corresponding 16S rRNA gene GenBank nucleotide accession numbers and closest taxonomic relationship

| S. No. | Location | Coordinates | Source | Isolates | Accession No. | Identification |
|---|---|---|---|---|---|---|
| 1 | Baratang | 12°07′N 92°47′E | Volcano mud | NBRISH6 | KP300814 | *Ochrobactrum* sp. |
| | | | | NBRISH11 | KC357774 | *P. stutzeri* |
| | | | | NBRISH26 | KX263306 | *Pseudomonas* sp. |
| 2 | Smith Island | 13°18′N 93°04′E | Soil | NBRISH10 | KC357773 | *P. stutzeri* |
| 3 | Baratang | 12°92′N 92°90′E | Lime stone cave | NBRISH13 | KC357775 | *P. stutzeri* |
| | | | | NBRISH14 | KC357776 | *P. stutzeri* |
| 4 | Uttra | 12°21′N 92°46′E | Sand | NBRISH16 | KX263305 | *Pseudomonas* sp. |
| 5 | Baratang | 12°07′N 92°47′E | Lichen adhered to stone in cave | NBRISH4 | KP300813 | *Pseudomonas guariconensis* |
| 6 | Sippighat | 11°66′N 92°73′E | Soil | – | – | – |
| 7 | Wandoor beach | 11°35′N 92°36′E | Sand | – | – | – |

HiMedia, India) with the same initial inoculum as above. The inoculated NB was incubated with shaking at 180 rpm at 28 °C (except for temperature stress) and samples were spotted on to NA medium up to 10 days to check on the survival of the bacterial isolates.

### Biochemical and other qualitative test for screened bacterial isolates

For preliminary identification of the screened bacterial isolates, a number of qualitative biochemical tests were performed with a KB003 Hi25$^{TM}$ identification kit (HiMedia, India). Each Hi25$^{TM}$ kit is optimized for qualitative colorimetric identification of bacteria by utilising carbohydrate source and other conventional biochemical tests. In addition, the selected isolates (initial inoculum approximately $7.5 \times 10^7$ CFU ml$^{-1}$) were examined for the following: Acyl Homoserine Lactone (AHL) bioassay using the Bioreporter strain *Agrobacterium tumefaciens* A136 (Singh et al. 2012); mannitol utilisation, motility and nitrate reduction using mannitol motility nitrate medium (MMNM;M1320, HiMedia, India); Gram staining (K 001-1KT Gram stains-kit, HiMedia, India); activity of cellulase (Cattelan et al. 1999), amylase and protease (Smibert and Krieg 1994); gelatinase and ACC deaminase (Penrose and Glick 2003); auto-aggregation (%); production of siderophores (Meyer and Abdallah 1978) and auxin (Brick et al. 1991); and phosphate solubilisation (Nautiyal 1999).

### Temporal quantitative estimation of survivability, PGP and other attributes under abiotic stress of selected isolates

The potential PGP isolates were subjected to temporal evaluation of their fitness for survival, PGP traits (phosphate solubilisation; auxin production), formation of biofilm, production of EPS and alginate under different abiotic stresses such as temperature (40 °C), salt (500 mM NaCl) and drought (PEG) up to 3 days after bacterial inoculation at 24 h intervals. The initial inoculum used was $\sim 7.5 \times 10^7$ CFU ml$^{-1}$. Briefly, their ability to tolerate the above stresses was determined by counting the respective colony forming unit (CFU ml$^{-1}$). Similarly, quantitative estimation of biofilm formation (Khan et al. 2012), EPS (Titus et al. 1995) and alginate (Mishra et al. 2012) production (μg ml$^{-1}$) by selected isolates under the different stress conditions was performed. For biofilm formation, the bacteria were stained with 0.1% crystal violet followed by washing with 95% ethanol. After washing, OD was measured at 590 nm using a spectrophotometer (EVOLUTION201, Thermo Scientific, USA). With regards to EPS production by isolates, an equal volume of phenol (0.5 M) and sulphuric acid (9.8 M) was used to determine the amount of EPS produced in 24 h old bacterial cultures at an absorbance of 490 nm using a spectrophotometer. Alginate production by selected isolates was quantified using equal volumes of borate sulphuric acid (9.8 M) and carbozale reagent (0.1% in ethanol)

added to 24 h old bacterial cultures, followed by measurement of the absorbance at 500 nm using a colourimeter. In order to estimate the solubilisation of phosphate (μg $ml^{-1}$) by bacterial isolates, NBRIP medium (Nautiyal 1999) was used as substrate. The solubilised phosphate was then quantified by the Molybdate Blue Method (Fiske and Subbarow 1925) on the 1st, 2nd and 3rd day of incubation at an absorbance of 660 nm using a spectrophotometer. For quantification of auxin production, the selected bacterial isolates were inoculated in NB supplemented with tryptophan as a substrate and incubated for 3 days at 30 °C. At intervals of 24 h up to 3 days, orthophosphoric acid followed by Salkowski's reagent (01 ml of 0.5 M $FeCl_3$ in 35% Perchloric acid) was added to the centrifuged supernatant of the cultures. The absorbance of the resultant pink colour was measured at 530 nm using a colourimeter (Srivastava et al. 2012).

## Evaluation of bacterial isolates for their ability of plant growth promotion under plant test

Following the screening of bacterial isolates based on their PGP and other attributes under abiotic stress in vitro, the selected isolates were tested for their PGP capability in plant tests using maize (*Zea mays*) as a model plant in plastic pot conditions (15 cm in diameter). Experiments were conducted in a completely randomised block design with 12 replicates using non-sterile 2 mm sieved field soils (2.5 kg soil per pot) from the CSIR-National Botanical Research Institute, Lucknow. For seed preparation, pre-sterilised seeds were mixed with each bacterial cell suspension ($\sim 10^{8-9}$ CFU $ml^{-1}$) until uniformly coated, while uninoculated control seeds were treated with water (Nautiyal 1997). 30 days after sowing, maize plants were harvested and data were recorded pertaining to shoot and root length (cm), shoot and root dry weight (g), and number of leaves per plant per treatment.

## DNA extraction and 16S rRNA gene PCR amplification of potentially selected isolates

Bacterial isolates (24 h old culture) were subjected to DNA extraction using a Wizard bacterial genomic DNA isolation kit (Promega, USA) following the manufacturer's instructions. The extracted DNA was used to partially amplify 16S genes (Dastager et al. 2009). Briefly, PCR was carried out in 20 μl volume consisting of 1.0 U *Taq* polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 mM of each dNTPs, 10 μM of forward (8f) primer (5′-AGAGTTTGATCCTGGCTCAG-3′) and 10 μM of reverse (1392R) primer (5′-ACGGGCGGTGTGTAC-3′) and 20–30 ng of bacterial genomic DNA. PCR-grade water was usied as a negative control during PCR. The obtained amplicons underwent electrophoresis compared with 100 bp plus DNA ladder (Thermo Scientific, USA) visualised using image acquisition software (Universal Hood III, Bio-Rad, USA). PCR was performed in a thermocycler (SureCycler 8800, Agilent Technologies, USA) with the following conditions: initial denaturation at 94 °C for 3 min; 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 58 °C, and 60 s elongation at 72 °C; and a 10 min final elongation. Subsequently, PCR amplicons were then purified using QIAquick® PCR purification kit (Qiagen, USA) and sent for sequencing (3730XL ABI Applied Biosystems, USA).

## BLAST search and phylogenetic analysis

After sequencing of PCR amplicons, partial 16S rRNA sequences were matched with available reference sequences in the NCBI database. Subsequently, multiple sequence alignment by CLUSTAL W was done, with editing using Bioedit version 7.0.5 (Hall 1999). For phylogenetic analysis, reference sequences representing near neighbours were retrieved from the NCBI database. The Maximum Composite Likelihood method was employed to determine the evolutionary distance between the sequences (Tamura et al. 2004). A phylogenetic tree was reconstructed using MEGA 5.0 software with the Neighbour Joining statistical algorithm, with bootstrap analysis based on 1000 random samples (Tamura et al. 2011).

## Statistical analyses

Initially, means were tested for homogeneity of variance to evaluate the variation among obtained values. Further, these means were compared by analysis of variance (ANOVA), followed by the Tuckey's highest significant difference (HSD) test to determine significance ($p \leq 0.05$).

## Results

### Isolation and screening of bacteria based on exopolysaccharide and alginate production along with abiotic stress tolerance ability

A total of 200 different bacterial isolates were obtained by growing them on NA. These isolates were screened for the concentration of EPS and alginate, setting a lower critical limit of 1500 μg $ml^{-1}$ for EPS and 250 μg $\mu l^{-1}$ for alginate (Fig. S1 and S2). We found 51 isolates with high EPS production ability and 22 isolates with high alginate production. These isolates were subsequently tested for their abiotic stress tolerance ability. With regard to temperature stress, we observed that 97 and 80% of the bacterial isolates could tolerate up to 40 and 45 °C respectively (data not shown). With regards to salt stress, 92% of the isolates were able to tolerate 1 M salt concentration while only 34% showed tolerance towards 2 M salt. With reference to drought stress, 82 and 38% of the bacterial isolates were capable of tolerating 45 and 60% PEG concentration respectively (data not shown). These levels of screening reduced the selected potentially PGP isolates to eight (Table 1). None of these were recovered from soil from Sippighat and sand from Wandoor beach locations (Table 1).

### Qualitative test for screened bacterial isolates

The eight selected bacterial isolates were subjected to a range of qualitative tests consisting of AHL bioassay, activities of cellulase, amylase, protease, gelatinase and ACC deaminase, motility, auto-aggregation (%), production of siderophores and auxin, and phosphate solubilisation. All the eight isolates were found to be Gram-negative, motile and nitrate reducers. In addition, they demonstrated cellulase, amylase, protease, gelatinase and ACC deaminase activities along with phosphate solubilisation, auxin production and AHL positive activity (Table S1). Four isolates (NBRISH6, NBRISH10, NBRISH14 and NBRISHS26) were found to be capable of siderophore production. In auto-aggregation tests, NBRISH14 gave the maximum value for auto-aggregation (58%), while NBRISH4 was found to exhibit the lowest auto-aggregation ability (22%, Table S1).

### Temporal quantitative estimation of PGP, Biofilm formation, EPS and alginate production attributes under abiotic stress of selected isolates

With regards to the survival of bacterial isolates for up to three days, it was observed that the abiotic stress affected the CFU counts drastically. Regarding temperature (40 °C) stress, all the isolates except NBRISH6 showed high temporal variations in terms of $\log_{10}$ CFU count when compared with their respective controls (data not shown). NBRISH6 presented the lowest temporal fluctuations in colony count (11% lower at day 01; 09% lower at day 02; 16% lower at day 03) when compared with that of controls. Similar results were obtained with regards to salt (500 mM NaCl) and drought (PEG) stresses, wherein all the bacterial isolates except NBRISH6 showed wide temporal variations upon comparison with those of corresponding controls(data not shown).

With regards to quantification of phosphate solubilisation (at 24 h intervals up to three days), all the eight bacterial isolates were found to be resistant to stress (temperature, salt and drought) dependent fluctuations over time (Table 2). Under temperature (40 °C) stress, NBRISH6 was observed to be the most resistant isolate having 36% (day 1), 35% (day 2) and 39% (day 3) lower levels of phosphate solubilisation than that of the respective controls. Upon consideration of salt (500 mM NaCl) and drought (PEG) stress, we found that bacterial isolates NBRISH4 and NBRISH26 exhibited least variation among the remaining bacterial isolates for phosphate solubilisation over three days when compared with of the respective controls (Table 2).

Considering auxin production, high temporal variation in the quantity was obtained from the eight bacterial isolates over time (Table 2). In the context of temperature (40 °C) and drought (PEG) stress, NBRISH4 appeared to be the least compromised bacterial isolate for auxin production ability in comparison to controls. On the other hand, bacterial isolate NBRISH10 (09.22 ± 0.72 μg $ml^{-1}$ at day one, 26.87 ± 0.99 μg $ml^{-1}$ at day two and 36.97 ± 1.17 μg $ml^{-1}$ at day three) demonstrated lowest variation among all the other isolates for auxin production under salt (500 mM NaCl) stress compared with the corresponding controls (19.81 ± 0.82 μg $ml^{-1}$ at day one, 51.66 ± 1.43

**Table 2** Auxin production and phosphate solubilisation of selected isolates at different stress conditions for up to three days

| Isolates | Day 1 | | | |
|---|---|---|---|---|
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Auxin production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 28.04 ± 1.35a | 9.62 ± 0.45c | 12.33 ± 1.07c | 23.16 ± 1.23b |
| NBRISH6 | 25.53 ± 2.86a | 13.4 ± 1.35b | 13.80 ± 1.52b | 22.61 ± 1.12a |
| NBRISH10 | 19.81 ± 0.82b | 15.96 ± 0.74c | 9.22 ± 0.72b | 31.68 ± 1.63a |
| NBRISH11 | 26.77 ± 2.58a | 23.73 ± 1.27a | 13.68 ± 0.48b | 29.90 ± 1.78a |
| NBRISH13 | 26.60 ± 0.46b | 40.80 ± 1.20a | 12.52 ± 1.88c | 38.88 ± 1.00a |
| NBRISH14 | 25.5 ± 2.28b | 39.79 ± 1.20a | 10.39 ± 1.07c | 20.91 ± 0.92b |
| NBRISH16 | 28.66 ± 1.07a | 17.13 ± 0.73b | 12.26 ± 0.75c | 30.83 ± 1.40a |
| NBRISH26 | 59.48 ± 2.18a | 14.74 ± 1.19c | 9.09 ± 0.38d | 23.59 ± 1.25b |
| Phosphate solubilization (μg $ml^{-1}$) | | | | |
| NBRISH4 | 35.3 ± 0.61a | 10.11 ± 0.069b | 10.25 ± 0.45b | 10.73 ± 0.32b |
| NBRISH6 | 29.81 ± 1.23a | 19.15 ± 0.42b | 22.25 ± 1.15b | 20.58 ± 0.0.40b |
| NBRISH10 | 27.19 ± 1.36a | 17.77 ± 0.39b | 16.63 ± 0.56b | 17.36 ± 0.22b |
| NBRISH11 | 24.40 ± 1.11a | 17.22 ± 0.85b | 13.72 ± 0.37c | 16.67 ± 0.0.15b |
| NBRISH13 | 15.73 ± 0.58bc | 14.78 ± 0.53c | 17.37 ± 0.70ab | 18.08 ± 0.13a |
| NBRISH14 | 23.44 ± 1.31a | 19.72 ± 0.04b | 17.41 ± 0.30b | 16.78 ± 0.24b |
| NBRISH16 | 33.68 ± 0.94a | 11.58 ± 0.84c | 10.75 ± 0.84c | 16.99 ± 0.76b |
| NBRISH26 | 31.79 ± 2.02a | 13.58 ± 0.78b | 9.35 ± 0.48b | 12.47 ± 0.64b |
| Isolates | Day 2 | | | |
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Auxin production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 48.31 ± 1.45a | 20.98 ± 0.40c | 16.01 ± 0.96d | 33.54 ± 0.46b |
| NBRISH6 | 47.35 ± 1.89a | 37.89 ± 0.60b | 35.23 ± 1.22b | 35.27 ± 1.69b |
| NBRISH10 | 51.66 ± 1.43b | 100.56 ± 2.45a | 26.87 ± 0.99c | 51.91 ± 1.67b |
| NBRISH11 | 39.65 ± 2.40c | 90.44 ± 1.75a | 31.90 ± 0.76d | 64.47 ± 0.81b |
| NBRISH13 | 35.30 ± 0.74a | 28.66 ± 1.03a | 31.93 ± 0.82a | 35.37 ± 1.37a |
| NBRISH14 | 38.81 ± 1.91a | 28.47 ± 1.23b | 36.61 ± 1.99a | 38.26 ± 1.15a |
| NBRISH16 | 45.44 ± 2.27a | 16.96 ± 0.65c | 14.56 ± 1.25c | 29.41 ± 0.63b |
| NBRISH26 | 30.72 ± 3.93a | 16.73 ± 1.72b | 11.52 ± 1.41b | 25.87 ± 1.74a |
| Phosphate solubilization (μg $ml^{-1}$) | | | | |
| NBRISH4 | 54.98 ± 1.44a | 17.40 ± 0.89b | 15.85 ± 0.84b | 16.42 ± 0.50b |
| NBRISH6 | 51.46 ± 1.30a | 33.28 ± 0.85b | 30.16 ± 0.61b | 32.28 ± 1.24b |
| NBRISH10 | 30.83 ± 0.28a | 19.29 ± 0.83c | 16.75 ± 0.30d | 25.68 ± 0.38b |
| NBRISH11 | 38.541 ± 0.42a | 17.78 ± 0.48c | 14.24 ± 0.36d | 20.65 ± 0.205b |
| NBRISH13 | 18.08 ± 0.16b | 18.49 ± 0.65b | 16.67 ± 0.099c | 24.63 ± 0.43a |
| NBRISH14 | 33.08 ± 0.50a | 21.57 ± 0.63c | 18.75 ± 0.46d | 23.81 ± 0.45b |
| NBRISH16 | 52.24 ± 0.05a | 20.80 ± 0.35c | 15.66 ± 0.15d | 25.17 ± 0.55b |
| NBRISH26 | 62.48 ± 2.59a | 27.93 ± 0.50b | 18.605 ± 0.14c | 18.31 ± 0.57c |

**Table 2** continued

| Isolates | Day 3 | | | |
|---|---|---|---|---|
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Auxin production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 78.75 ± 2.36a | 16.75 ± 1.13c | 19.56 ± 0.49c | 42.93 ± 1.79b |
| NBRISH6 | 86.46 ± 1.70a | 30.83 ± 0.49d | 38.22 ± 0.55c | 51.74 ± 0.89b |
| NBRISH10 | 103.06 ± 2.22a | 33.56 ± 1.78c | 36.97 ± 1.17c | 76.71 ± 1.86b |
| NBRISH11 | 27.52 ± 1.33a | 41.91 ± 0.54b | 38.42 ± 0.63c | 65.53 ± 0.60a |
| NBRISH13 | 52.36 ± 1.44a | 26.97 ± 0.45d | 36.33 ± 0.56c | 41.44 ± 0.35b |
| NBRISH14 | 68.20 ± 0.57a | 20.66 ± 0.45c | 41.64 ± 0.88b | 42.11 ± 0.98b |
| NBRISH16 | 42.97 ± 1.23a | 14.09 ± 0.87c | 30.96 ± 1.01b | 43.51 ± 1.33a |
| NBRISH26 | 27.37 ± 1.84b | 12.13 ± 0.64d | 17.28 ± 0.82c | 41.98 ± 0.85a |
| Phosphate solubilization (μg $ml^{-1}$) | | | | |
| NBRISH4 | 67.7 ± 0.91a | 36.21 ± 0.48b | 23.28 ± 0.38c | 24.64 ± 0.97c |
| NBRISH6 | 80.49 ± 1.21a | 48.73 ± 0.86b | 39.18 ± 0.63c | 42.83 ± 1.42c |
| NBRISH10 | 50.56 ± 0.50a | 20.27 ± 0.80c | 19.66 ± 0.37c | 33.77 ± 0.69b |
| NBRISH11 | 45.69 ± 0.79a | 20.36 ± 1.23c | 18.65 ± 0.56c | 31.02 ± 1.70b |
| NBRISH13 | 41.85 ± 0.49a | 25.32 ± 0.52c | 21.51 ± 1.14c | 35.31 ± 1.39ab |
| NBRISH14 | 51.36 ± 1.20a | 23.51 ± 0.28c | 23.35 ± 0.69c | 31.98 ± 0.38b |
| NBRISH16 | 77.72 ± 0.76a | 34.88 ± 0.28c | 28.85 ± 0.82b | 32.26 ± 0.21b |
| NBRISH26 | 72.67 ± 1.19a | 38.59 ± 1.35b | 28.59 ± 1.17c | 27.00 ± 0.55c |

Mean ± standard errors were compared by analysis of variance (ANOVA), followed by the Tuckey's test. Means were considered of three replicates. Statistically significant differences were then determined at $p \leq 0.05$, using the SPSS ver 20 and were denoted with mean ± standard error followed with different letters

μg $ml^{-1}$ at day two and 103.06 ± 2.22 μg $ml^{-1}$ at day three) (Table 2).

In a similar manner, we observed a high degree of variation in the values obtained for biofilm formation by the eight bacterial isolates over time (Table 3). Specifically under temperature (40 °C) stress, NBRISH26 outperformed the remaining isolates regarding consistency of biofilm formation (76% more at day one, 38% lower at day two and 43% lower at day three) when compared with their respective controls. Considering the biofilm formation ability between the isolates under salt (500 mM NaCl) stress, NBRISH11 appeared to be the most stable isolate when compared with the corresponding controls. In the context of drought (PEG) stress, NBRISH16 was found to be the most resistant (0.138 ± 0.009 OD at day one, 0.431 ± 0.010 OD at day two and 0.207 ± 0.002 OD at day three) isolate tested, showing least variation in the OD for biofilm formation over time (Table 3).

Upon considering EPS production, we found that NBRISH6 performed consistently under temperature (40 °C) stress demonstrating lowest temporal variation (1951.43 ± 5.97 μg $ml^{-1}$ at day one, 2529.63 ± 5.31 μg $ml^{-1}$ at day two and 1720.23 ± 5.90 μg $ml^{-1}$ at day three) among the eight bacterial isolates when compared with the respective controls (Table 4). However in salt (500 mM NaCl) and drought (PEG) stress, NBRISH4 exhibited maximum reistance among all the bacterial isolates tested in terms of EPS production ability.

With regards to alginate production ability, it was found that NBRISH4 exhibited the least temporal fluctuations under temperature (40 °C) stress when compared with the corresponding controls (Table 4). NBRISH14 also demonstrated lowest variation in its ability for alginate production under salt (500 mM NaCl) stress over time. In the context of drought (PEG) stress, NBRISH10 showed the highest resistance towards temporal variations in the values obtained for alginate production (Table 4).

Overall, NBRISH4, following by NBRISH6, has performed better in vitro over time for their different characteristics under most of the abiotic stresses.

**Table 3** Biofilm formation by the selected isolates under different stress conditions for up to three days

| Isolates | Day 1 | | | |
|---|---|---|---|---|
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Biofilm OD at 590 nm | | | | |
| NBRISH4 | 0.115 ± 0.02c | 0.664 ± 0.018a | 0.239 ± 0.003b | 0.230 ± 0.002b |
| NBRISH6 | 0.225 ± 0.007b | 0.495 ± 0.013a | 0.182 ± 0.019b | 0.419 ± 0.034a |
| NBRISH10 | 0.369 ± 0.05b | 0.741 ± 0.025a | 0.219 ± 0.039c | 0.459 ± 0.002b |
| NBRISH11 | 0.254 ± 0.01b | 0.432 ± 0.020a | 0.117 ± 0.030c | 0.346 ± 0.053ab |
| NBRISH13 | 0.095 ± 0.004b | 0.535 ± 0.045a | 0.176 ± 0.06b | 0.208 ± 0.026b |
| NBRISH14 | 0.130 ± 0.01c | 0.380 ± 0.012a | 0.249 ± 0.056b | 0.397 ± 0.002a |
| NBRISH16 | 0.225 ± 0.01b | 0.393 ± 0.022a | 0.220 ± 0.013b | 0.138 ± 0.009c |
| NBRISH26 | 0.234 ± 0.009c | 0.411 ± 0.036a | 0.269 ± 0.030bc | 0.353 ± 0.001ab |
| Colony forming unit (CFU $ml^{-1}$) | | | | |
| NBRISH4 | 9.20 ± 0.05b | 7.13 ± 0.05c | 7.35 ± 0.07c | 10.12 ± 0.13a |
| NBRISH6 | 9.23 ± 0.03a | 8.22 ± 0.03b | 9.13 ± 0.03a | 8.24 ± 0.04b |
| NBRISH10 | 8.30 ± 0.05c | 6.60 ± 0.09d | 9.28 ± 0.04b | 10.27 ± 0.10a |
| NBRISH11 | 10.20 ± 0.17a | 6.75 ± 0.04b | 10.62 ± 0.15a | 10.37 ± 0.05a |
| NBRISH13 | 9.23 ± 0.07b | 8.17 ± 0.08c | 7.81 ± 0.08c | 10.22 ± 0.04a |
| NBRISH14 | 10.10 ± 0.25b | 6.72 ± 0.11c | 11.40 ± 0.07a | 10.25 ± 0.02b |
| NBRISH16 | 9.20 ± 0.14a | 5.18 ± 0.04c | 9.51 ± 0.04a | 8.42 ± 0.05b |
| NBRISH26 | 8.30 ± 0.09a | 5.37 ± 0.10c | 7.18 ± 0.04b | 7.43 ± 0.10b |
| Isolates | Day 2 | | | |
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Biofilm OD at 590 nm | | | | |
| NBRISH4 | 0.349 ± 0.067a | 0.444 ± 0.006a | 0.362 ± 0.015a | 0.320 ± 0.003a |
| NBRISH6 | 0.858 ± 0.020a | 0.679 ± 0.007b | 0.476 ± 0.002b | 0.533 ± 0.001c |
| NBRISH10 | 0.861 ± 0.031a | 0.589 ± 0.075b | 0.455 ± 0.003b | 0.505 ± 0.048b |
| NBRISH11 | 0.840 ± 0.037a | 0.277 ± 0.013c | 0.338 ± 0.014c | 0.473 ± 0.004b |
| NBRISH13 | 0.876 ± 0.055a | 0.528 ± 0.072b | 0.270 ± 0.014c | 0.371 ± 0.012bc |
| NBRISH14 | 0.832 ± 0.17a | 0.258 ± 0.019b | 0.22 ± 0.004b | 0.342 ± 0.015b |
| NBRISH16 | 0.734 ± 0.09a | 0.294 ± 0.031b | 0.365 ± 0.003b | 0.431 ± 0.010b |
| NBRISH26 | 0.528 ± 0.08a | 0.327 ± 0.017c | 0.363 ± 0.022ab | 0.438 ± 0.019ab |
| Colony forming unit (CFU $ml^{-1}$) | | | | |
| NBRISH4 | 10.28 ± 0.10a | 8.63 ± 0.05c | 5.26 ± 0.06d | 9.22 ± 0.04b |
| NBRISH6 | 9.33 ± 0.07a | 8.49 ± 0.10b | 9.39 ± 0.06a | 9.23 ± 0.05a |
| NBRISH10 | 10.60 ± 0.05b | 9.17 ± 0.04c | 11.30 ± 0.07a | 9.31 ± 0.04c |
| NBRISH11 | 10.22 ± 0.11b | 8.32 ± 0.05c | 11.44 ± 0.05a | 10.37 ± 0.07b |
| NBRISH13 | 9.92 ± 0.15ab | 8.12 ± 0.08c | 9.53 ± 0.10b | 10.32 ± 0.06a |
| NBRISH14 | 9.50 ± 0.09c | 9.23 ± 0.04c | 10.61 ± 0.06a | 10.23 ± 0.05b |
| NBRISH16 | 10.20 ± 0.12a | 7.44 ± 0.04c | 9.53 ± 0.15b | 9.32 ± 0.11b |
| NBRISH26 | 10.03 ± 0.24a | 8.34 ± 0.05c | 7.22 ± 0.03d | 9.32 ± 0.06b |

**Table 3** continued

| Isolates | Day 3 | | | |
|---|---|---|---|---|
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Biofilm OD at 590 nm | | | | |
| NBRISH4 | 0.187 ± 0.064b | 0.453 ± 0.022a | 0.123 ± 0.003b | 0.092 ± 0.0039b |
| NBRISH6 | 0.55 ± 0.040a | 0.589 ± 0.007a | 0.237 ± 0.001b | 0.211 ± 0.002b |
| NBRISH10 | 0.571 ± 0.008a | 0.575 ± 0.009a | 0.278 ± 0.002a | 0.23 ± 0.001a |
| NBRISH11 | 0.462 ± 0.09a | 0.260 ± 0.016ab | 0.188 ± 0.015b | 0.267 ± 0.005ab |
| NBRISH13 | 0.438 ± 0.006b | 0.535 ± 0.046a | 0.095 ± 0.005c | 0.115 ± 0.002c |
| NBRISH14 | 0.47 ± 0.04a | 0.283 ± 0.005b | 0.125 ± 0.003c | 0.115 ± 0.002c |
| NBRISH16 | 0.277 ± 0.8a | 0.275 ± 0.011a | 0.188 ± 0.004a | 0.207 ± 0.002a |
| NBRISH26 | 0.458 ± 0.06a | 0.263 ± 0.01b | 0.11 ± 0.003c | 0.093 ± 0.003c |
| Colony forming unit (CFU $ml^{-1}$) | | | | |
| NBRISH4 | 10.10 ± 0.12a | 6.98 ± 0.07c | 5.13 ± 0.04d | 9.22 ± 0.04b |
| NBRISH6 | 9.27 ± 0.10b | 7.80 ± 0.07c | 9.44 ± 0.09b | 10.18 ± 0.09a |
| NBRISH10 | 10.41 ± 0.04a | 10.65 ± 0.06a | 9.25 ± 0.05b | 9.12 ± 0.04b |
| NBRISH11 | 9.40 ± 0.07b | 8.64 ± 0.15c | 11.43 ± 0.09a | 9.22 ± 0.04b |
| NBRISH13 | 9.28 ± 0.04b | 8.13 ± 0.03c | 10.35 ± 0.08a | 9.12 ± 0.05b |
| NBRISH14 | 8.52 ± 0.06c | 7.21 ± 0.03d | 10.60 ± 0.05a | 9.14 ± 0.04b |
| NBRISH16 | 8.15 ± 0.04c | 6.70 ± 0.08d | 9.20 ± 0.03b | 10.08 ± 0.06a |
| NBRISH26 | 9.23 ± 0.05b | 5.66 ± 0.03d | 6.58 ± 0.04c | 9.76 ± 0.14a |

Mean ± standard errors were compared by analysis of variance (ANOVA), followed by the Tuckey's test. Means were considered of three replicates. Statistically significant differences were then determined at $p \leq 0.05$, using the SPSS ver 20 and were denoted with means ± standard error followed with different letters

## Plant tests with bacterial isolates for the assessment of their PGP ability

Plants with individual bacterial treatments demonstrated higher root and shoot length (RL & SL), root and shoot fresh and dry weight (RFW, SFW, RDW and SDW) and number of leaves per plant (NLPP) when compared with control (Figs. 1, 2, 3, S1). We found that plants treated with strain NBRISH6 showed non-significant higher values compared with most of the other isolates but significantly higher values for all the parameters recorded compared to NBRISH4 (RFW and SFW) and controls (Fig. 1). On comparison with controls, NBRISH6 bacterial treatment to the plant showed 44 and 56% increase in RL and SL respectively (Fig. S1). Similarly, in comparison to controls, NBRISH6 increased the RFW and SFW by 97 and 144% respectively. Further, NBRISH6 treated plants were found to have increased RDW and SDW by 94 and 157% respectively, compared with controls (0.54 ± 0.16 g of RDW and 0.53 ± 0.13 g of SDW) (Fig. 2). In the context of NLPP, NBRISH6 treated plants showed the greatest number of leaves (5.33 ± 0.74) followed by NBRISH26 treated plants (5.16 ± 0.68) when compared with those in controls (3 ± 0.57) (Fig. 1).

## Molecular identification and phylogenetic analysis of potential isolates

All eight potential isolates were subjected to identification based on partial 16S rRNA gene sequences which revealed all the bacterial isolates to belong to *Pseudomonas* spp. except for NBRISH6 that belongs to *Ochrobactrum* sp. (Table 1). Out of the seven isolates which belonged to *Pseudomonas* spp., four (NBRISH10, NBRISH11, NBRISH13 and NBRISH14) of them appeared to represent *Pseudomonas stutzeri*, while NBRISH4 was characterised as *Pseudomonas guariconensis*. All the sequences were classified to the genus level with confidence >80%. Sequences of the eight bacterial isolates

**Table 4** Exo-polysaccharide and alginate production by selected isolates at different stress conditions for up to three days

| Isolates | Day 1 | | | |
|---|---|---|---|---|
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Exo-polysachharide production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 1032.73 ± 2.00a | 446.21 ± 2.17b | 443.93 ± 1.58b | 325.68 ± 2.34c |
| NBRISH6 | 1951.43 ± 5.97a | 752.45 ± 1.52d | 1623 ± 1.98b | 1057.41 ± 4.37c |
| NBRISH10 | 1652.33 ± 3.40a | 667.54 ± 2.53d | 1167.7 ± 2.04b | 1066.89 ± 1.75c |
| NBRISH11 | 2069.06 ± 4.77a | 612.74 ± 2.62d | 1274.36 ± 1.32b | 938.14 ± 1.48c |
| NBRISH13 | 1084.36 ± 4.66b | 451.38 ± 4.52d | 1474.4 ± 9.18a | 1014.41 ± 2.88c |
| NBRISH14 | 1139.3 ± 5.29b | 407.06 ± 3.51d | 1277.6 ± 1.49a | 980.52 ± 2.28c |
| NBRISH16 | 1432.2 ± 2.39a | 279.78 ± 5.27d | 715.5 ± 2.07c | 744.11 ± 2.89b |
| NBRISH26 | 1474.73 ± 3.24a | 669.79 ± 2.87b | 531.82 ± 2.23d | 618.28 ± 1.51c |
| Alginate production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 105.64 ± 1.06b | 13.25 ± 1.55d | 25.76 ± 0.40c | 130.64 ± 2.66a |
| NBRISH6 | 195.34 ± 2.61a | 61.64 ± 1.55c | 50.25 ± 0.73d | 148.05 ± 1.41b |
| NBRISH10 | 232.81 ± 3.33a | 120.52 ± 2.48c | 58.4 ± 1.08d | 159.63 ± 1.94b |
| NBRISH11 | 171.36 ± 2.46a | 112.78 ± 1.38c | 62.26 ± 0.86d | 129.53 ± 2.93b |
| NBRISH13 | 258.69 ± 4.66a | 107.35 ± 1.31b | 49.47 ± 0.29c | 114.51 ± 2.94b |
| NBRISH14 | 150.82 ± 2.26a | 113.20 ± 0.87c | 74.3 ± 0.25d | 142.54 ± 1.49b |
| NBRISH16 | 121.45 ± 1.92a | 63.28 ± 2.69c | 47.3 ± 0.51d | 111.66 ± 2.06b |
| NBRISH26 | 92.96 ± 4.17a | 88.17 ± 1.16a | 21.09 ± 0.37c | 76.57 ± 2.34b |
| Isolates | Day 2 | | | |
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Exo-polysachharide production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 1668.3 ± 5.28a | 505.22 ± 3.91d | 996.9 ± 3.47b | 663.37 ± 2.30c |
| NBRISH6 | 2529.63 ± 5.31a | 860.00 ± 2.43d | 1807.23 ± 4.17b | 1706.86 ± 2.90c |
| NBRISH10 | 2231.9 ± 9.69a | 844.24 ± 2.02c | 2247.63 ± 5.10a | 1858.77 ± 2.77b |
| NBRISH11 | 2242.9 ± 3.11a | 1010.72 ± 2.88d | 2097.86 ± 2.44b | 1759.74 ± 0.46c |
| NBRISH13 | 1566.26 ± 7.76b | 705.75 ± 2.64c | 1882.63 ± 2.66a | 1594.72 ± 3.43b |
| NBRISH14 | 1550.53 ± 4.93b | 1115.75 ± 2.87d | 1476.36 ± 8.10c | 1728.45 ± 1.44a |
| NBRISH16 | 1535.86 ± 4.03a | 680.15 ± 2.07d | 1053.36 ± 8.61c | 1168.91 ± 4.34b |
| NBRISH26 | 1710.83 ± 5.39a | 484.35 ± 2.99d | 661.08 ± 3.31c | 973.12 ± 1.51b |
| Alginate production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 126.88 ± 2.57b | 10.43 ± 0.80d | 42.65 ± 0.32c | 162.74 ± 1.60a |
| NBRISH6 | 304.016 ± 1.93a | 188.65 ± 1.16c | 203.8 ± 0.92b | 193.24 ± 1.51c |
| NBRISH10 | 296.78 ± 3.65a | 185.95 ± 2.34b | 192.76 ± 1.81b | 178.56 ± 4.11b |
| NBRISH11 | 224.30 ± 3.32b | 141.22 ± 1.90d | 207.81 ± 2.81c | 254.21 ± 2.23a |
| NBRISH13 | 293.56 ± 2.74a | 157.12 ± 1.90c | 145.65 ± 2.01d | 248.96 ± 2.07b |
| NBRISH14 | 232.21 ± 1.83a | 187.04 ± 1.56b | 111.6 ± 1.38c | 229.01 ± 0.96a |
| NBRISH16 | 150.11 ± 3.74a | 105.96 ± 2.19b | 78.5 ± 0.79c | 142.06 ± 2.08a |
| NBRISH26 | 137.38 ± 1.53b | 123.64 ± 1.25c | 70.18 ± 0.65d | 186.54 ± 3.51a |

**Table 4** continued

| Isolates | Day 3 | | | |
|---|---|---|---|---|
| | Control | Temperature (40 °C) | Salt (250 mM) | Drought (45% PEG) |
| Exo-polysachharide production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 1543.93 ± 3.67a | 490.47 ± 3.16c | 379.03 ± 1.05d | 522.91 ± 1.28b |
| NBRISH6 | 1720.23 ± 5.90a | 726.22 ± 4.79c | 560.6 ± 3.44d | 1450.68 ± 4.10b |
| NBRISH10 | 1847.23 ± 6.92a | 911.9 ± 2.38c | 825.06 ± 4.71d | 1656.55 ± 3.66b |
| NBRISH11 | 2020.86 ± 3.41a | 1695.30 ± 3.15b | 817.8 ± 2.27d | 1576.18 ± 3.23c |
| NBRISH13 | 2098.86 ± 3.67a | 1282.66 ± 3.19b | 814.26 ± 1.77d | 1229.75 ± 2.32c |
| NBRISH14 | 1978.4 ± 2.33a | 421.1 ± 2.80d | 735.56 ± 2.05c | 1022.04 ± 2.41b |
| NBRISH16 | 1626.16 ± 6.91a | 947.64 ± 4.57b | 411.36 ± 1.34d | 593.85 ± 2.43c |
| NBRISH26 | 1383.23 ± 4.78a | 323.05 ± 1.69c | 208.94 ± 1.67d | 403.15 ± 3.69b |
| Alginate production (μg $ml^{-1}$) | | | | |
| NBRISH4 | 188.12 ± 2.45a | 20.70 ± 0.97d | 39.69 ± 0.84c | 49.51 ± 2.08b |
| NBRISH6 | 308.51 ± 2.20a | 192.18 ± 3.08b | 159.41 ± 0.48c | 157.49 ± 4.80c |
| NBRISH10 | 281.49 ± 2.77a | 177.42 ± 2.101b | 122.37 ± 0.59d | 153.91 ± 1.83c |
| NBRISH11 | 270.85 ± 2.87a | 178.91 ± 1.89c | 96.97 ± 1.26d | 194.66 ± 1.96b |
| NBRISH13 | 163.16 ± 7.50a | 162.80 ± 1.13a | 101.16 ± 3.42b | 144.60 ± 2.92a |
| NBRISH14 | 204.95 ± 3.19a | 180.71 ± 2.65b | 101.3 ± 1.55d | 159.68 ± 2.02c |
| NBRISH16 | 151.59 ± 1.85a | 113.75 ± 1.20b | 75.27 ± 0.88d | 98.41 ± 1.08c |
| NBRISH26 | 122.34 ± 3.08a | 122.78 ± 1.14a | 37.27 ± 0.45c | 104.91 ± 4.02b |

Means ± standard errors were compared by analysis of variance (ANOVA), followed by the Tuckey's test. Means were considered of three replicates. Statistically significant differences were then determined at $p \leq 0.05$, using the SPSS ver 20 and were denoted with means ± standard error followed with different letters

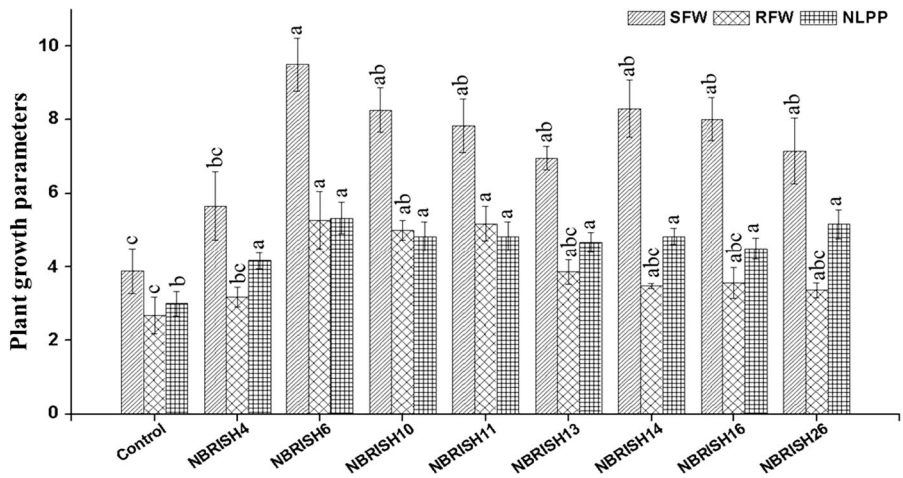



**Fig. 1** Shoot and root fresh weight (SFW and RFW; expressed as g in weight) and number of leaves per plant (NLPP; expressed in numbers) of maize plants inoculated with different bacterial isolates under unsterilised soils in vivo. Mean values for 12 replicates are shown. *Errors bars* represent standard errors. *Different letters* above the bars represent significant differences according to analysis of variance (ANOVA), followed by the Tuckey's test ($p \leq 0.05$)

were submitted to GenBank with the accession numbers shown in Table 1. The phylogeny of these bacterial isolates was further explored by constructing a phylogenetic tree using closely related and representative sequences from NCBI. After phylogenetic reconstruction, it was observed that the isolates were

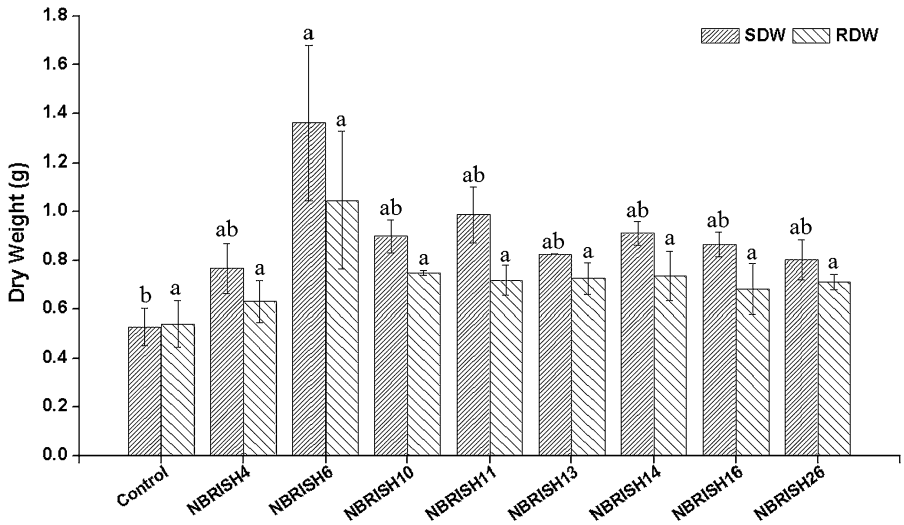



**Fig. 2** Shoot and root dry weight (SRW and RRW; expressed as g in weight) of maize plants inoculated with different bacterial isolates under unsterilised soils in vivo. Mean values for 12 replicates are shown. *Errors bars* represent standard errors. *Different letters* above the bars represent significant differences according to analysis of variance (ANOVA), followed by the Tuckey's test ($p \leq 0.05$)

recovered in two distinct clusters comprising *Pseudomonas* spp. (7 isolates) and *Ochrobactrum* spp. (NBRISH6) (Fig. 4). The cluster of *Pseudomonas* spp. further divided into two groups. The sequences of accession number KC357773, KC357774, KC357775 and KC357776 corresponding to the isolates NBRISH10, NBRISH11, NBRISH13 and NBRISH14 respectively formed a separate clade with *P. stutzeri* with a bootstrap support value of 100% (Fig. 3). Similarly, accession no. KP300813 representing bacterial isolate NBRISH4 formed a separate clade with *P. guariconensis* with a bootstrap support value of 100%. Two bacterial isolates, NBRISH16 (KX263305) and NBRISH26 (KX263306), were found to form a separate clade nearest to multiple *Pseudomonas* spp. (Fig. 4). Finally, the most notable bacterial isolate based on overall plant growth promotion abilities, NBRISH6 (KP300814), was found to be closely related to *Ochrobactrum intermedium* with a bootstrap support value of 99%.

## Discussion

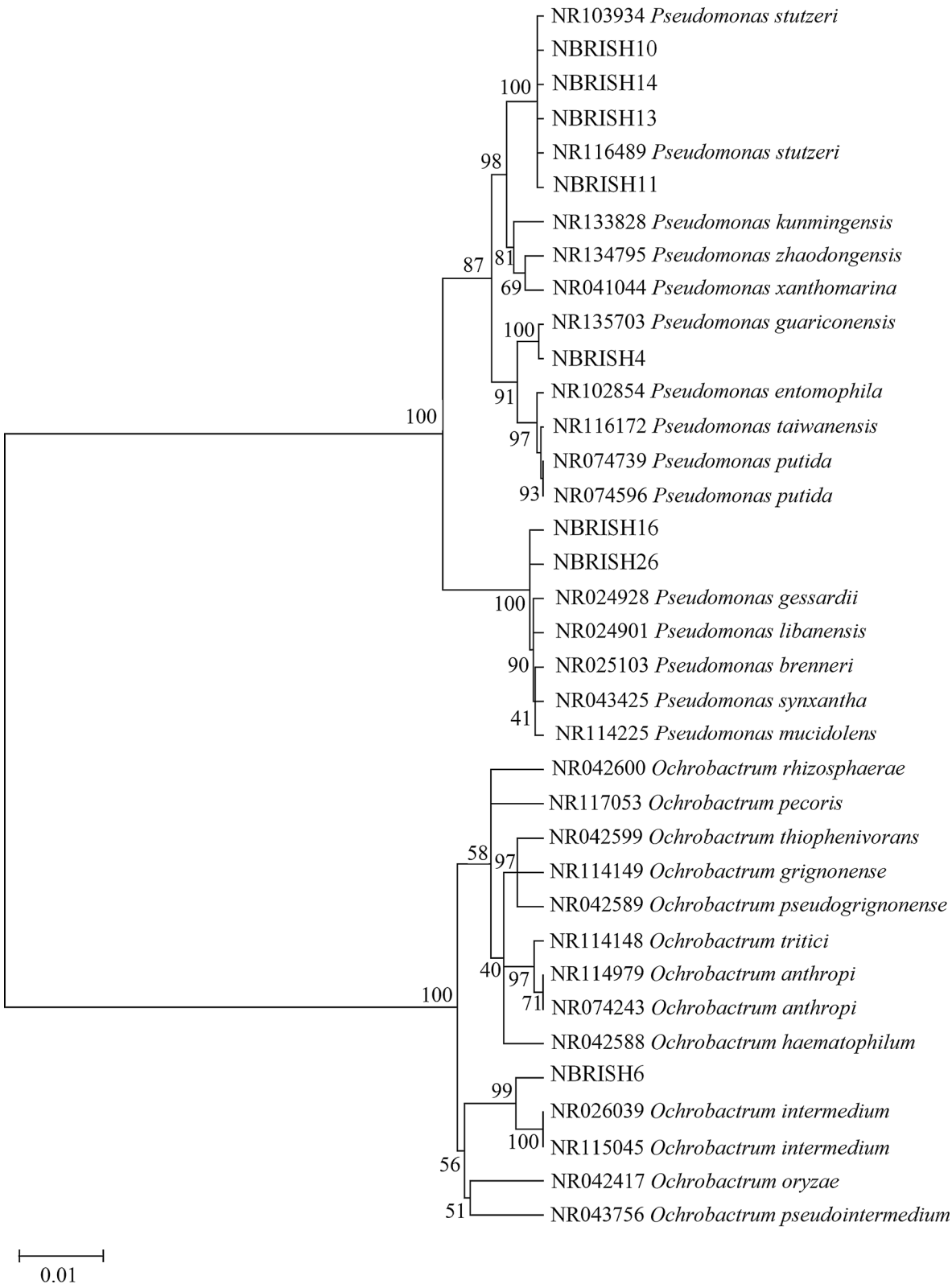
Salinity, drought and high temperature impacts deeply on our agro-ecosystems every year by creating uncultivable land and therefore affects crop production significantly. To overcome this issue, soil microbes with multiple abiotic stress mitigating traits have been proposed as a solution (Chaudhry et al. 2015; Yadav et al. 2015). However, even though these microbes may show successful abiotic stress tolerant qualities in vitro, they fail to perform either in situ conditions or, even if they do, they do not promote plant growth as expected of them. Similarly, soil microbes exhibiting PGP traits in vitro either lose their features in situ or cannot tolerate abiotic stress to achieve success in terms of PGP (Podile et al. 2013; Chauhan and Nautiyal 2010; Kloepper and Beauchamp 1992). Therefore, there is a need for microbes that possess multiple PGP as well as abiotic stress mitigating characteristics to provide stress protection and health benefits to plants.

In this regard, we have tried to isolate, characterise and screen bacteria from extreme environments such as locations nearby to volcanos in the Andaman and Nicobar islands, India based on their dual PGP and abiotic stress alleviating traits. Out of an initial 200 bacterial isolates, we selected eight efficient ones belonging to the phylum Proteobacteria namely, *Pseudomonas* spp. (NBRISH4, NBRISH10, NBRISH11, NBRISH13, NBRISH14, NBRISH16 and NBRISH26) and an *Ochrobactrum* sp. (NBRISH6) (Table 1). Previous studies (Yadav et al. 2015; Amaresan et al. 2014; Venkadesaperumal et al. 2014; Nishiyama et al. 1998) with culture dependent and independent approaches did not find any *Pseudomonas* sp. and *Ochrobactrum* sp. in volcanic sites or nearby areas. The earlier studies (Yadav et al. 2015; Amaresan et al. 2014; Venkadesaperumal et al. 2014) found *Bacillus*, *Escherichia*, *Ralstonia*, *Staphylococcus, Pantoea agglomerans* and *Exiguobacterium* sp. as

most common isolates exhibiting PGP traits from the volcano sites in the Andaman and Nicobar Islands, India. Notwithstanding the application of *Pseudomonas* sp. (Meintanis et al. 2006; Gao et al. 2016; Chakrabarty 1981) and *Ochrobactrum* sp. (Ortega-González et al. 2015; Mei et al. 2010) in biodegradation, their roles in the context of PGP are less well documented. Our study has been focused towards application of *Pseudomonas* and *Ochrobactrum* isolates from extreme environments (locations nearby to the sites of volcanos, Andaman and Nicobar Islands, India) as PGP. However, previous studies (Abiala et al. 2015; Laditi et al. 2012) suggested that the application of indigenous soil microbes in their native habitats may perform better than the 'exotic' isolates. A similar observation was made by Requena et al. (1997) wherein they found the native arbuscular mycorrhizal fungi and rhizobacteria exceeded in PGP performance when compared with their 'exotic' counterparts. This might be due to the fact that the local bioinoculants are well adapted to the regional conditions. In contrasting, our isolates were not imported commercial ones, yet they can be considered 'exotic' and showed enhancement of PGP, in particular length, fresh and dry matter, and leaves of plant when compared with controls (Figs. 1, 2, 3, S1). These PGP properties might indicate that the microbes considered in the present study were well-adapted to the extreme conditions developed in locations near volcanic sites. A possible explanation might be that microbes use polyphosphates as an energy source under extreme environments for their survival and therefore, when exposed to longer-term stress, microbes are able to adapt to unfavorable conditions (Seufferheld et al. 2008). Zhu et al. (2007) observed that the 'exotic' microbes (arbuscular mycorrhizal fungi) performed at par with increasing acidic pH level of soil while the performance of indigenous microbes (arbuscular mycorrhizalM fungi) was compromised. Similarly, Rodriguez et al. (2008) observed that microbes (symbiotic fungi) confer habitat-specific abiotic (salt and heat) stress tolerance to the establishment of plants under high stress conditions.

With regards to different characteristics of the bacterial isolates in vitro, we found no single isolate performed consistently better under any stress conditions (Tables 2, 3, 4). However, the *Pseudomonas* spp. exhibited better PGP, biofilm, alginate and EPS traits in various stress conditions. On the other hand, the *Ochrobactrum* sp. (NBRISH6) demonstrated better survival only in terms of colony count (CFU) under each stress. *Pseudomonas* and *Ochrobactrum* spp. are less known for their PGP attributes such as phosphate solubilisation (Oteino et al. 2015; Meng et al. 2014) and indole acetic acid (IAA) production (Meng et al. 2014; Bharucha et al. 2013). A possible mechanism for phosphate solubilisation is the secretion of organic acids by soil microbes to solubilise the phosphate complexes in soil and make them available to plants (Oteino et al. 2015; Meng et al. 2014). Further, IAA production by these soil microbes differentiates and proliferate the plant tissue and play an indirect role in the suppression of the root-associated pathogens (Khare and Arora 2010). Apart from IAA and phosphate solubilisation, these isolates also exhibited significant EPS and alginate production along with biofilm formation (Bogino et al. 2013; Qurashi and Sabri 2012; Redmile-Gordon et al. 2014). These characteristics provide competitive advantages to the producers through enhanced quorum sensing, colony adhesion, syntrophy, indirect defense against predation, solute transport, resistance towards antibiotics secreted by other rhizospheric soil resident microbes, and tolerance towards desiccation and heavy metals (Bogino et al. 2013; Qurashi and Sabri 2012; Redmile-Gordon et al. 2014).

Among the eight isolates studied here, our expectation that microbes would perform equally well in vitro and in vivo was not supported, except for strain NBRISH6. On considering PGP traits, isolate NBRISH4 demonstrated better in vitro phosphate solubilisation under salt stress, and auxin production under both temperature and salt stress but did not replicate the same performance in vivo. In contrast, the bacterial isolate NBRISH6that presented maximum phosphate solubilisation in vitro under temperature stress only showed maximum plant growth in vivo in terms of length, fresh and dry weights, and leaves count (Table 2; Figs. 1, 2, S1). In a similar observation, Cardinale et al. (2015) reported that the isolates having multiple PGP-activities in vitro did not promote plant growth in vivo. *Curtobacterium flaccumfaciens* demonstrated only two out of six PGP activities but increased barley growth up to 300% (Cardinale et al. 2015). However, their suggestion that the pure culture assay based screening of microbes for PGP traits is unsuitable (Cardinale et al. 2015), remains limitative in nature. Our findings for isolate


NR103934 Pseudomonas stutzeri
NBRISH10
NBRISH14
NBRISH13
NR116489 Pseudomonas stutzeri
NBRISH11
NR133828 Pseudomonas kunmingensis
NR134795 Pseudomonas zhaodongensis
NR041044 Pseudomonas xanthomarina
NR135703 Pseudomonas guariconensis
NBRISH4
NR102854 Pseudomonas entomophila
NR116172 Pseudomonas taiwanensis
NR074739 Pseudomonas putida
NR074596 Pseudomonas putida
NBRISH16
NBRISH26
NR024928 Pseudomonas gessardii
NR024901 Pseudomonas libanensis
NR025103 Pseudomonas brenneri
NR043425 Pseudomonas synxantha
NR114225 Pseudomonas mucidolens
NR042600 Ochrobactrum rhizosphaerae
NR117053 Ochrobactrum pecoris
NR042599 Ochrobactrum thiophenivorans
NR114149 Ochrobactrum grignonense
NR042589 Ochrobactrum pseudogrignonense
NR114148 Ochrobactrum tritici
NR114979 Ochrobactrum anthropi
NR074243 Ochrobactrum anthropi
NR042588 Ochrobactrum haematophilum
NBRISH6
NR026039 Ochrobactrum intermedium
NR115045 Ochrobactrum intermedium
NR042417 Ochrobactrum oryzae
NR043756 Ochrobactrum pseudointermedium
100
98
87
81
69
100
91
100
97
93
100
90
41
58
97
40
97
71
100
99
100
56
51
0.01

◀**Fig. 3** Growth representation of *Zea mays* plants inoculated with selected isolates. *A* represent Control; *B* represent NBRISH4; *C* represent NBRISH6; *D* represent NBRISH10; *E* represent NBRISH11; *F* represent NBRISH13; *G* represent NBRISH14; *H* represent NBRISH16; *I* represent NBRISH26

NBRISH6 (*Ochrobactrum* sp.) with maximum CFU in vitro under each abiotic stress (temperature, salt and drought) suggests that it may resist fluctuations under natural conditions too and therefore, presumably promote plant growth in vivo. This may explain why NBRISH6 increased the growth of maize plants in vivo more than the remaining isolates and controls (Figs. 1, 2, 3, S1). Although speculative, CFU might be a suitable in vitro survival metric for microbes to reflect their performance in vivo.

## Conclusions

This study establishes that *Pseudomonas* spp. and *Ochrobactrum* sp. from an extreme environment (locations near volcanos in Andaman and Nicobar islands, India) exhibiting tolerance towards different stress (temperature, salt and drought) conditions possess multiple PGP attributes. These findings suggest that further tests of the efficacy of isolates under field conditions with abiotic stress are warranted, to deduce their specific characteristics and the underlying mechanisms of PGP and stress alleviation. Knowledge of microbes from extreme environments having multiple plant beneficial and abiotic stress lowering characteristics is expected to enhance crop productivity on a sustainable basis in difficult or stress environments.


**Fig. 4** Phylogenetic relationships based on the 16S rRNA gene sequences using the neighbor-joining method (MEGA 6). The sequences are from the GenBank database and the present study. The *numbers* above each node on the tree indicates the percentages of bootstrap sampling derived from 1000 replications. *Bar* infers nucleotide substitutions per nucleotide

**Acknowledgements** The study was conducted using the operating funds of the network project Plant Microbe and Soil Interactions (PMSI) (BSC-0117) funded by Council of Scientific and Industrial Research, New Delhi, India. Authors are thankful to the Director, CSIR-NBRI, Lucknow for providing necessary resources to conduct this study.

## References

Abiala MA, Odebode AC, Hsu SF, Blackwood CB (2015) Phytobeneficial properties of bacteria isolated from the rhizosphere of maize in southwestern Nigerian soils. Appl Environ Microbiol 81:4736–4743. doi:10.1128/AEM.00570-15

Amaresan N, Kumar K, Sureshbabu K, Madhuri K (2014) Plant growth-promoting potential of bacteria isolated from active volcano sites of Barren Island, India. Lett Appl Microbiol 58:130–137. doi:10.1111/lam.12165

Barea JM (2015) Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J Soil Sci Plant Nutr 15:261–282

Bharucha U, Patel K, Trivedi UB (2013) Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). Agric Res 2:215–221. doi:10.1007/s40003-013-0065-7

Bogino PC, Oliva MDLM, Sorroche FG, Giordano W (2013) The role of bacterial biofilms and surface components in plant-bacterial associations. Int J Mol Sci 14:15838–15859. doi:10.3390/ijms140815838

Brick JM, Bostock RM, Silversone SE (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Cardinale M, Ratering S, Suarez C, Montoya AMZ, Geissler-Plaum R, Schnell S (2015) Paradox of plant growth promotion potential of rhizobacteria and their actual promotion effect on growth of barley (*Hordeum vulgare* L.) under salt stress. Microbiol Res 181:22–32. doi:10.1016/j.micres.2015.08.002

Cattelan AJ, Hartel PG, Furhmann JJ (1999) Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680. doi:10.2136/sssaj1999.6361670

Chakrabarty AM (1981) Microorganisms having multiple compatible degradative energy-generating plasmids and preparation thereof. US patent 4,259,444

Chaudhry V, Bhatia A, Bharti SK, Mishra SK, Chauhan PS, Mishra A, Sidhu OP, Nautiyal CS (2015) Metabolite profiling reveals abiotic stress tolerance in Tn5 mutant of *Pseudomonas putida*. PLoS ONE 10:e0113487. doi:10.1371/journal.pone.0113487

Chauhan PS, Nautiyal CS (2010) The *purB* gene controls rhizosphere colonization by *Pantoea agglomerans*. Lett Appl Microbiol 50:205–210. doi:10.1111/j.1472-765X.2009.02779

Chien A, Edgar DB, Trela JM (1976) Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus acquaticus*. J Bacteriol 127:1550–1557

Dastager SG, Lee JC, Ju YJ, Park DJ, Kim CJ (2009) *Leifsonia kribbensis* sp. nov., isolated from soil. Int J Syst Evol Microbiol 59:18–21. doi:10.1099/ijs.0.001925-0

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375–400

Gadhave KR, Hourston JE, Gange AC (2016) Developing soil microbial inoculants for pest management: can one have too much of a good thing? J Chem Ecol 42:348–356. doi:10.1007/s10886-016-0689-8

Gao Y-Z, Liu H, Chao H-J, Zhou N-Y (2016) Constitutive expression of a Nag-like dioxygenase gene through an internal promoter in the 2-chloronitrobenzene catabolism gene cluster of *Pseudomonas stutzeri* ZWLR2-1. Appl Environ Microbiol 82:3461–3470. doi:10.1128/AEM.00197-16

Gopalakrishnan S, Sathya A, Vijayabharathi R, Varshney RK, Gowda CLL, Krishnamurthy L (2015) Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech 5:355–377. doi:10.1007/s13205-014-0241

Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7:96–102. doi:10.4172/1948-5948.1000188

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598. doi:10.1007/s13213-010-0117-1

Khan N, Mishra A, Chauhan PS, Sharma YK, Nautiyal CS (2012) *Paenibacillus lentimorbus* enhances growth of chickpea (*Cicer arietinum* L.) in chromium-amended soil. Antonie Van Leeuwenhoek 101:453–459. doi:10.1007/s10482-011-9637-3

Khare E, Arora NK (2010) Effect of indole-3-acetic acid (IAA) produced by *Pseudomonas aeruginosa* in suppression of charcoal rot disease of chickpea. Curr Microbiol 61:64–68. doi:10.1007/s00284-009-9577-6

Kloepper JW, Beauchamp CJ (1992) A review of issues related to measuring of plant roots by bacteria. Can J Microbiol 38:1219–1232. doi:10.1139/m92-202

Laditi MA, Nwoke OC, Jemo M, Abaidoo RC, Ogunjobi AA (2012) Evaluation of microbial inoculants as biofertilizers for the improvement of growth and yield of soybean and maize crops in savanna soils. Afr J Agric Res 7:405–413. doi:10.5897/AJAR11.904

Mei GY, Yan XX, Turak A, Luo ZQ, Zhang LQ (2010) AidH, an alpha/beta-hydrolase fold family member from an *Ochrobactrum* sp. strain, is a novel *N*-acylhomoserine lactonase. Appl Environ Microbiol 76:4933–4942. doi:10.1128/AEM.00477-10

Meintanis C, Chalkou KI, Kormas KA, Karagouni AD (2006) Biodegradation of crude oil by thermophilic bacteria isolated from a volcano island. Biodegradation 17:3–9. doi:10.1007/s10532-005-6495-6

Meng X, Yan D, Long X, Wang C, Liu Z, Rengel Z (2014) Colonization by endophytic *Ochrobactrum* anthropi Mn1 promotes growth of Jerusalem artichoke. Microb Biotechnol 7:601–610. doi:10.1111/1751-7915.12145

Meyer JM, Abdallah MA (1978) The florescent pigment of *Pseudomonas* fluorescens biosynthesis, purification and physical-chemical properties. J Gen Microbiol 107:319–328. doi:10.1099/00221287-107-2-319

Mishra S, Mishra A, Chauhan PS, Mishra SK, Kumari M, Niranjan A, Nautiyal CS (2012) *Pseudomonas putida* NBRIC19 dihydrolipoamide succinyltransferase (*SucB*) gene controls degradation of toxic allelochemicals produced by *Parthenium hysterophorus*. J Appl Microbiol 112:793–808. doi:10.1111/j.1365-2672.2012.05256

Nautiyal CS (1997) A method for selection and characterization of rhizosphere-competent bacteria of chickpea. Curr Microbiol 34:12–17. doi:10.1007/s002849900136

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. doi:10.1111/j.1574-6968.1999.tb13383

Nishiyama M, Watanabe Y, Marumoto T (1998) Bacterial 16S rDNA sequences in immature volcanic ash soil on volcanoes Mt. Sakurajima and Mt. Fugen in Japan determined by PCR amplification. Soil Sci Plant Nutr 44:711–715. doi:10.1080/00380768.1998.10414498

Ortega-González DK, Cristiani-Urbina E, Flores-Ortíz CM, Cruz-Maya JA, Cancino-Díaz JC, Jan-Roblero J (2015) Evaluation of the removal of pyrene and fluoranthene by *Ochrobactrum anthropi Fusarium* sp. and their coculture. Appl Biochem Biotechnol 175:1123–1138. doi:10.1007/s12010-014-1336

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front Microbiol 6:745. doi:10.3389/fmicb.2015.00745

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant 118:10–15. doi:10.1034/j.1399-3054.2003.00086

Podile AR, Vukanti RVNR, Sravani A, Kalam S, Dutta S, Durgeshwar P, Rao VP (2013) Root colonization and quorum sensing are the driving forces of plant growth promoting rhizobacteria (PGPR) for growth promotion. Proc Natl Acad Sci, India, Sect B Biol 80:407–413. doi:10.16943/ptinsa/2014/v80i2/55117

Qurashi AW, Sabri AN (2012) Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. Braz J Microbiol 43:1183–1191. doi:10.1590/S1517-838220120003000046

Redmile-Gordon MA, Brookes PC, Evershed RP, Goulding KWT, Hirsch PR (2014) Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biol Biochem 72:163–171. doi:10.1016/j.soilbio.2014.01.025

Requena N, Jimenez I, Toro M, Barea JM (1997) Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in mediterranean semi-arid ecosystems. New Phytol 136:667–677. doi:10.1046/j.1469-8137.1997.00786

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416. doi:10.1038/ismej.2007.106

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491. doi:10.1126/science.2448875

Seufferheld MJ, Alvarez HM, Farias ME (2008) Role of polyphosphates in microbial adaptation to extreme environments. Appl Environ Microbiol 74:5867–5874. doi:10.1128/AEM.00501-08

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131. doi:10.1016/j.sjbs.2014.12.001

Singh RB (2013) Climate change and abiotic stress management in India. In: Tuteja N, Gill SS (eds) Climate change and plant abiotic stress tolerance, vol 1. Wiley, Weinheim, pp 57–77. doi:10.1002/9783527675265.ch03

Singh BN, Singh HB, Singh A, Singh BR, Mishra A, Nautiyal CS (2012) *Lagerstroemia speciosa* fruit extract modulates quorum sensing-controlled virulence factor production and biofilm formation in *Pseudomonas aeruginosa*. Microbiol 158:529–538. doi:10.1099/mic.0.052985-0

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology, 3rd edn. ASM Press, Washington DC, pp 607–654

Srivastava S, Yadav A, Seem K, Mishra S, Chaudhary V, Nautiyal CS (2008) Effect of high temperature on *Pseudomonas putida* NBRI10987 biofilm formation and expression of stress sigma factor RpoS. Curr Microbiol 56:453–457. doi:10.1007/s00284-008-9105-0

Srivastava S, Chaudhry V, Mishra A, Chauhan PS, Rehman A, Yadav A, Tuteja N, Nautiyal CS (2012) Gene expression profiling through microarray analysis in *Arabidopsis thaliana* colonized by *Pseudomonas putida* MTCC5279, a plant growth promoting rhizobacterium. Plant Signal Behav 7:235–245. doi:10.4161/psb.18957

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101:11030–11035. doi:10.1073/pnas.0404206101

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. doi:10.1093/molbev/msr121

Titus S, Gasnkar N, Srivastava KB, Karande AA (1995) Exopolymer production by a fouling marine bacterium *Pseudomonas alcaligenes*. Indian J Mar Sci 24:45–48

Venkadesaperumal G, Amaresan N, Kumar K (2014) Plant growth promoting capability and genetic diversity of bacteria isolated from mud volcano and lime cave of Andaman and Nicobar Islands. Braz J Microbiol 45:1271–1281. doi:10.1590/S1517-83822014000400018

World Economic Forum (2011) Realizing a new vision for agriculture: a roadmap for stakeholders. World Economic Forum, Davos

Yadav AN, Verma P, Kumar M, Pal KK, Dey R, Gupta A, Padaria JC, Gujar GT, Kumar S, Suman A, Prasanna R,

Saxena AK (2015) Diversity and phylogenetic profiling of niche-specific Bacilli from extreme environments of India. Ann Microbiol 65:611–629. doi:10.1007/s13213-014-0897-9

Zhu H-H, Yao Q, Sun X-T, Hu Y-L (2007) Colonization, ALP activity and plant growth promotion of native and exotic arbuscular mycorrhizal fungi at low pH. Soil Biol Biochem 39:942–950. doi:10.1016/j.soilbio.2006.11.006