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ORIGINAL ARTICLE

Demonstrating the potential of abiotic stress-tolerant *Jeotgalicoccus huakuii* NBRI 13E for plant growth promotion and salt stress amelioration

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Abstract

The present study aimed to demonstrate the potential of abiotic stress-tolerant *Jeotgalicoccus huakuii* NBRI 13E for plant growth promotion and salt stress amelioration. NBRI 13E was characterized for abiotic stress tolerance and plant growth-promoting (PGP) attributes under normal and salt stress conditions. Phylogenetic comparison of NBRI 13E was carried out with known species of the same genera based on 16S rRNA gene. Plant growth promotion and rhizosphere colonization studies were determined under greenhouse conditions using maize, tomato, and okra. Field experiment was also performed to assess the ability of NBRI 13E inoculation for improving growth and yield of maize crop in alkaline soil. NBRI 13E demonstrated abiotic stress tolerance and different PGP attributes under in vitro conditions. Phylogenetic and differential physiological analysis revealed considerable differences in NBRI 13E as compared with the reported species for *Jeotgalicoccus* genus. NBRI 13E colonizes in the rhizosphere of the tested crops, enhances plant growth, and ameliorates salt stress in a greenhouse experiment. Modulation in defense enzymes, chlorophyll, proline, and soluble sugar content in NBRI 13E-inoculated plants leads to mitigate the deleterious effect of salt stress. Furthermore, field evaluation of NBRI 13E inoculation using maize was carried out with recommended 50 and 100% chemical fertilizer controls, which resulted in significant enhancement of all vegetative parameters and total yield as compared to respective controls. *Jeotgalicoccus huakuii* NBRI 13E is reported for the first time for its ability to develop a bioinoculant formulation for stress amelioration and improved crop productivity.

Keywords Abiotic stress · Defense enzymes · *Jeotgalicoccus huakuii* · Maize · PGP attributes

Introduction

Soil salinity is a global problem increasing its breadth by 0.25–0.5 million hectares (Mha) every year. The irrigated agricultural land with increasing soil salinity has adversely affected the crop production by more than 34 Mha (Rubio et al. 2017). Notably, salinity stress is the primary concern of the arid and semiarid area leading to reduced plant growth and yield which might seriously affect the relative proportions of food supply and demand in the future (Li et al. 2016; Negrão et al. 2017). Salinity mainly affects photosynthesis by reduction of the contents of photosynthetic pigments, which consequently changes light absorbance (Ashraf and Harris 2013). Also, a decrease in plant biomass, leaf area, and growth has been observed in different crops under salt stress (Bharti et al. 2013; Giuffrida et al. 2013). There have been numerous reports about the correlation between salt and alkaline stress (Bromham et al. 2013; Bui 2013; Bui et al. 2014). The presence of excess soluble salts in the soil of arid and semiarid areas limits the production of most crops including vegetables such as tomato, okra, and maize (AVRDC 2006; Farooq et al. 2015; Albaladejo et al. 2017). Salinity (high soil NaCl concentrations) often co-exists with alkalinity (high soil pH) in the soil due to the occurrence of sodium carbonates (Rengasamy 2010). Degradation of soil physical condition by increasing the soil pH is

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considered to be another significant cause of salinity which can affect plant growth (Bui et al. 2014; Machado and Serralheiro 2017). Higher pH (alkaline) is toxic and can adversely affect physiological and biochemical processes, including mineral uptake, photosynthesis, membrane integrity, and yield of plants (Chen et al. 2012; Gerhardt et al. 2017). Therefore, lineages occupying these environments have to evolve strategies to cope with both alkalinity and salt stress (Bui 2013).

Addressing severe global issues like food security by the use of chemical fertilizers has raised the concern in the public domain as its application may intensify soil salinization (Rubio et al. 2017). Several researchers have reported that application of plant growth-promoting (PGP) microbes with multifarious PGP attributes could be used to develop potential alternative technology for abiotic stress amelioration (Tiwari et al. 2016; Misra et al. 2017). Plant growth-promoting rhizobacteria (PGPR) have been reported for playing a significant role in plant growth promotion by indole acetic acid (IAA) production, phosphate (P) solubilization, biofilm formation, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (Ambardar and Vakhlu 2013; Chakraborty et al. 2013; Siddikee et al. 2015; Misra et al. 2017). Other essential traits of PGPR are exopolysaccharide (EPS) and alginate (Alg) production which provides a competitive advantage to PGPR against desiccation in extreme environments by water conservation and scavenging essential nutrients (Sandhya and Ali 2015; Mishra et al. 2017). PGPR treatment has imparted growth promotion in various crops including okra, maize, and tomato through the increased shoot and root length and shoot fresh and dry weight, under salt stress condition (Fan et al. 2016; Li and Jiang 2017; Nazir et al. 2017). PGPR also protect the plant against the aftermath of salt stress by preventing inhibition of photosynthesis and modulating defense enzyme machinery (Tiwari et al. 2016; Gerhardt et al. 2017).

However, certain PGPR turned out to be unsuccessful under saline stress because of their either inability to colonize the root system or adapt to the saline conditions (Paul and Nair 2008). Therefore, for the successful application of PGPR under saline condition, it is a prerequisite that bacterial strains should be isolated from native salt-affected soils as well as compatible to the rhizosphere of multiple hosts (Sharma et al. 2015; Vaishnav et al. 2016; Gontia-Mishra et al. 2017; Misra et al. 2017).

The present study was aimed to evaluate the abiotic stress tolerance and multiple PGP attributes under saline stress of *Jeotgalicoccus huakuii* (NBRI 13E). We have conducted the greenhouse experiment to examine the effect of NBRI 13E inoculation to foster the growth promotion in different hosts, i.e., okra (*Abelmoschus esculentus*), maize (*Zea mays*) and, tomato (*Solanum lycopersicum*) under control and saline stress conditions. Apart from saline stress, NBRI 13E was further exposed to degraded alkaline soil using maize as a host plant. To the best of our knowledge, this is the first report for abiotic stress tolerance with multiple PGP attributes of *J. huakuii* strain for conferring plant fitness under stressed soil conditions.

Materials and methods

Abiotic stress tolerance ability of NBRI 13E

A plant growth-promoting and salt-tolerant *J. huakuii* NBRI 13E (NBRI 13E) (GenBank accession no. KX495292) was isolated from the arable soil of central zone (UP), India (Misra et al. 2017). In the present study, NBRI 13E was subjected to temporal evaluation for its ability to tolerate different abiotic stresses, namely salt (NaCl; 0.5 and 1 M), drought (30 and 45% PEG 6000), pH (9 and 11), and temperature (37 and 40 °C). The bacteria under the mentioned abiotic stresses were grown in nutrient medium at 28 °C (except for temperature stress) for 24 h with continuous shaking at 180 rpm. The ability to tolerate abiotic stress was determined by counting the respective colony forming unit (Log_{10} CFU ml^{-1}) (Mishra et al. 2017).

Quantitative estimation of PGP and other attributes under normal and salt stress condition of NBRI 13E

NBRI 13E was quantitatively evaluated for P solubilization, IAA production, biofilm formation, and ACC deaminase activity under normal (0 M NaCl) as well as salt (0.5 and 1 M NaCl) stressed condition. Production of EPS and Alg by NBRI 13E under the normal and salt-stressed conditions was also estimated to understand its potential to utilize for protecting the host plant under stressed conditions. Strain NBRI 13E was further characterized by qualitative assessment of biochemical tests using KB003 Hi25TM identification kit (HiMedia, India).

In order to estimate the solubilization of phosphate (μg ml^{-1}) by NBRI 13E, NBRIP medium was used as substrate. The solubilized phosphate was then quantified by molybdenum blue method at an absorbance of 660 nm using a spectrophotometer (Nautiyal 1999). In the case of quantification of auxin production, NBRI 13E was inoculated in nutrient broth (NB) supplemented with tryptophan as a substrate and incubated at 28 °C. After 24 h of incubation, orthophosphoric acid followed by Salkowski's reagent was added to the centrifuged supernatant of the culture. The absorbance of the resultant pink color was measured at 530 nm using a colorimeter (Bric et al. 1991). In the case of biofilm formation, bacterial inoculum was stained with 0.1% crystal violet followed by washing with 95% ethanol. After washing, optical density (OD) was measured at 590 nm using a spectrophotometer (Evolution 201, Thermo Scientific, USA) (Srivastava et al. 2008). The ACC deaminase activity was performed as described earlier with slight modifications (Penrose and Glick

2003). With regard to EPS production by NBRI 13E, an equal volume of phenol and sulfuric acid was used to determine the amount of exopolysaccharide produced in 24-h-old bacterial culture at the absorbance of 490 nm using a spectrophotometer (Titus et al. 1995). Quantitative assessment of Alg production was determined using the method as described by Mishra et al. (2012).

Phylogenetic characterization of NBRI 13E by using 16S rRNA gene analysis

The partial 16S rRNA gene sequence of NBRI 13E (Misra et al. 2017; GenBank accession no. KX495292.1) was aligned with available nearest neighbor sequences from the NCBI database using ClustalW of the MEGA 7 software package (Kumar et al. 2016). The method of Jukes and Cantor (1969) was used to calculate evolutionary distances. The phylogenetic dendrogram was constructed by the neighbor-joining method, and tree topologies were evaluated by performing bootstrap analysis of 1000 datasets using MEGA 7 (molecular evolutionary genetic analysis) (Kumar et al. 2016).

Assessment of NBRI 13E for its ability of plant growth promotion under greenhouse condition

Following the characterization of NBRI 13E based on its PGP and other attributes under normal and salt stress conditions in vitro, the bacterial strain was also tested for its capability of PGP under plant test using okra, maize, and tomato as model plants in plastic pot condition (15 cm in diameter). The evaluation of seed germination assay under different salt concentrations (0, 50, 100, 150, and 200 mM) has led us to select 100 mM as the lowest critical limit for germination of tested host crop seeds. Experiments were conducted in a completely randomized block design with 18 plant replicates equally distributed in six pots (three plant replicates per pot) containing 2.0 mm sieved unsterilized field soils (2.0 kg soil per pot) of CSIR-National Botanical Research Institute, Lucknow, India (latitude/longitude 11°24′ N/79°44′ E). Similarly, okra (*A. esculentus* "Anamika") and maize (*Z. mays* "Maharaja") seeds were bacterized as described by Nautiyal (1997), while for tomato (*S. lycopersicum* "Pusa ruby"), the method illustrated by Dixit et al. (2016) was followed. Plants were grown under natural greenhouse conditions and the treatments for each host plant with respect to bacterial strain were as follows: control (uninoculated; no salt), salt (uninoculated; 100 mM NaCl), NBRI 13E (*J. huakuii*), and NBRI 13E+S (*J. huakuii* treated with 100 mM NaCl). Soil moisture was maintained to 20% with water in the control and salt treatments and 48-h grown bacterial culture (NBRI 13E) (~ $10^{8–9}$ CFU ml^{-1}) in bacteria (NBRI 13E) and bacteria with salt (NBRI 13E+S) treatments. Plants in all the treatments were grown in parallel and harvested at the same time. In a salt-stressed set (salt and NBRI 13E+S), 100 mM salt (NaCl) treatment was given according to Nautiyal et al. (2013).

Plant vegetative parameters and biochemical and antioxidative assays

For vegetative parameters, three replicates of okra, maize, and tomato plants were harvested, and data were recorded for the shoot and root length (cm), number of leaves per plant, and plant fresh and dry weight (g).

For estimation of chlorophyll (total) and carotenoid content in the leaves, we chose three replicates of the plants under each treatment (control, NBRI 13E, salt, and NBRI 13E+S) (Arnon 1949). To quantify the proline content, we took leaves from three plants pertaining to each treatment (control, NBRI 13E, salt, and NBRI 13E+S). The determination of proline content was done from fresh leaves (three replicates concerning each treatment) using the method of Bates et al. (1973). Sugar estimation in greenhouse-grown plants was performed using fresh leaves (three replicates pertaining to each treatment) as per the protocol of Dubois et al. (1956).

Estimation of defense enzymes of leaf samples was done using established standard protocols; 500 mg of leaves (three replicates pertaining to each treatment) were homogenized in 1 ml of extraction buffer (100 mM potassium phosphate buffer [pH 7.0] containing 0.1 mM EDTA and 1% polyvinylpyrrolidone [*w*/*v*] at 4 °C). The homogenate was centrifuged at 15,000×*g* for 15 min at 4 °C and the supernatants were stored at − 80 °C. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured as previously described (Beauchamp and Fridovich 1971). Briefly, 3 ml mixture was prepared to contain 100 μl of enzyme extract, 13 mM methionine, 75 μM nitrobluetetrazolium (NBT), 40 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, and 02 μM riboflavin in that order. In the case of blank, the mixture containing the enzyme extract was kept in the dark, whereas those without the protein (control) and with the enzyme (treatments) were maintained in the light. The reaction was started by switching on the light for 30 min. The absorbance was measured at 560 nm. The activity of SOD is the measurement of NBT reduction by mixture devoid of protein under light minus NBT reduction by the mixture containing protein. One unit of activity is the quantum of enzyme required to inhibit half of the initial NBT reduction under the light and expressed as units mg^{-1} protein g^{-1} FW. Catalase (CAT; EC 1.11.1.6) activity was determined as described (Aebi 1984). The rate of decline in absorbance was measured during 3 min at 240 nm in the reaction mixture consisting of 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2, and 100 μl of enzyme extract. For the control, the mixture considered was 50 mM sodium phosphate buffer (pH 7.0) and 100 μl of protein, whereas for the blank, it was

50 mM sodium phosphate buffer (pH 7.0) and 20 mM H_2O_2. The expression of enzyme activity was in H_2O_2 (μmol) separated in min mg^{-1} FW. The ascorbate peroxidase (APX; EC 1.11.1.3) was assayed by estimating the reduction in absorbance (290 nm; an absorbance coefficient 2.8 mM^{-1} cm^{-1}) for the rate of oxidation of ascorbate (Nakano and Asada 1981). In short, the reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, and 0.1 mM EDTA. The reaction was initiated by adding either enzyme extract (treatments) or hydrogen peroxide (control). The decline in absorbance was recorded from 10 to 30 s after the start of the reaction. The expression of the enzyme activity was in μmol of ascorbate oxidized min^{-1} g^{-1} FW. Guaiacol peroxidase (EC 1.11.1.7) activity was determined (Hemeda and Klein 1990) colorimetrically at 470 nm using 1% guaiacol (*v*/*v*; as a donor), 0.3% H_2O_2 (as a substrate), and 50 mM phosphate buffer (pH 6.6). The protein was added to initiate the reaction, whereas ethanol was added to serve as a control. The oxidation of guaiacol (extinction coefficient 26.6 mM^{-1} cm^{-1}) was monitored with the increase in absorbance. The activity of the enzyme was expressed in terms of μmol of guaiacol oxidized min^{-1} g^{-1} FW. The polyphenol oxidase (EC 1.10.3.1) assay was performed (Patra and Mishra 1979) with the mixture consisting of 300 μmol phosphate buffer (pH 6.8), 5 μmol pyrogallol, and enzyme extract for 5 min; 5% (*v*/*v*) H_2SO_4 was used to stop the reaction followed by centrifugation for 15 min at 3000×*g*. Enzyme activity was measured as an increase in the amount of purpurogallin formed in the reaction at 420 nm. The expression of the polyphenol oxidase activity was in terms of μg polyphenol oxidase (PPO) mg^{-1} protein g^{-1} FW.

Tracking of NBRI 13E in the rhizosphere of okra, maize, and tomato

In order to monitor the rhizosphere competence ability of NBRI 13E on plant roots grown in nonsterilized soils, a spontaneous rifampicin-resistant (Rif^R) strain of NBRI 13E was isolated on NA plates, containing 250 μg rifampicin ml^{-1} (from Sigma Chemical Co., St. Louis, MO, USA) as described earlier (Nautiyal 1997). One gram of roots of the host plants was thoroughly washed with tap water for 2 min to remove all loosely adhering soil particles, followed by washing with sterile 0.85% NaCl (*w*/*v*) and macerated with a mortar and pestle. Heterogeneous rhizosphere bacterial population was recovered by serial dilution plating of the homogenate on NA plates and NA plates amended with 50 μg rifampicin ml^{-1} for NBRI 13E. Average rhizosphere colonization of NBRI 13E (Log_{10} CFU/g root dry weight) was determined from three plants at different time intervals (0, 15, 30, and 45 days after inoculation, DAI). No naturally occurring Rif^R bacteria were observed when root homogenates of uninoculated controls were plated from nonsterilized soils.

Field experiment

A field experiment was conducted to evaluate the efficacy of NBRI 13E in enhancing growth and yield of maize (*Z. mays* "Maharaja") crop in highly alkaline soil (pH 10.2) at Banthara Research Centre, Lucknow, India (latitude 26° 42′ N, longitude 80° 49′ E). The treatments tested were control, NBRI 13E, 50% NPK, and 100% NPK. The recommended doses of N, P, and K were applied at the rate of 120, 60, and 40 kg ha^{-1} and considered as NPK (100%) treatment. Moreover, half of the aforesaid mentioned dose of N, P, and K was considered as NPK (50%), while NBRI 13E treatment carries bacterial treatment along with NPK (50%). However, no N, P, and K were applied to the control. FYM (farmyard manure) was applied in all the treatments having 25 t ha^{-1} as basal dose. Seed bacterization was carried out as described by Nautiyal (1997), while seeds were treated with water in the case of control, 100% NPK, and 50% NPK. Each treatment was replicated thrice following a randomized complete block design (RCBD). Plot size of 7.5 m^2 (2.5 m × 3.0 m) was used for each treatment. There were six rows in each plot, and ten seeds were sown in a single row. Plant-to-plant distance was 20 cm, and row-to-row distance was maintained as 60 cm. Seed rate was used as 20–22 kg ha^{-1}. The sources of N, P, and K were urea, diammonium phosphate (DAP), and sulfate of potash (SOP), respectively. The crop was irrigated with canal water. All recommended agronomic and plant protection measures were followed. The crop was harvested at maturity and data regarding plant and cob growth parameters (plant height, plant fresh weight and plant dry weight, number of cob per plant, cob weight, number of rows per cob, number of seeds per row, total seeds per cob, seed weight per cob, 100 seed weight) and yield of maize were recorded. For data collection, six plants per plot were randomly selected.

Statistical analysis

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 Duncan test to determine significance ($p \leq 0.05$). The SPSS statistical software (ver 20) was used for the statistical analysis.

Results

Abiotic stress tolerance ability of NBRI 13E

NBRI 13E was subjected to evaluate its abiotic stress tolerance abilities under in vitro conditions. At 0.5 and 1.0 M NaCl, NBRI 13E has shown survival till day 10 with 6.24 and 5.26 Log_{10} CFU ml^{-1}, respectively (Fig. 1). While at 30 and 45% PEG 6000, NBRI 13E could survive with 5.57 and

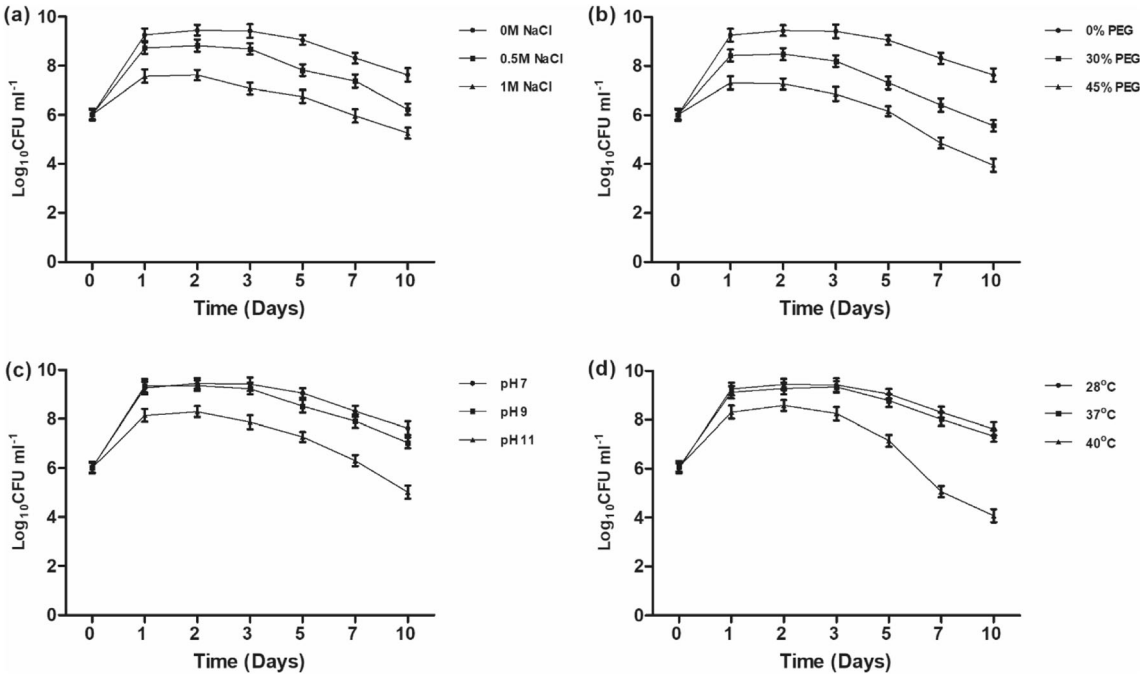

Fig. 1 Temporal growth assessment of *Jeotgalicoccus huakuii* (NBRI 13E) under different abiotic stress conditions: **a** salt, **b** drought, **c** pH, and **d** temperature; 0 M NaCl, 0% PEG, pH 7, and 28 °C represent control for the experiment under the mentioned stress condition. Error bars are the standard error of the means ($n = 3$)

3.95 Log_{10} CFU ml^{-1}, respectively (Fig. 1). Moreover, at pH 9 and 11 and 37 and 40 °C, NBRI 13E has demonstrated its survival with 7.05 and 5.02 Log_{10} CFU ml^{-1} and 7.34 and 4.08 Log_{10} CFU ml^{-1}, respectively (Fig. 1). Overall, NBRI 13E has demonstrated its ability to withstand abiotic stress with minimum (3.97%; 37 °C and 8.46%; pH 9) and maximum (93.06%; 45% PEG 6000 and 87%; 40 °C) decrease of growth pattern as compared to control till the tenth day.

Quantitative estimation of PGP and other attributes under normal and salt stress conditions of NBRI 13E

Quantitative estimation of biofilm formation by NBRI 13E showed significant difference across different salt stress conditions (s 1). Maximum biofilm formation capability of NBRI 13E was exhibited under 0.5 M NaCl condition, while the minimum was demonstrated in 1 M NaCl (Table 1).

In the case of IAA production, NBRI 13E was observed to have no significant difference between normal and 0.5 M NaCl condition but exhibited significant reduction under 1 M salt stress condition (Table 1). IAA production ability of NBRI 13E was found to be the highest under normal condition, but it demonstrated slight (4.15%) reduction under 0.5 M NaCl stress and intense decrease (79.00%) under 1 M NaCl stress when compared with normal condition.

Regarding phosphate solubilization in the current study, we observed that NBRI 13E exhibited a significant difference in phosphate solubilization ability under stress and normal conditions. It demonstrated the highest phosphate solubilization ability under 0.5 M NaCl condition, and least activity was observed under 1 M NaCl condition (Table 1).

Further, NBRI 13E was subjected to quantitative estimation of ACC deaminase activity under control and salt (0.5 and 1 M NaCl) stress conditions. NBRI 13E displayed the highest ACC deaminase activity under 0.5 M NaCl stress condition, while the lowest was observed under the control condition (Table 1). Surprisingly, NBRI 13E showed enhancement (19.01 and 6.39%) rather than reduction for ACC deaminase activity under 0.5 and 1 M NaCl stress in comparison to control.

Upon considering EPS production, we found that NBRI 13E exhibited the highest EPS production under 0.5 M NaCl condition, while under 1 M NaCl, it accounted for the least EPS production (Table 1). Although NBRI 13E documented a significant difference among all the three treatments for EPS production, its activity was startlingly enhanced under 0.5 M NaCl condition by 11.66%, while it declined by 27.97% under 1 M NaCl condition in comparison to control.

With regard to alginate production ability, it has been found that NBRI 13E showed a significant difference among all the three treatments for alginate production and has recorded the highest alginate production capacity under 0.5 M NaCl condition, whereas it exhibited the lowest alginate production capacity under 1 M NaCl condition (Table 1). NBRI 13E used in this study was also evaluated for qualitative estimation of biochemical properties and found to be positive for only catalase and arginine activity (Supplementary Table S1).

The physiological characteristics of NBRI 13E were found to be consistent with other bacterial species of the genus

Table 1 Plant growth-promoting (PGP) attributes of *Jeotgalicoccus huakuii* (NBRI 13E) under different salt (NaCl) stress (0, 0.5, and 1 M) condition

Plant growth-promoting traits	0 M NaCl	0.5 M NaCl	1 M NaCl
Biofilm[1]	0.84 ± 0.01^{b}	0.94 ± 0.00^{c}	0.29 ± 0.00^{a}
IAA[2]	105.47 ± 1.90^{b}	101.27 ± 1.63^{b}	22.21 ± 0.16^{a}
P-solubilization[3]	25.15 ± 0.07^{c}	23.94 ± 0.12^{b}	11.11 ± 0.16^{a}
ACC deaminase[4]	0.71 ± 0.02^{a}	0.88 ± 0.00^{b}	0.76 ± 0.02^{a}
EPS[5]	582.83 ± 1.93^{b}	659.75 ± 1.12^{c}	455.43 ± 1.77^{a}
Alg[6]	388.48 ± 1.15^{b}	586.72 ± 1.15^{c}	378.08 ± 1.25^{a}

PGP attributes are expressed as mean values of three replicates ± SE which were compared by analysis of variance (ANOVA), followed by the Duncan test. Statistically significant differences were then determined at $p \leq 0.05$, using the SPSS ver 20.0 and represented by different letters. Values in the rows with the same letter are not significantly different ($p \leq 0.05$) by the Duncan test

[1] Biofilm was measured at an optical density (OD) at 590 nm

[2] Indole acetic acid (IAA) production is expressed as μg ml^{-1}

[3] Phosphate (P) solubilization is expressed as μg ml^{-1}

[4] ACCdeaminase activity is expressed as nmol α-ketobutyrate mg $protein^{-1}$ h^{-1}

[5] Exopolysaccharide (EPS) production is expressed as μg ml^{-1}

[6] Alginate (Alg) production is expressed as μg ml^{-1}

Jeotgalicoccus (Supplementary Table S1). For example, growth at 37 °C and 250, 500, and 1000 mM NaCl, catalase activity was similar to those of *J. huakuii*, *Jeotgalicoccus marinus*, *Jeotgalicoccus halotolerans*, and *Jeotgalicoccus halophilus*. By contrast, NBRI 13E could be distinguished from other members of the genus *Jeotgalicoccus* which do not possess the ability to survive at high pH (11) and under drought stress condition (PEG 30 and 45%). Also, NBRI 13E demonstrated various PGP attributes such as IAA and siderophore production, P solubilization, ACC deaminase activity, and biofilm formation which have not been determined for other members of the genus *Jeotgalicoccus* (Supplementary Table S1).

16S rRNA gene-based phylogenetic analysis of NBRI 13E

Plant growth-promoting rhizobacteria NBRI 13E showed the closest homology with the species of *Jeotgalicoccus* genera (Fig. 2). Based on 16S rRNA gene sequence analysis, strain NBRI 13E did not form a coherent cluster with *J. marinus* JSM 076033T and *J. huakuii* NY-2. 16S rRNA gene sequence comparisons revealed that strain NBRI 13E was related most closely to *J. huakuii* NY-2 and *J. marinus* JSM 076033T (99.9% similarity), followed by *J. halotolerans* YKJ-101 and *J. halophilus* C1-52 (96%), *Jeotgalicoccus psychrophilus* YKJ-115 and *Jeotgalicoccus aerolatus* MPA-33 (95%), and *Salinicoccus salitudinis* YIM-C678 and *Salinicoccus kunmingensis* YIM Y15 (93%) (Fig. 2). Phylogenetic analysis based on 16S rRNA gene sequences thus revealed that strain NBRI 13E was related most closely to the type strains of the four recognized species of the genus *Jeotgalicoccus* and formed a distinct subclade with these four taxa in the phylogenetic trees that was supported by a high bootstrap resampling value (Fig. 2). Species of the genera *Salinicoccus*, *Nosocomiicoccus*, and *Macrococcus* were the next nearest relatives of the novel strain, but they formed quite separate and robust clusters (Fig. 2). However, *Jeotgalicoccus*, *Salinicoccus*, *Nosocomiicoccus*, and *Macrococcus* all belong to the same family Staphylococcaceae under class Bacilli and division Firmicutes. The nucleotide sequence of NBRI 13E has been obtained from GenBank under the accession number KX495292.1.

Assessment of plant growth promotion and rhizosphere colonization by NBRI 13E under greenhouse conditions

Effect of NBRI 13E inoculation on vegetative parameters of okra, maize, and tomato

The three crop plants okra (*A. esculentus*), maize (*Z. mays*), and tomato (*S. lycopersicum*) were evaluated for their growth promotion in the presence and absence of NBRI 13E under control and salt-stressed conditions. Results revealed that inoculation with NBRI 13E has significantly enhanced the overall plant biomass of all three crop plants as compared to the respective uninoculated controls under both normal and salt-stressed conditions.

In the case of okra, plants treated with NBRI 13E demonstrated significantly higher growth promotion in terms of shoot length, root length, and plant fresh and dry weight when compared with the control, while they showed nonsignificant enhancement in terms of the number of leaves (Table 2 and Fig. 3). Under salt stress, NBRI 13E significantly enhanced the shoot length, root length, number of leaves, plant fresh weight, and plant dry weight by 11.10, 5.95, 33.33, 29.27, and 15.38%, respectively.

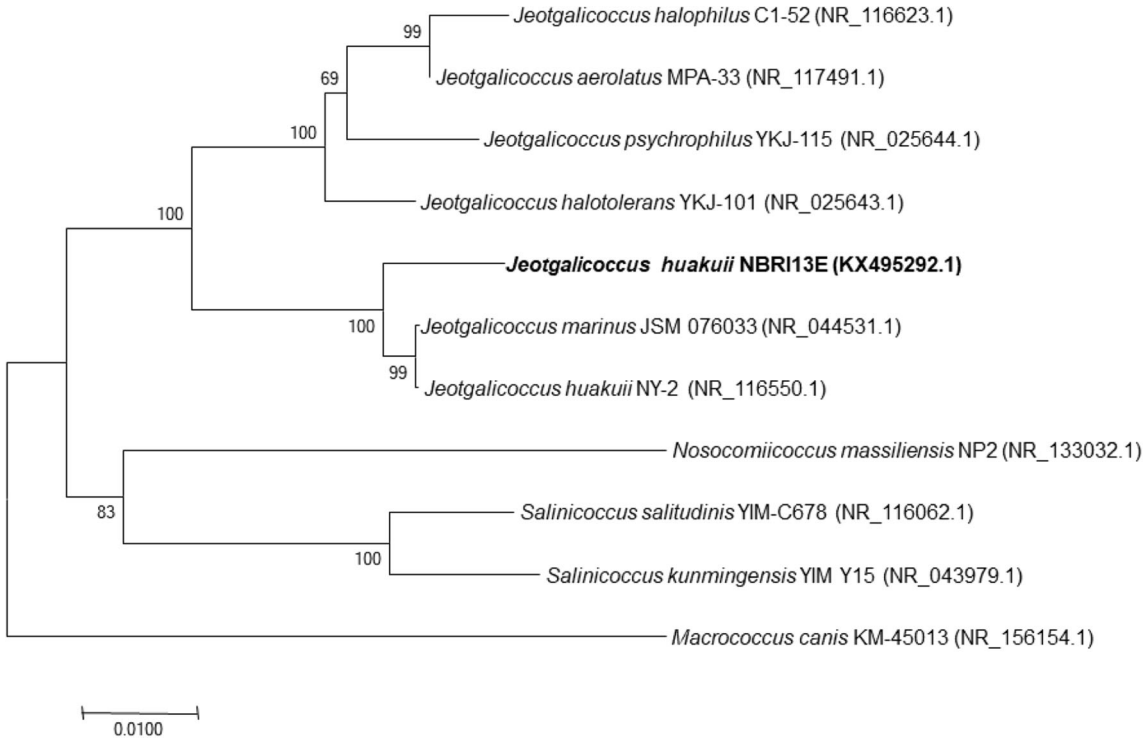

Fig. 2 Evolutionary relationships of NBRI 13E. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes–Cantor method and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1565 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016)

Similarly, in the case of maize, NBRI 13E-inoculated plants exhibited a significant growth enhancement in terms of shoot length and plant fresh weight in comparison to the control, whereas they displayed nonsignificant difference for root length, number of leaves, and plant dry weight (Table 2 and Fig. 3). Further, NBRI 13E-treated plants under salt stress were found to have increased shoot length, root length, number of leaves, plant fresh weight, and plant dry weight by 29.89, 19.93, 33.25, 34.58, and 27.14%, respectively, compared with the salt-stressed plants.

Likewise, in the case of tomato, we observed that plants having NBRI 13E treatment showed significant growth promotion in terms of the shoot and root length and plant fresh and dry weight and nonsignificant growth promotion in terms of the number of leaves (Table 2 and Fig. 3). However, under salt stress, NBRI 13E-treated plants displayed enhanced shoot and root length by 34.70 and 25.62% and plant fresh and dry weight by 34.41 and 48.77%, respectively.

Effect of NBRI 13E inoculation on chlorophyll, proline, and soluble sugar content of okra, maize, and tomato

Biochemical assays (total chlorophyll, carotenoid, proline, and total soluble sugar) were performed to access the response of NBRI 13E treatment toward nonenzymatic properties of three crops under normal and salt stress conditions.

In the case of okra, we observed that NBRI 13E-treated plants have significantly higher values for total chlorophyll and carotenoid content when compared to the untreated normal and salt control (Fig. 4). NBRI 13E exhibited maximum potential to enhance total chlorophyll and carotenoid content by 54.02 and 13.90% under salt stress condition. Our findings also showed a significant difference for proline as well as total soluble sugar accumulation in both treated and untreated plants under normal and salt stress conditions (Fig. 4). In comparison to salt control plants, NBRI 13E-inoculated plants accumulated 18.04% less proline. Under salt stress condition, NBRI 13E-treated plants also showed high (47.96%) total soluble sugar content.

Similarly, in the current study, plants receiving NBRI 13E treatment showed significant enhancement in terms of total chlorophyll and carotenoid for maize under both stress and nonstress conditions (Fig. 4). NBRI 13E-treated plants demonstrated higher values for total chlorophyll and carotenoid by 53.12 and 56.16% in comparison to control plants under salt stress condition. Also, NBRI 13E inoculation helped plants to accumulate less (55.27%) proline under salt stress as compared to salt control plants. Total soluble sugar content was also found to be increased by 27.42% in NBRI 13E-treated plants as compared to salt stress control plants.

Table 2 Measurement of vegetative parameters of okra, maize, and tomato plants

Treatment	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh plant wt. (g)	Dry plant wt. (g)
Okra (*Abelmoschus esculentus*)					
Control	15.76 ± 0.26^{c}	10.23 ± 0.27^{b}	3.00 ± 0.00^{b}	2.32 ± 0.08^{b}	0.62 ± 0.02^{b}
Salt	13.13 ± 0.18^{a}	9.00 ± 0.26^{a}	2.00 ± 0.00^{a}	2.03 ± 0.02^{a}	0.55 ± 0.02^{a}
NBRI 13E	16.87 ± 0.28^{d}	10.50 ± 0.17^{c}	3.00 ± 0.00^{b}	3.28 ± 0.02^{d}	0.72 ± 0.02^{c}
NBRI 13E+S	14.77 ± 0.15^{b}	9.57 ± 0.01^{ab}	3.00 ± 0.00^{b}	2.87 ± 0.02^{c}	0.65 ± 0.02^{b}
Maize (*Zea mays*)					
Control	42.07 ± 1.67^{b}	24.13 ± 0.55^{c}	3.67 ± 0.33^{b}	5.58 ± 0.04^{b}	0.69 ± 0.02^{b}
Salt	30.73 ± 0.52^{a}	17.27 ± 0.49^{a}	2.67 ± 0.33^{a}	3.86 ± 0.03^{a}	0.51 ± 0.03^{a}
NBRI 13E	54.73 ± 0.87^{c}	24.27 ± 0.30^{c}	4.33 ± 0.33^{b}	6.42 ± 0.03^{d}	0.72 ± 0.02^{b}
NBRI 13E+S	43.83 ± 0.84^{b}	21.57 ± 0.44^{b}	4.00 ± 0.00^{b}	5.90 ± 0.03^{c}	0.70 ± 0.02^{b}
Tomato (*Solanum lycopersicum*)					
Control	27.53 ± 0.13^{b}	9.53 ± 0.22^{a}	12.00 ± 0.58^{b}	11.25 ± 0.03^{b}	4.44 ± 0.02^{b}
Salt	18.50 ± 0.15^{a}	9.00 ± 0.21^{a}	8.67 ± 0.33^{a}	7.70 ± 0.02^{a}	2.30 ± 0.02^{a}
NBRI 13E	28.57 ± 0.23^{c}	11.13 ± 0.22^{b}	12.33 ± 0.33^{b}	11.65 ± 0.03^{c}	4.58 ± 0.02^{c}
NBRI 13E+S	28.33 ± 0.29^{c}	12.10 ± 0.21^{c}	12.33 ± 0.33^{b}	11.74 ± 0.03^{c}	4.49 ± 0.02^{b}

Parameters are expressed as mean values of three replicates ± SE which were compared by analysis of variance (ANOVA), followed by the Duncan test. Statistically significant differences (signified by different letters) were then determined at $p \leq 0.05$, using the software SPSS ver 20.0. Values in the columns with the same letter are not significantly different ($p \leq 0.05$) by the Duncan test. Four different treatments refer to control (uninoculated; no salt), salt (uninoculated; 100 mM NaCl), NBRI 13E (*Jeotgalicoccus huakuii*), and NBRI 13E+S (*Jeotgalicoccus huakuii* treated with 100 mM NaCl)

In the same way, we found that NBRI 13E treatment demonstrated a significant increase for total chlorophyll and carotenoid in tomato under normal and salt stress conditions (Fig. 4). We found that plants inoculated with NBRI 13E showed enhancement in total chlorophyll and carotenoid content by 37.28 and 32.48%, respectively, in comparison to salt stress control plants. Moreover, under salt stress condition, NBRI 13E treatment increased the proline accumulation in plants by 32.48% as compared to control plants. NBRI 13E-treated plants also exhibited 43.09% more total soluble sugar in comparison to control plants under salt stress.

Effect of NBRI 13E inoculation on defense enzymes of okra, maize, and tomato

Simultaneously, we carried out defense enzyme assay like SOD, APX, guaiacol peroxidase (GPX), CAT, and PPO to analyze quenching of accumulated H_2O_2 and other oxidative stress in plants. Salt-treated plants of all the three crops were observed to have significant maximum activity for all the antioxidant enzymes considered (Fig. 5). However, NBRI 13E treatment has significantly lowered the activity for all the antioxidant enzymes under salt stress (NBRI 13E+S) when compared with only salt treatment. SOD, APX, GPX, CAT, and PPO activity was reduced by 30.74, 44.17, 27.32, 11.44, and 41.09%, respectively, in okra plants under salt stress treated with NBRI 13E when compared to only salt-treated plants (Fig. 5). Moreover, NBRI 13E treatment demonstrated 64.97, 86.55, 59.77, 87.30, and 65.15% decrement for SOD, APX, GPX, CAT, and PPO activity in maize plants under salt stress condition (Fig. 5). Similarly, tomato plants under salt stress exhibited 10.78, 20.95, 8.56, 7.23, and 24.87% reduction in SOD, APX, GPX, CAT, and PPO activity in NBRI 13E treatment (Fig. 5).

Colonization of NBRI 13E in okra, maize, and tomato rhizosphere under greenhouse conditions

Colonization of NBRI 13E and heterogeneous bacterial population in okra, maize, and tomato rhizospheres under greenhouse conditions were monitored. The population of NBRI 13E increased in the rhizosphere of okra, maize, and tomato from 0 to 15 DAI by 9.52, 14.30, and 13.49% in nonsterilized soil. NBRI 13E exhibited maximum colonization and survivability in maize with 7.30 and 6.10 Log_{10} CFU g^{-1} till 45 DAI under normal and salt stress conditions. Under salt stress condition, NBRI 13E demonstrated survivability till day 45 with 5.02, 5.48, and 5.30 Log_{10} CFU g^{-1} in the rhizosphere soil of okra, maize, and tomato, respectively (Fig. 6). However, the population of NBRI 13E decreased maximum by 17.00% and minimum by 15.27% in tomato and maize rhizosphere under salt stress at 45 DAI. After 30 DAI, colonization of NBRI 13E was 7.00 Log_{10} CFU g^{-1} rhizosphere soil, throughout the growing period for all the three crops under normal condition.

Fig. 3 Evaluation of *Jeotgalicoccus huakuii* (NBRI 13E) on growth promotion of (**a**) okra, (**b**) maize, and (**c**) tomato

Heterogeneous bacterial populations during the same period were in the range 7.05–7.35 Log_{10} CFU g^{-1} root in okra, maize, and tomato rhizospheres receiving NBRI 13E treatment.

Evaluation of NBRI 13E inoculation on Z. mays under field condition

Further, the efficacy of NBRI 13E was evaluated for enhancing vegetative parameters and yield of maize crop under field condition. We found that NBRI 13E inoculation demonstrated significant enhancement over control for all the parameters observed in this study. In this regard, the NBRI 13E-treated plants were observed to have maximum height and fresh and dry weight (Table 3). Similarly, NBRI 13E treatment resulted in maximum cob weight, no. of rows per cob, no. of seeds per row, total seeds per cob, and seed weight per cob with significant difference among all the treatments (Table 3). However, NBRI 13E showed no significant difference with 100% NPK in the case of 100 seed weight. Moreover, NBRI 13E significantly enhanced the total yield of maize crop by 38.69 and 33.86% when compared with the control and 50% NPK, while a nonsignificant increase (1.69%) was represented in comparison to 100% NPK.

Discussion

Jeotgalicoccus have been reported as a halotolerant strain by several researchers (Alves et al. 2008; Guo et al. 2010; Liu et al. 2011; Mokashe et al. 2015; Misra et al. 2017), but their utilization as bioformulation based on multiple PGP attributes under stress condition is still under infancy. The results of the phylogenetic analysis supported the affiliation of NBRI 13E to the genus *Jeotgalicoccus* having distinct subclade formation with other closest relatives. NBRI 13E differed markedly from the six reported species of a *Jeotgalicoccus* genus based on 16S rRNA gene-based phylogenetic analysis and also demonstrated abiotic stress tolerance and various PGP traits (Fig. 2, Supplementary Table S1).

Therefore, the present study was conducted to demonstrate the potential of *J. huakuii* (NBRI 13E), as not only enhancing the crop productivity under control conditions but also exhibiting a positive response under stressed conditions. Under in vitro conditions, the PGP strain NBRI 13E has demonstrated multiple PGP attributes, i.e., biofilm production, ACC deaminase activity, EPS, and alginate production under normal and stressed conditions (Table 1), although several researchers have observed the PGP properties of bacterial isolates under different abiotic stress conditions (Mishra et al. 2016; Sharma et al. 2015).

Strains from the genera *Jeotgalicoccus* have been reported for IAA production. The increasing concentration of salt corresponds to the decrease in IAA production. In our study, we have reported that NBRI 13E was able to produce IAA under control conditions, but it reduces with the increasing concentrations of salt. Earlier, Mukasheva et al. (2016) reported a strain of *J. halotolerans* which produces maximum IAA, while another strain is unable to produce IAA (Yadav et al. 2016). Similar to *Jeotgalicoccus*, there are several other genera which have demonstrated a gradual decline in IAA production corresponding to increasing NaCl concentration (Tank and Saraf 2010; Li et al. 2017; Sarkar et al. 2017; Soleimani et al. 2017).

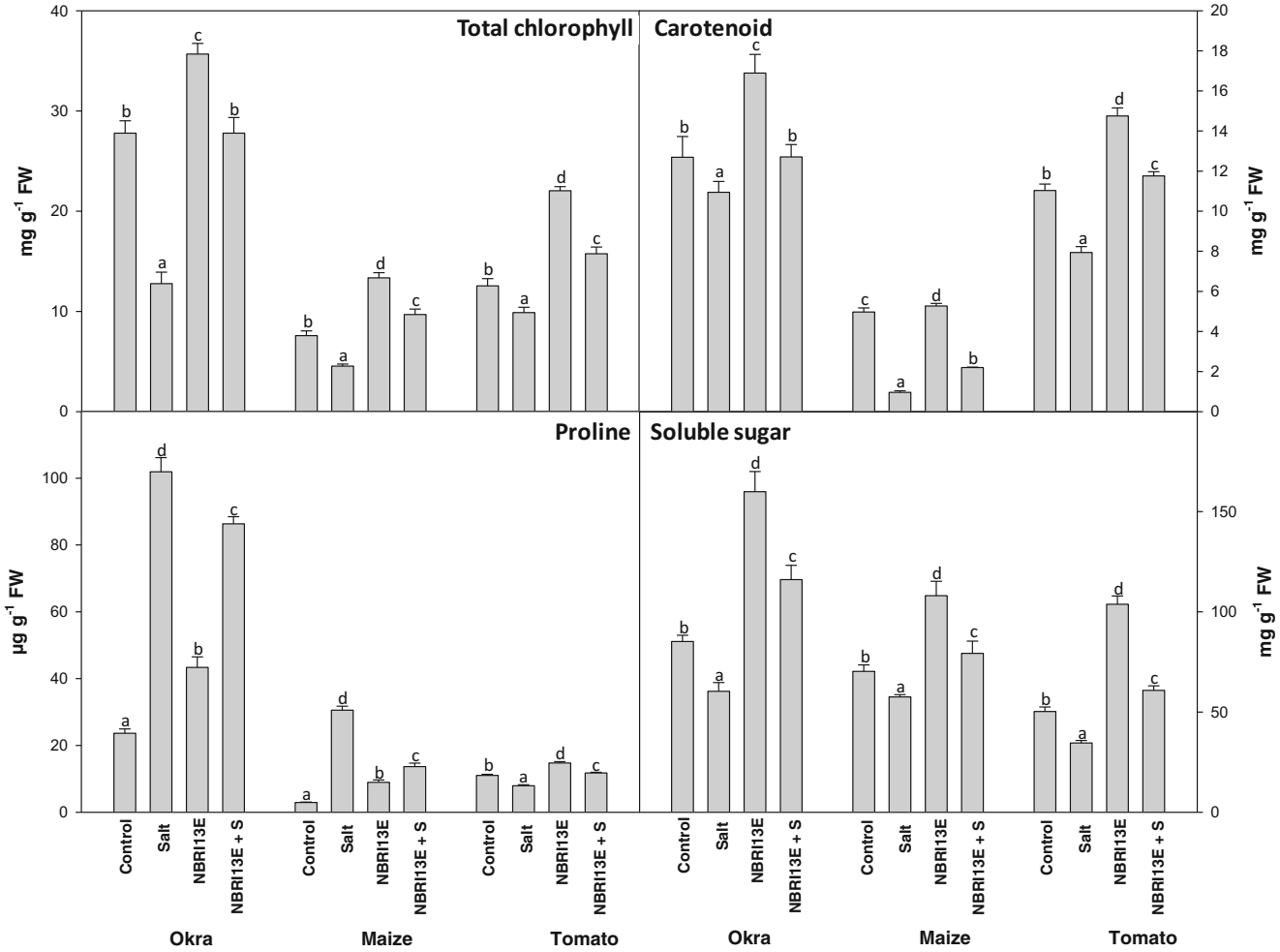

Fig. 4 Chlorophyll, proline, and soluble sugar in okra, maize, and tomato leaves. The values shown here are the mean of three replicates. Errors bars represent standard errors. Different letters above the bars represent significant differences according to the analysis of variance (ANOVA), followed by the Duncan test ($p \leq 0.05$) applied using the software SPSS ver 20.0

Phosphate solubilization under salt stress by NBRI 13E has been reported for the first time in the present study. Earlier, Yadav et al. (2016) reported the lack of P solubilization attribute by *J. halotolerans*. In the presence of increasing salt concentrations, the P solubilization ability of NBRI 13E decreases under in vitro conditions. A similar trend of reduction of P solubilization was also reported by Sadeghi et al. (2012) and Mishra et al. (2017).

It has been reported that biofilm formation helps bacteria to withstand harsh conditions by allowing them to tolerate various abiotic stresses (Kamjumphol et al. 2013). In this perspective, NBRI 13E demonstrated significantly enhanced biofilm-forming ability under 500 mM NaCl concentration as compared to the control condition (Table 1). Notwithstanding, our findings were in corroboration with the findings of Philips et al. (2017) and Pumirat et al. (2017) which stated that biofilm formation could be strongly induced under salt (NaCl) stress.

Further, on considering PGP attributes of NBRI 13E, ACC deaminase activity was found maximum at 0.5 M NaCl concentration. Similar to our results, an increase in the ACC deaminase activity in the presence of salt (NaCl) was also observed in other studies (Tank and Saraf 2010; Suarez et al. 2015; Li et al. 2017).

Exopolysaccharide production has been reported as another essential trait for PGPR which allows them to grow under water-stressed conditions (Sandhya et al. 2009). NBRI 13E exhibited higher EPS and alginate production under 0.5 M NaCl concentration as compared to the control (Table 1). Our findings related to higher EPS production under salt (NaCl) stress was supported by several researchers (Priester et al. 2006; Sheng et al. 2006; Sandhya and Ali 2015). In contrast to our report, Mishra et al. (2017) and Sarkar et al. (2017) have demonstrated a significant decrease in EPS and alginate production under salt stress condition.

Apart from the PGP attributes, abiotic stress tolerance is another essential characteristic of any PGPR toward developing its formulation for plant growth promotion and stress management. Therefore, NBRI 13E was also evaluated for

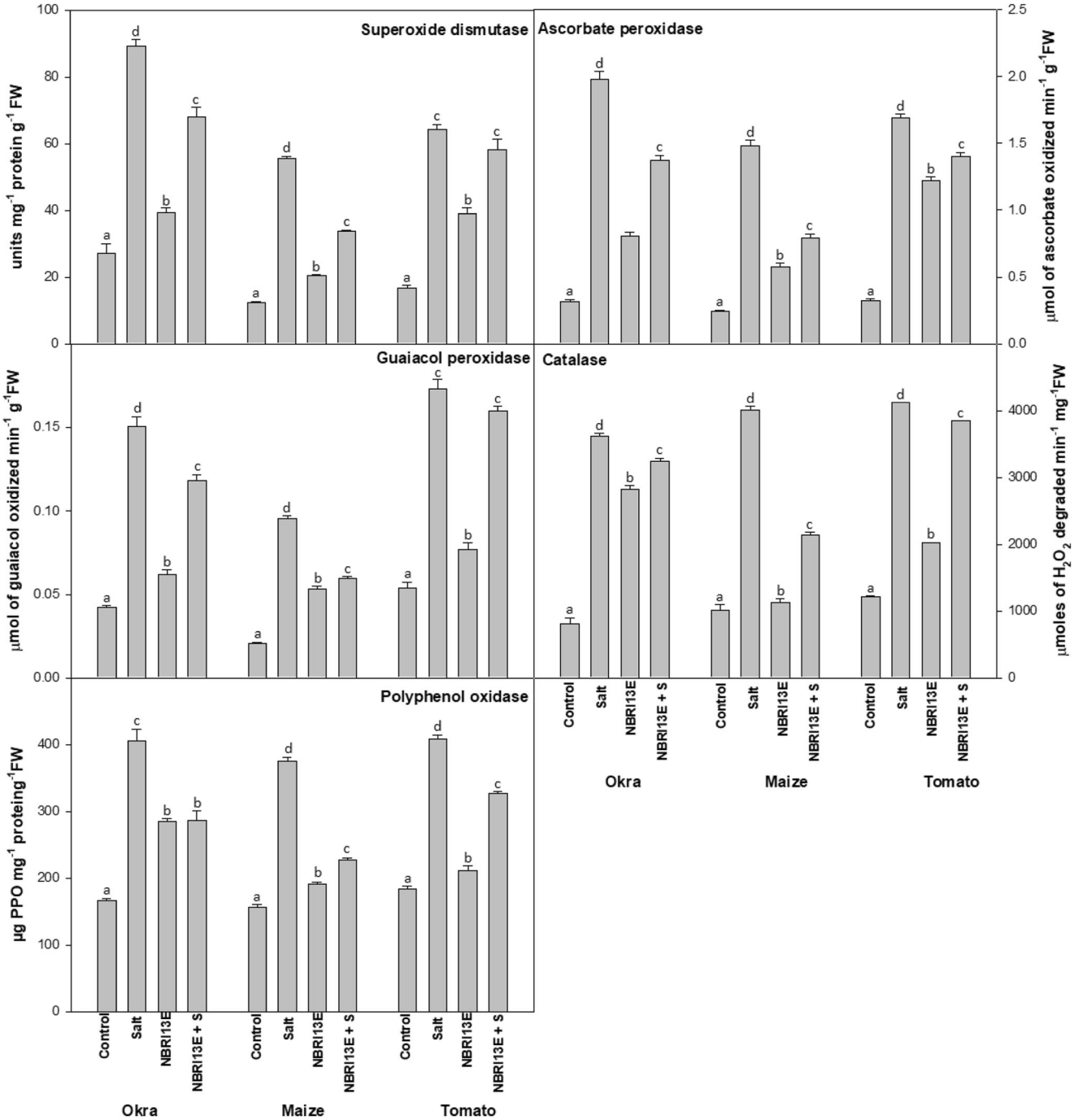

Fig. 5 Defense enzyme activities in okra, maize, and tomato leaves. The values shown here are the mean of three replicates. Errors bars represent standard errors. Different letters above the bars represent significant differences according to the analysis of variance (ANOVA), followed by the Duncan test ($p \leq 0.05$) applied using the software SPSS ver 20.0

salt, drought temperature, and pH stress under in vitro condition. Results demonstrated the ability of NBRI 13E to survive under harsh conditions, which are in accordance with the earlier report on *Jeotgalicoccus* by Guo et al. (2010) to represent its optimal growth at 3–8% NaCl concentration. Similarly, in the present study, NBRI 13E exhibited better survivability under 0.5 M (2.9%) NaCl concentration after its temporal (up to 10 days) evaluation of viability under salt stress condition (Fig. 1), although no earlier other than the present study has reported the survivability of *Jeotgalicoccus* sp. under other (drought and high alkaline pH) abiotic stresses. In the present study, NBRI 13E have also been evaluated for its phytobeneficial impact by mitigating salt stress on okra, maize, and tomato in terms of vegetative, biochemical, and defense

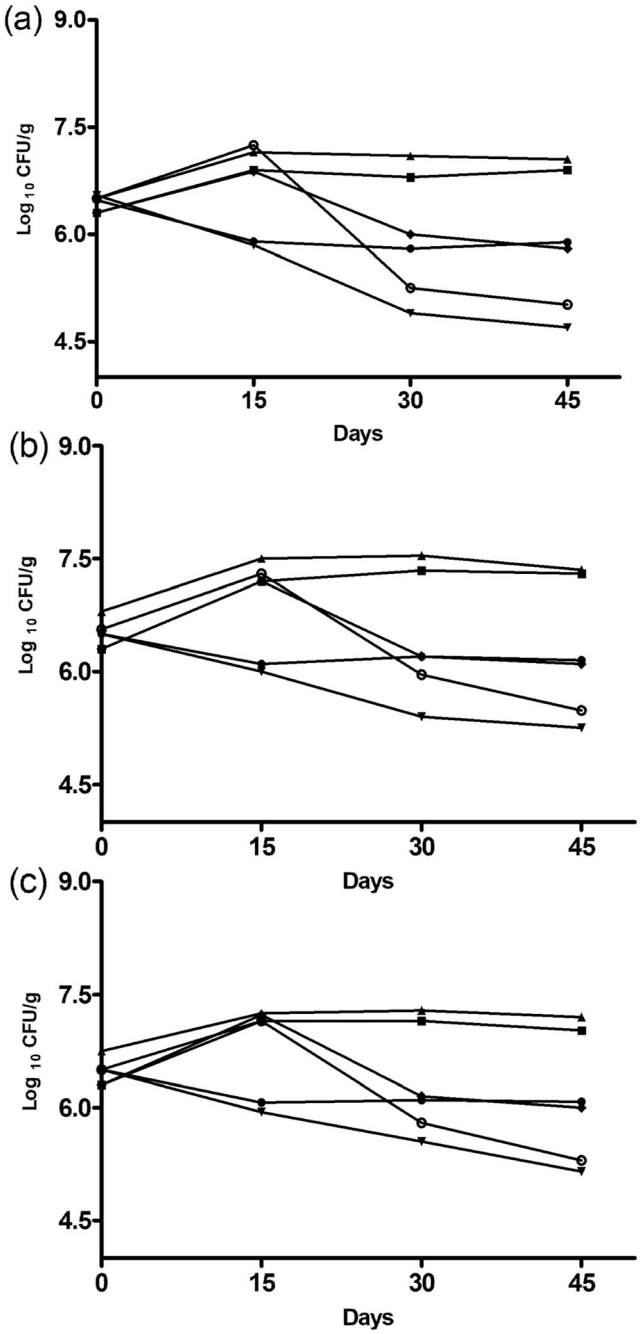

Fig. 6 Survival and competence in the rhizosphere of **a** okra, **b** maize, and **c** tomato by *Jeotgalicoccus huakuii* (NBRI 13E). The different symbols refer to (●) Heterotrophic bacteria (uninoculated control), (■) NBRI 13E and (▲) heterotrophic bacteria (inoculated with NBRI 13E), (▼) Heterotrophic bacteria (uninoculated control with salt), and (♦) NBRI 13E and (○) heterotrophic bacteria (inoculated with NBRI 13E+ S). Error bars are the standard error of the means ($n = 3$)

enzyme parameters under greenhouse condition (Table 2; Figs. 3, 4, and 5). Earlier, also, we have reported salt stress ameliorating the effect of NBRI 13E on rice seedling under in vitro conditions (Misra et al. 2017). Surprisingly, no earlier study demonstrated plant growth promotion and salt stress amelioration by *Jeotgalicoccus* salt stress under greenhouse condition. However, *J. halotolerans* is the only species which has been reported for significantly enhancing the root length of soybean and barley under control conditions (Mukasheva et al. 2016).

Inoculation of NBRI 13E has also demonstrated a significant increment of total chlorophyll, carotenoid, and total soluble sugar content while reducing proline accumulation under salt stress condition (Fig. 4). However, there are reports of similar effects due to inoculation of PGPR in different hosts, but none of them are from *Jeotgalicoccus* genera under salt stress conditions (Ullah and Bano 2015; Vurukonda et al. 2016; Li and Jiang 2017). Our results of NBRI 13E mediating less proline accumulation in maize plants are in contrast to Li et al. (2017) and Yasmin et al. (2017) which showed that *Enterobacter cloacae* and *Pseudomonas* sp. could induce the synthesis of more proline in canola seedlings and maize plants under salt (100 mM NaCl) and drought stress. Previous reports related to salt stress alleviation have well established the role of EPS production by binding the excess Na^+, therefore making it unavailable to plants under saline stress (Upadhyay et al. 2011; Balsanelli et al. 2014; Choudhary et al. 2016; Forni et al. 2017). Therefore, enhanced EPS production by NBRI 13E under salt stress could be the rationale behind our findings related to decreased proline content in NBRI 13E-treated plants (except tomato) under salt stress. Also, the response mechanisms related to increased proline accumulation are likely to incur a high cost to plants' productivity (Bistgani et al. 2017). For that reason, NBRI 13E could prove to be beneficial PGPR in maintaining the integrity of plants without compromising fitness. Similar to our results, Fukami et al. (2018) showed that proline contents in maize plant were significantly reduced due to the inoculation of PGPR, indicating a reduced need for the synthesis of these molecules under saline stress. Our findings were also supported by several studies showing a decrease in proline content of PGPR-treated plants under salt stress (Singh and Jha 2016; Tiwari et al. 2016; Curá et al. 2017).

The result for antioxidant enzymes in our study for the three host plants was significantly reduced in NBRI 13E-inoculated treatment under salt stress conditions (Fig. 5). Earlier, it has been reported that plants under abiotic stress have high ROS quenching activity, while inoculation of PGPR reduces the antioxidant activities (Vardharajula et al. 2011; Kang et al. 2014; Tiwari et al. 2016). Moreover, in contrast to our findings, Ullah and Bano (2015) and Habib et al. (2016) observed that inoculation of other PGPR had increased the antioxidants' activity in host plants under salt stress, although it has been previously stated that the reduced level of oxidative stress enzymes suggested a low level of stress experienced by PGPR-inoculated plants (Kang et al. 2014). Therefore, NBRI 13E-mediated reduction in defense enzyme activities demonstrated its role in protecting the host plants under salt stress conditions.

Table 3 Effect of *Jeotgalicoccus huakuii* (NBRI 13E) on plant parameters of maize under field condition

Parameters	Control	NBRI 13E	50% NPK	100% NPK
Plant height (cm)	178.50 ± 1.70^{a}	239.17 ± 1.17^{d}	210.94 ± 1.49^{b}	228.17 ± 1.55^{c}
Plant fresh weight (g)	345.27 ± 0.01^{a}	501.50 ± 0.03^{c}	395.72 ± 0.02^{ab}	404.28 ± 0.01^{b}
Plant dry weight (g)	171.05 ± 0.01^{a}	263.22 ± 0.01^{c}	173.00 ± 0.01^{a}	228.39 ± 0.01^{b}
No. of cob per plant	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}
Cob weight (g)	152.22 ± 6.41^{a}	281.39 ± 7.93^{d}	203.67 ± 6.38^{b}	252.89 ± 6.96^{c}
No. of rows per cob	14.33 ± 0.33^{a}	17.44 ± 0.22^{d}	15.67 ± 0.33^{b}	16.56 ± 0.22^{c}
No. of seeds per row	26.78 ± 1.00^{a}	38.44 ± 0.51^{d}	32.17 ± 0.86^{b}	35.78 ± 0.74^{c}
Total seeds per cob	387.67 ± 21.83^{a}	671.22 ± 13.95^{d}	505.67 ± 21.06^{b}	594.44 ± 19.35^{c}
Seed weight per cob	107.64 ± 7.81^{a}	173.42 ± 4.00^{d}	127.31 ± 5.37^{b}	154.47 ± 4.84^{c}
100 seed weight (g)	25.20 ± 0.21^{a}	26.23 ± 0.19^{b}	25.27 ± 0.23^{a}	25.92 ± 0.22^{b}
Total yield (t h^{-1})	5.07 ± 0.08^{a}	8.27 ± 0.08^{c}	5.47 ± 0.08^{b}	8.13 ± 0.08^{c}

Parameters are expressed as mean values of 18 replicates ± SE which were compared by analysis of variance (ANOVA), followed by the Duncan test. Statistically significant differences (signified by different letters) were then determined at $p \leq 0.05$, using the software SPSS ver 20.0. Four different treatments refer to control (uninoculated; no NPK), NBRI 13E (*Jeotgalicoccus huakuii*; 50% NPK), 50% NPK (uninoculated; half of the recommended NPK dose), and 100% NPK (uninoculated; recommended NPK dose)

Growth and colonization of NBRI 13E in the rhizosphere of okra, maize, and tomato were observed throughout the experiment. Along with heterotrophic bacterial population, NBRI 13E has established itself to colonize and compete with the same titer as of native heterotrophic bacterial population under normal as well as salt stress condition. Similar to these results, earlier reports have demonstrated the colonization ability of bacterial strains in the rhizosphere of different crop plants (Chauhan and Nautiyal 2010; Mishra et al. 2011; Mendis et al. 2018).

Furthermore, based on our results from the greenhouse experiments, we have selected maize as host to evaluate NBRI 13E for enhancing maize plant growth and yield under naturally challenged field condition. Our results from the field trial demonstrated that NBRI 13E inoculation resulted in better plant growth and increased yield when compared with the control and 50 and 100% recommended NPK (Table 3). Similar to our results, the application of indigenous PGPR along with recommended doses of chemical fertilizer could significantly increase the maize yield (Gupta et al. 2017; Sood et al. 2018).

Therefore, the current study draws necessary inference on indigenous *J. huakuii* (NBRI 13E) for its characterization as potential bioinoculant formulation under normal and abiotic stress conditions. Our findings demonstrated that NBRI 13E imparted phytobeneficial effect in terms of enhanced photosynthetic pigments and less proline accumulation along with reduced antioxidant enzymes by using three different host plants. We also found that application of NBRI 13E compromised the amount of chemical fertilizer to achieve the enhanced maize yield as that of the recommended dose under degraded soil condition. Therefore, the study highlights the effectiveness of NBRI 13E for attaining better maize yield by conjoint application of PGPR and 50% chemical (NPK) fertilizer under a degraded condition which results to be cost-effective as well as a preventive measure to conserve soil fertility.

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Compliance with ethical standards

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

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References

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105:121–126

Albaladejo I, Meco V, Plasencia F, Flores FB, Bolarin MC, Egea I (2017) Unravelling the strategies used by the wild tomato species *Solanum pennellii* to confront salt stress: from leaf anatomical adaptations to molecular responses. Environ Exper Bot 135:1–12

Alves M, Nogueira C, Ana AM, Chung AP, Morais PV, da Costa MS (2008) *Nosocomiicoccus ampullae* gen. nov., sp. nov., isolated from the surface of bottles of saline solution used in wound cleansing. Int J Syst Evol Microbiol 58:2939–2944

Ambardar S, Vakhlu J (2013) Plant growth promoting bacteria from *Crocus sativus* rhizosphere. World J Microbiol Biotechnol 29: 2271–2279

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: an overview. Photosynthetica 51:163–190

AVRDC (2006) Vegetables matter. In: AVRDC – The World Vegetable Center. Shanhua, Taiwan

Balsanelli E, de Baura VA, Pedrosa FD, de Souza EM, Monteiro RA (2014) Exopolysaccharide biosynthesis enables mature biofilm formation on abiotic surfaces by *Herbaspirillum seropedicae*. PLoS One 9:e110392

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276–287

Bharti N, Yadav D, Barnawal D, Maji D, Kalra A (2013) *Exiguobacterium oxidotolerans*, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennell under primary and secondary salt stress. World J Microbiol Biotechnol 29:379–387

Bistgani ZE, Siadat SA, Bakhshandeh A, Pirbalouti AG, Hashemi M (2017) Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. Crop J. https://doi.org/10.1016/j.cj.2017.04.00

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

Bromham L, Saslis-Lagoudakis CH, Bennett TH, Flowers TJ (2013) Soil alkalinity and salt tolerance: adapting to multiple stresses. Biol Lett 9:20130642

Bui EN (2013) Soil salinity: a neglected factor in plant ecology and biogeography. J Arid Environ 92:14–25

Bui EN, Thornhill A, Miller JT (2014) Salt- and alkaline-tolerance are linked in Acacia. Biol Lett 10:20140278

Chakraborty U, Chakraborty BN, Chakraborty AP, Dey PL (2013) Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World J Microbiol Biotechnol 29: 789–803

Chauhan PS, Nautiyal CS (2010) The purB gene controls rhizosphere colonization by *Pantoea agglomerans*. Lett Appl Microbiol 50: 205–210

Chen S, Xing J, Lan H (2012) Comparative effects of neutral salt and alkaline salt stress on seed germination, early seedling growth and physiological response of a halophyte species *Chenopodium glaucum*. Afr J Biotechnol 11:9572–9581

Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A (2016) Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. J Plant Growth Regul 35: 276–300

Curá JA, Franz DR, Filosofía JE, Balestrasse KB, Burgueño LE (2017) Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. bacteria increases the tolerance of maize to drought stress. Microorganisms 5:1–16

Dixit R, Agrawal L, Gupta S, Kumar M, Yadav S, Chauhan PS, Nautiyal CS (2016) Southern blight disease of tomato control by 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing *Paenibacillus lentimorbus* B-30488. Plant Signal Behav 11: e1113363

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Fan P, Chen D, He Y, Zhou Q, Tian Y, Gao L (2016) Alleviating salt stress in tomato seedlings using *Arthrobacter* and *Bacillus megaterium* isolated from the rhizosphere of wild plants grown on saline–alkaline lands. Int J Phytoremediation 18:1113–1121

Farooq M, Hussain M, Wakeel A, Siddique KHM (2015) Salt stress in maize: effects, resistance mechanisms, and management. A review. Agron Sustain Dev 35:461–481

Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410:335–356

Fukami J, Cerezini P, Hungria M (2018) *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express 8:73

Gerhardt KE, MacNeill GJ, Gerwing PD, Greenberg BM (2017) Phytoremediation of salt-impacted soils and use of plant growth-promoting rhizobacteria (PGPR) to enhance phytoremediation. In: Ansari AA (ed) Phytoremediation. Springer International Publishing, pp 19–51

Giuffrida F, Scuderi D, Giurato R, Leonardi C (2013) Physiological response of broccoli and cauliflower as affected by NaCl salinity. Acta Hortic 1005:435–441

Gontia-Mishra I, Sapre S, Kachare S, Tiwari S (2017) Molecular diversity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing PGPR from wheat (*Triticum aestivum* L.) rhizosphere. Plant Soil 414:213–227

Guo XQ, Li R, Zheng LQ, Lin DQ, Sun JQ, Li SP, Li WJ, Jiang JD (2010) *Jeotgalicoccus huakuii* sp. nov., a halotolerant bacterium isolated from seaside soil. Int J Syst Evol Microbiol 60:1307–1310

Gupta S, Kaushal R, Spehia RS, Pathania SS, Sharma V (2017) Productivity of capsicum influenced by conjoint application of isolated indigenous PGPR and chemical fertilizers. J Plant Nutr 40: 921–927

Habib SH, Kausar H, Saud HM (2016) Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. Biomed Res Int 2016:6284547

Hemeda HM, Klein BP (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J Food Sci 55:184–185

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic, New York, pp 21–132

Kamjumphol W, Chareonsudjai S, Chareonsudjai P, Wongratanacheewin S, Taweechaisupapong S (2013) Environmental factors affecting *Burkholderia pseudomallei* biofilm formation. Southeast Asian J Trop Med Public Health 44:72–81

Kang S, Khan AL, Waqas M, You Y, Kim J, Kim J, Hamayun M, Lee I (2014) Plant growth promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. J Plant Interact 9:673–682

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874

Li HQ, Jiang XW (2017) Inoculation with plant growth-promoting bacteria (PGPB) improves salt tolerance of maize seedling. Russ J Plant Physiol 64:235–241

Li P, Wu J, Qian H (2016) Regulation of secondary soil salinization in semi-arid regions: a simulation research in the Nanshantaizi area along the Silk Road, northwest China. Environ Earth Sci 75:698

Li H, Lei P, Panga X, Li S, Xu H, Xu Z, Feng X (2017) Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterobacter cloacae* HSNJ4. Appl Soil Ecol 119:26–34

Liu WY, Jiang LL, Guo CJ, Yang SS (2011) *Jeotgalicoccus halophilus* sp. nov., isolated from salt lakes. Int J Syst Evol Microbiol 61:1720–1724

Machado RMA, Serralheiro RP (2017) Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae 3:30

Mendis HC, Thomas VP, Schwientek P, Salamzade R, Chien J-T, Waidyarathne P, Kloepper J, Fuente LDL (2018) Strain-specific quantification of root colonization by plant growth promoting rhizobacteria *Bacillus firmus* I-1582 and *Bacillus amyloliquefaciens* QST713 in non-sterile soil and field conditions. PLoS One 13: e0193119

Mishra A, Chauhan PS, Chaudhry V, Tripathi M, Nautiyal CS (2011) Rhizosphere competent *Pantoea agglomerans* enhances maize (*Zea mays*) and chickpea (*Cicer arietinum* L.) growth, without altering the rhizosphere functional diversity. Antonie Van Leeuwenhoek 100:405–413

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

Mishra SK, Khan MH, Misra S, Kant VK, Khare P, Srivastava S, Chauhan PS (2017) Characterization of *Pseudomonas* spp. and *Ochrobactrum* sp. isolated from volcanic soil. Antonie Van Leeuwenhoek 110:253–270

Misra S, Dixit VK, Khan MH, Mishra SK, Dviwedi G, Yadav S, Lehri A, Chauhan PS (2017) Exploitation of agro-climatic environment for selection of 1aminocyclopropane-1-carboxylic acid (ACC) deaminase producing salt-tolerant indigenous plant growth promoting rhizobacteria. Microbiol Res 205:25–34

Mokashe N, Chaudhari A, Patil U (2015) Optimal production and characterization of alkaline protease from newly isolated halotolerant *Jeotgalicoccus* sp. Biocatal Agric Biotechnol. https://doi.org/10.1016/j.bcab.2015.01.003

Mukasheva T, Berzhanova R, Ignatova L, Omirbekova A, Brazhnikova Y, Sydykbekova R, Shigaeva M (2016) Bacterial endophytes of Trans-Ili Alatau region's plants as promising components of a microbial preparation for agricultural use. Acta Biochim Pol 63:321–328

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

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

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270

Nautiyal CS, Srivastava S, Chauhan PS, Seem K, Mishra A, Sopory SK (2013) Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol Biochem 66:1–9

Nazir Q, Akhtar MJ, Imran M, Arshad M, Hussain A, Mahmood S, Hussain S (2017) Simultaneous use of plant growth promoting rhizobacterium and nitrogenous fertilizers may help in promoting growth, yield, and nutritional quality of okra. J Plant Nutr 40: 1339–1350

Negrão S, Schmöckel SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot 119:1–11

Patra HK, Mishra M (1979) Pyrophosphatase, peroxidase and polyphenol oxidase activities during leaf development and senescence. Plant Physiol 63:318–323

Paul D, Nair S (2008) Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. J Basic Microbiol 48:378–384

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

Philips J, Rabaey K, Lovley DR, Vargas M (2017) Biofilm formation by *Clostridium ljungdahlii* is induced by sodium chloride stress: experimental evaluation and transcriptome analysis. PLoS One 12: e0170406

Priester JH, Olson SG, Webb SM, Neu MP, Hersman LE, Holden PA (2006) Enhanced exopolymer production and chromium stabilization in *Pseudomonas putida* unsaturated biofilms. Appl Environ Microbiol 72:1988–1996

Pumirat P, Vanaporn M, Boonyuen U, Indrawattana N, Rungruengkitkun A, Chantratita N (2017) Effects of sodium chloride on heat resistance, oxidative susceptibility, motility, biofilm and plaque formation of *Burkholderia pseudomallei*. Microbiology Open 6:e493

Rengasamy P (2010) Soil processes affecting crop production in salt-affected soils. Aust J Soil Res 37:613–620

Rubio MB, Hermosa R, Vicente R, Gómez-Acosta FA, Morcuende R, Monte E, Bettiol W (2017) The combination of *Trichoderma harzianum* and chemical fertilization leads to the deregulation of phytohormone networking, preventing the adaptive responses of tomato plants to salt stress. Front Plant Sci 8:294

Sadeghi A, Karimi E, Dahaji PA, Javid MG, Dalvand Y, Askari H (2012) Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. World J Microbiol Biotechnol 28:1503–1509

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sandhya V, Ali SZ (2015) The production of exopolysaccharide by *Pseudomonas putida* GAP-P45 under various abiotic stress conditions and its role in soil aggregation. Microbiology 84:512–519

Sandhya V, Ali SKZ, Minakshi G, Gopal R, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAPP45. Biol Fertil Soils 46:17–26

Sarkar A, Ghosh PK, Pramanik K, Mitra S, Soren T, Pandey S, Mondal MH, Maiti TK (2017) A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Res Microbiol. https://doi.org/10.1016/j.resmic.2017.08.005

Sharma A, Singh P, Kumar S, Kashyap PL, Srivastava AK, Chakdar H, Singh RN, Kaushik R, Saxena AK, Sharma AK (2015) Deciphering diversity of salt-tolerant bacilli from saline soils of eastern Indo-Gangetic plains of India. Geomicrobiol J 32:170–180

Sheng GP, Yu HQ, Yue Z (2006) Factors influencing the production of extracellular polymeric substances by *Rhodopseudomonas acidophila*. Int Biodeter Biodegr 58:89–93

Siddikee MA, Sundaram S, Chandrasekaran M, Kim K, Selvakumar G, Sa T (2015) Halotolerant bacteria with ACC deaminase activity alleviate salt stress effect in canola seed germination. J Korean Soc Appl Biol Chem 58:237–241

Singh RP, Jha PN (2016) The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). PLoS One 11: e0155026

Soleimani R, Alikhani HA, Towfighi H, Khavazi K, Pourbabaee AA (2017) Isolated bacteria from saline-sodic soils alter the response of wheat under high adsorbed sodium and salt stress. Int J Environ Sci Technol 14:143–150

Sood G, Kaushal R, Chauhan A, Gupta S (2018) Effect of conjoint application of indigenous PGPR and chemical fertilizers on productivity of maize (*Zea mays* L.) under mid hills of Himachal Pradesh. J Plant Nutr 41:297–303

Srivastava S, Yadav A, Seem K, Mishra S, Chaudhary V, Nautiyal CS (2008) Effect of high temperature on *Pseudomonas putida* NBRI0987 biofilm formation and expression of stress sigma factor RpoS. Curr Microbiol 56:453–457

Suarez C, Cardinale M, Ratering S, Steffens D, Jung S, Montoya AMZ, Geissler-Plaum R, Schnell S (2015) Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare* L.) under salt stress. Appl Soil Ecol 95:23–30

Tank N, Saraf M (2010) Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J Plant Interact 5:51–58

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

Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant Physiol Biochem 99:108–117

Ullah S, Bano A (2015) Isolation of plant-growth-promoting rhizobacteria from rhizospheric soil of halophytes and their impact on maize (*Zea mays* L.) under induced soil salinity. Can J Microbiol 61:307–313

Upadhyay SK, Singh JS, Singh DP (2011) Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. Pedosphere 21:214–222

Vaishnav A, Varma A, Tuteja N, Choudhary DK (2016) PGPR-mediated amelioration of crops under salt stress. In: Choudhary DK (ed) Plant-microbe interaction: an approach to sustainable agriculture. Springer Nature Pte Ltd., Singapore, pp 205–226

Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6:1–14

Vurukonda SSKP, Vardharajula S, Shrivastava M, Ali SKZ (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24

Yadav AN, Sachan SG, Verma P, Saxena AK (2016) Bioprospecting of plant growth promoting psychrotrophic bacilli from the cold dessert of north western Indian Himalayas. Indian J Exp Biol 54:142–150

Yasmin H, Nosheen A, Naz R, Bano A, Keyani R (2017) L-tryptophan-assisted PGPR-mediated induction of drought tolerance in maize (*Zea mays* L.). J Plant Interact 12:567–578