Annals of Microbiology (2018) 68:525–535
https://doi.org/10.1007/s13213-018-1358-7

ORIGINAL ARTICLE

Bacteria endemic to saline coastal belt and their ability to mitigate the effects of salt stress on rice growth and yields

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Received: 15 April 2018 / Accepted: 12 July 2018 / Published online: 20 July 2018

Abstract

Increase in soil salinity adversely affects the metabolism and lowers the yield of rice (*Oryza sativa* L). Application of plant growth-promoting rhizobacteria (PGPR) to ameliorate the effects of salt stress on sensitive rice can be both effective and sustainable. In this study, 20 bacterial strains were isolated from the soil of saline-prone regions of Satkhira, north of the Sundarbans in coastal Bangladesh. Three bacteria among these grew well in the presence of 3 M salt (NaCl) and were Gram positive and non-motile. Their 16S rRNA sequence revealed that they belong to the *Halobacillus* genus. Two of them were identified as *Halobacillus dabanensis strain SB-26* and the other one as *Halobacillus* sp. *GSP 34*. A couple of mechanisms by which these microbes could play beneficial role if associated with plants, such as nitrogen fixation and indole acetic acid (IAA) production, were identified. The two bacterial strains showed positive results for nitrogen fixation and indole acetic acid (IAA) activity under salt stress. Their effect on the physiology and yield of a farmer popular but sensitive BRRI dhan 28 rice variety was investigated under both control and salt stress. At the seedling stage, inoculated plants had significantly greater root length, shoot height, total weight, chlorophyll content, but lower electrolyte leakage both in control and salt stress (0, 40, and 80 mM). Performance of the plants was even better when both bacteria were used in combination. At the reproductive stage, the plants also showed better phenology in presence of the inoculated bacteria. Under stress (50 mM NaCl), these plants showed significantly greater plant height, lower spikelet damage, and yield reduction compared to untreated plants. The identified *Halobacillus* strains can therefore be used to improve the yield of rice by exploiting their plant growth promotion activities in coastal areas affected by moderate salinity, such as those with an ionic conductivity of up to 5 dS m^{-1}.

Keywords Salt-tolerant bacteria · Salt-sensitive rice variety · Plant growth-promoting rhizobacteria (PGPR) · Nitrogen fixation · Indole acetic acid (IAA)

Introduction

Climate change-induced increase in soil salinity accompanied by the steady rise in the total population has made food security a serious challenge (Misra 2014) with reported decline crop production worldwide (Barnawal et al. 2014). In Bangladesh, salinity intrusion causes the scenario exacerbate in coastal regions. Recent studies have predicted that soil salinity will exceed 4 dS/m by 2050 resulting in decrease in production of high yielding rice varieties by 15.6% in coastal districts (Dasgupta et al. 2014; Dasgupta et al. 2018). Apart from this, osmotic pressure and toxic effect deriving from salinization alter the microbial community though reducing the biodiversity, microbial activity, and microbial biomass (de Souza Silva and Fay 2012; Yan et al. 2015). However, it has been also reported that taxonomically, diverse bacteria around roots showed modified physiological and structural characteristics in the saline environment and in turn contributed positively to plant growth (Paul and Nair 2008).

Rice is an essential staple food for more than the half of the world's population (Redfern et al. 2012). But the damage caused by salinity at different stages of its growth makes it highly sensitive among major cereals (Hoang et al. 2016). Salt stress alters several genes and their products at

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-018-1358-7) contains supplementary material, which is available to authorized users.

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transcriptional and translational level in rice through reducing infiltration and hydraulic conductivity of soil (Nautiyal et al. 2013; Nackley et al. 2015). As a result, it restrains plant growth and productivity by promoting osmotic stress, water deficit, and stomatal closure. As a consequence, there is decline in photosynthetic rates and biomass accumulation (Bashan et al. 2014). To overcome this problem, genetic engineering and molecular breeding techniques have been used to increase productivity and its tolerance to biotic and abiotic stresses (Nautiyal et al. 2013). But developing transgenic plants to overcome stress is associated with high cost, yield penalty, loss of endogenous gene functions, and involves other regulatory issues (Singh et al. 2015). As an alternative for reducing the effects of salt stress, salt-tolerant bacteria can help plant growth by retaining their root colonization and biocontrol potential. These microbes can increase rooting processes and yields by as much as 10–15%. Some plant growth-promoting bacteria (PGPB) isolated from saline soil, like *Pseudomonas* sp. and *Bacillus* sp., are reported to have protective and growth promotion activity on, white clover (Han et al. 2014a), wheat (Kumar et al. 2014), and maize (Kuan et al. 2016), even in saline soils. So, microbe-mediated plant stress amelioration is gaining importance and has become a sustainable approach in salt stress management by improving growth and productivity (Barnawal et al. 2014).

Plant growth-promoting bacteria (PGPB) are those bacteria that live in close association with plant roots, augment plant growth as well as their yield, and protect them from different biotic and abiotic stresses (Souza et al. 2015). These bacteria have been reported to develop two mechanisms to counteract the changes in external osmolarity arising due to salinity. The first response is a 'salt-in' strategy, where rise in osmotic pressure causes maintenance of high intracellular salt concentration equivalent to the external one. The other strategy is to maintain low salt concentration, balanced by accumulation of uncharged, highly water-soluble organic compatible solutes, for example sugars and derivatives, proline, sugars, polyols and derivatives, glycine betaines, ectoines, and other quaternary ammonium compounds (QACs). This is called the "compatible solute strategy" (Paul and Lade 2014). These bacteria facilitate plant growth through direct (for example, nitrogen fixation, phosphate solubilization, siderophore activity, phytohormone production, and ACC deaminase activity) or indirect (e.g., antifungal activity and antibiotic production) mechanisms to overcome the effects of salt stress (Glick 2012).

It is therefore evident that plant growth-promoting bacteria (PGPB) can ameliorate the toxic effect of salt stress through one or more mechanisms. Exploitation of salt-tolerant bacteria to help plants grow can be an effective approach to mitigate salt stress (Shrivastava and Kumar 2015). The soils of coastal regions of Bangladesh are enriched with excess of magnesium, calcium, and sulfate (Lisa et al. 2004), which favor the inhabitation of microbial communities. So, our objectives were to isolate salt-tolerant bacteria from saline-prone regions of Satkhira, Bangladesh, characterize their effect on the plant growth- promoting activities, and analyze their effect on the performance of a sensitive rice variety at both seedling and reproductive stages under control and salt stressed conditions. Our findings show that locally adapted bacteria can help in rice growth and yield under moderate salt stress where soils have an iconic conductivity of up to 5 dS m^{-1}.

Methods and materials

Soil sampling and characterization of soil sample

A total of nine soil samples were collected in sterile polyethene bags from saline-prone regions of Satkhira, Bangladesh, and were transferred aseptically to the laboratory. The pH and salinity of the soil samples were measured by 1:2.5 ratio and 1:5 ratio (Corwin 2003). The (%) of organic carbon and (%) of total nitrogen were determined by Walkley and Black oxidation method (Walkley 1947) and Kjeldahl method (Kjeldahl 1883).

Isolation and screening of salt-tolerant bacteria

Different salt concentrations (250 to 3100 mM of NaCl) were used to standardize laboratory strain *E. coli DH5α*.

Freshly collected soil samples were allowed to settle down debris (if any) and were diluted in phosphate buffer saline (PBS) by serial dilution technique. Samples were diluted up to six times (10^{-1} to 10^{-6}) in sterile PBS solution before spreading and 100 μL from each dilution were plated on Luria Agar plates. The selected colony was sub-cultured into media supplemented with different salt concentrations (0.25 to 3.1 M). The plates were incubated at 37 °C for 24–48 h. The growing bacterial isolates were sub-cultured and obtained in the form of pure culture and preserved in glycerol broth at − 80 °C for further analysis.

Determination of highest degree of salt tolerance

Individual isolates were inoculated in 5-mL sterile LB (Luria Broth) containing 250, 500, 750, 1000, 1500, 2000, 2500, 2600, 2700, 2800, 2900, 3000, and 3100 mM of NaCl. Tubes were incubated at 37 °C for 48 h. After 48 h, growth of bacteria was measured at 600 nm and the concentration of NaCl at which no turbidity was observed by a spectrophotometer (SHIMADZU Spectrophotometer, Japan) was considered as the highest degree of salt tolerance for each bacterial isolates.

Characterization of bacterial isolates

Effect of temperature and pH on growth of bacterial isolates

The individual bacterial isolates were grown in Luria Broth (LB) and incubated separately at 30, 35, 37, and 40 °C temperature range. Ability of the isolates to grow in acid or alkaline media was studied by Luria Broth (LB) pH adjusted from 5 to 9 using 1 N HCl or 1 N NaOH. These were incubated overnight at 37 °C. Growth was recorded at 600 nm by a spectrophotometer.

Morphological and biochemical characterization of bacterial isolates

Morphological and biochemical characteristics of the bacterial isolates were examined according to the Bergey's Manual of Determinative Bacteriology (Gots et al. 1975) (Supplementary Fig. 2 and Table 1).

Molecular identification of bacterial isolates and phylogeny analysis

For genetic characterization, the total genomic DNA of selected isolates was extracted by a modified phenol/chloroform procedure of Sambrook and Russell (2001). A fragment of 16S rRNA gene was amplified by using following primers: 27F1 (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGCTACCTTGTTACGAC 3′). PCR analyses were carried out in a 25-μL reaction mixture containing 100 ng of genomic DNA, 100 μM of each dNTP, 2.4 ng of each primers, 1 unit of Taq Polymerase, 1.5 mM $MgCl_2$, DMSO 2.4%, and 1× PCR Buffer-$MgCl_2$ (Invitrogen, USA). PCR condition was as follows: 95 °C for 5 min (initial denaturation), followed by 35 cycles of 95 °C for 30 s, 60 °C for 50 s, 72 °C for 50 s, and final extension 72 °C for 10 min. PCR amplification was carried out in a thermocycler (GeneAtlas of Astec). Restriction digestions of amplified DNA from bacterial isolates were carried out for 1 h at 37 °C in 10-μL volumes with the following components: buffer, BSA (bovine serum albumin), restriction enzymes (*Rsa*I, *Mbo*I, *Hae*III, *Hind*III), and DNA.

The sequenced nucleotides were edited manually and compared against GenBank database using the NCBI BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST).

Characterization of plant growth promotion traits of bacterial isolates under salt stress

Nitrogen fixation activity of bacterial isolates

The sample bacteria were streaked on nitrogen-free media (Burk's media) for 1 week in an incubator at 37 °C and observed visually (Park et al. 2005). The media consisted of 1% dextrose, 0.041% potassium dihydrogen phosphate, 0.052% dipotassium hydrogen phosphate, 0.005% sodium sulfate, 0.02% calcium chloride, 0.01% magnesium sulfate (heptahydrate), 0.0005% ferrous sulfate (heptahydrate), 0.00025% $Na_2MoO_4{\cdot}2H_2O$, and 1.5% Agar in ddH_2O, pH = 7. Presence of nitrogen fixation genes was confirmed by PCR amplification of the *nifH* gene (subunit II of nitrogenase enzyme) using *nifH*F1: GCIWTYTAYGGIAARGGIG, *nifH*R1: AAICCR CCRCAIACIACRTC.

Determination of IAA (indole acetic acid) activity

Each bacterium was cultured in Luria Broth (LB) containing 0.2% (*w*/*v*) tryptophan and 3 M NaCl, and then incubated at 37 °C for 20 h at 200 rpm in an incubated shaker to accelerate late exponential phase. Bacteria cultures in the LB without NaCl were used as controls. The cultures were then centrifuged at 8000 rpm for 15 min at 4 °C in a refrigerated centrifuge. The supernatants were mixed with Salkowski's reagent (ratio 2:1) and left in the dark for 20 min. Salkowski's reagent was prepared by mixing 2-mL 0.5 M ferric chloride ($FeCl_3$), 49 mL water, and 49-mL 70% perchloric acid ($HClO_4$). The optical density of the resulting mixture was measured at an absorbance of 530 nm. The IAA concentration was determined using a standard curve of authentic IAA (Bric et al. 1991).

Effect of bacterial inoculation on seedling and reproductive stage of rice plants

Farmer popular but salt sensitive rice variety, BRRI dhan 28 (Hossain et al. 2013), was used for this experiment. Rice seeds were surface-sterilized by 99% ethanol followed by sodium hypochlorite for disinfection. Then, the rice seeds were washed with deionized water and kept at 37 °C for seed germination. Germinated seeds were transferred to Yoshida solution (Gregorio et al. 1997b). Bacterial isolates were cultivated in LB for 20 h to accelerate late exponential growth phase cells. The bacterial cells were collected by centrifugation and washed with 0.9% (*w*/*v*) NaCl and re-suspended with deionized water 10^8 CFU/mL (Nakbanpote et al. 2014). Plastic pots were prepared by mixing 2 kg of vermiculite and soil (1:1) and watered with Yoshida solution. For the seedling stage, plant roots were colonized in bacterial suspension for 45 min (Gopalakrishnan et al. 2012). Then, the seedlings were sown in the prepared plastic pots. For the reproductive stage, 1 mL of bacterial suspension was inoculated into the soil with nutrient solution near the roots after 15 days of sowing the plant. In each pot, two seedlings were planted at the same depth. Each pot was watered with nutrient solution (300 mL) once per week. One-milliliter bacterial suspension culture was inoculated into the soil with nutrient solution every 15 days in both seedling and reproductive stages. In case of salt stress treatments, the seedling stage plants were watered

to obtain final concentrations of 0, 40, and 80 mM NaCl dissolved in nutrient solution taking into account the dilution effect of the total volume of the soil. For the reproductive stage, plants were watered as above to obtain final concentrations of 0, 50, and 100 mM NaCl dissolved in nutrient solution (Bashan et al. 2014). Four replicates (pots) were used for control and five for salt-stressed plants.

Measurement of electrolyte leakage and chlorophyll content at seedling stage

Relative electrolyte leakage was measured according to Cao et al. (2007) and Parvin et al. (2015). The plant leaf segments were weighed (0.1 g) and taken in a bottle with 25 mL deionized water. The tubes were shaken on a gyratory shaker at room temperature for 2 h. The initial electrical conductivity (C1) of the solution was measured by using a conductivity detector. The leaf samples were then boiled in deionized water at 120 °C for 10 min to release all the electrolytes from the tissues completely. The final electrical conductivity (C2) of the resulting solution was recorded. The percentage of electrolyte leakage was calculated according to the formula: (C1/C2) × 100. Finally, statistical analysis was done by using *t* test.

For chlorophyll measurement, fresh leaves were cut into pieces and 100 mg put into a bottle containing 12 mL of 80% acetone. After 48 h, absorbance of leaf tissue extract was measured at wavelength 663 and 645 nm. The total amount of chlorophyll was calculated using formula: $[\{(0.00802 \times A_{663}) + (0.0202 \times A_{645})\} \times V/W\}]$; A = absorbance, V = volume, and W = weight (Jnandabhiram and Sailen Prasad 2012).

Measurement of yield parameters at reproductive stage

After 140 days, plants were assessed for yield parameters. Shoot weight, flag leaf weight, flag leaf length, flag leaf damage, plant height, total tillers, effective tillers, panicle length, panicle damage, unfilled grain weight, and filled grain weight were measured (Gregorio et al. 1997a).

Statistical analysis

All statistical analyses were done using Data Analysis ToolPak of Microsoft Office Excel 2013. The F test was performed to verify equal variance of the independent set of samples, and the Student's *t* test was performed based on the result assuming equal variance or unequal variance as applicable to compare significant differences ($p < 0.05$) between bacterial inoculated and non-inoculated plants both in control and saline conditions.

Results

Characterization of soil samples

Analysis of the two soil samples revealed that they were calcareous soil. That means it contained excessive amount of calcium carbonate ($CaCO_3$). The range of EC (electrical conductivity) of moderately saline soil is 4–8 dS/m (Redfern et al. 2012). The results of the physicochemical parameters of these soil samples including pH, EC, organic carbon, C, and N contents are given in the Table 1.

Isolation and screening of salt-tolerant bacteria

Initially, a total of 20 isolates were screened from soil of saline-prone regions. Laboratory strain *E. coli DH5α* was used as control and this strain grew up to 750 mM of NaCl. Three isolates were selected based on their high level of salt tolerance. The bacterial isolates were named to reflect their region of isolation and our laboratory (Satkhira and Plant Biotechnology) as SK-Pbt 01, SK-Pbt 02, and SK-Pbt 03. SK-Pbt 01 was isolated from soil sample 5, and SK-Pbt 02 and SK-Pbt 03 were isolated from soil sample 7. The three strains were cultivated in Luria Bertani (LB) broth that contained 3 M of NaCl. All the isolates were incubated at 37 °C for 48 h.

Effect of temperature and pH on bacterial growth

The bacteria showed maximum growth at 37 °C and moderate growth was observed at 35 °C. Slow growth was observed at 30 and 40 °C (Supplementary Fig. 1 A). The bacteria showed maximum growth at pH 7 and 8 and very moderate growth was observed at pH 6. At pH 5 and 9, bacterial growth was not observed (Supplementary Fig. 1 B). That means high acidic pH and alkaline pH retarded the growth of the bacterial isolates.

Table 1 Characteristics of soils used in this study

Soil sample	GPS location	pH	EC (dS/m)	Organic carbon (%)	Total N (%)
Sample 5	22°34′19.2″N 89°21′40.0″E	8.06	4.83	8.10	3.50
Sample 7	22°33′41.6″N 89°18′27.5″E	8.40	4.08	12.84	9.05

Molecular identification of bacterial isolates

The biochemical and the ARDRA analysis revealed that these 20 bacteria belonged to the same Genus. So, for molecular identification, the sequencing results of 16S rRNA gene of the three unknown bacteria—SK-Pbt 01, SK-Pbt 02, and SK-Pbt 03—were blasted using BLAST tool in National Center for Biotechnology Information (NCBI). It was found that there was a 99% match between the query sequences of unknown bacteria (SK-Pbt 01 and SK-Pbt 02) and the 16S ribosomal RNA gene sequence (MF671999) of *Halobacillus dabanensis strain SB-26*. The query sequences of unknown bacteria (SK-Pbt 03) matched with the 16S ribosomal RNA gene sequence (MF672000) of *Halobacillus* sp. *GSP-34* by 99%. These sequences are deposited in NCBI.

Nitrogen fixation activity in bacterial isolates under salt stress

PCR amplification of *nifH* gene

These three isolates were grown on nitrogen-free Burk's media (Fig. 1a), and the expected 650 bp band amplification was observed (Fig. 1b) in these isolates. So, it was confirmed that these bacteria were nitrogen fixers.

Indole acetic acid production in bacterial isolates under salt stress

The bacterial isolates were grown in the presence of L-Trp (L-Tryptophan) both in 3 M salt and without salt. The concentration of IAA produced was calculated by a standard curve. Among the three isolates, SK-Pbt 01 (25.07 μg/mL) produced the highest average amount of IAA followed by SK-Pbt 03 (24.47 μg/mL) and SK-Pbt 02 (19.58 μg/mL) in saline condition (Fig. 2a). So, in the presence of NaCl, bacterial efficiency for IAA synthesis was enhanced compared to the control condition, but they did not produce significant amount of IAA. All the isolates produced high quantity of IAA during their late exponential phase of growth.

Effect of bacterial inoculation on yield of rice plants at seedling stage

The plants inoculated with bacteria showed the lowest damage as well as performed better both in control and salt stress (40 and 80 mM) (Figs. 3a–c and 4a–e). The pH of the soil was maintained at 6.5–7.0 which is optimal for the growth of both bacteria and rice plants. And the ambient temperature was maintained at 37 °C as bacterial growth was maximum at this condition. In this condition, two individual bacteria along with the combination of SK-Pbt 02 and SK-Pbt 03 were found to be more efficient in promotion of shoot height, root length, total weight, chlorophyll content, and reduction in electrolyte leakage in both control and stress condition. Only under inoculation with both bacteria together, there was a significant reduction in electrolyte leakage and increased chlorophyll content under 40 and 80 mM salt stress. Since SK-Pbt 01 and SK-Pbt 02 were nearly identical, phenology of only SK-Pbt 02 is shown.

Effect of bacterial inoculation on yield of rice plants at reproductive stage

Bacteria-inoculated plants both in control and saline condition showed significantly better performance compared to non-inoculated plants for all six agronomic traits under control (0 mM NaCl) and continuous 50 mM NaCl stress in soil (Fig. 5a–d) and (Fig. 6a, b).

Measurement of agronomic traits after bacterial inoculation in both control condition and saline condition

The yield parameters of the bacterial inoculated plants both control and salt stress were assessed (Fig. 7a–c). SK-Pbt 02 and SK-Pbt 03 increased plant height to 61.67 and 63.8 cm compared to non-inoculated plants (59.3 cm) in control conditions and 59.6 and 62 cm compared to non-inoculated plants (56.7 cm) in saline condition. Bacterial inoculated plants have significantly higher percentage of spikelet fertility compared to non-inoculated plants both in control (0 mM NaCl) and saline (50 mM NaCl) conditions. SK-Pbt 02 and SK-Pbt 03 showed significantly improved spikelet fertility rate, from

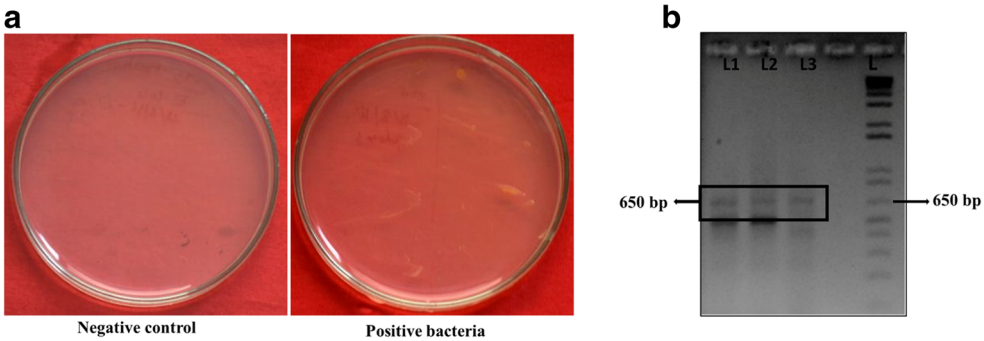

Fig. 1 **a** Bacteria grown on Burk's media. **b** PCR amplification of *nifH* gene. L1: SK-Pbt 01; L2: SK-Pbt 02; L3: SK-Pbt 03; L: 1 kb^{+}

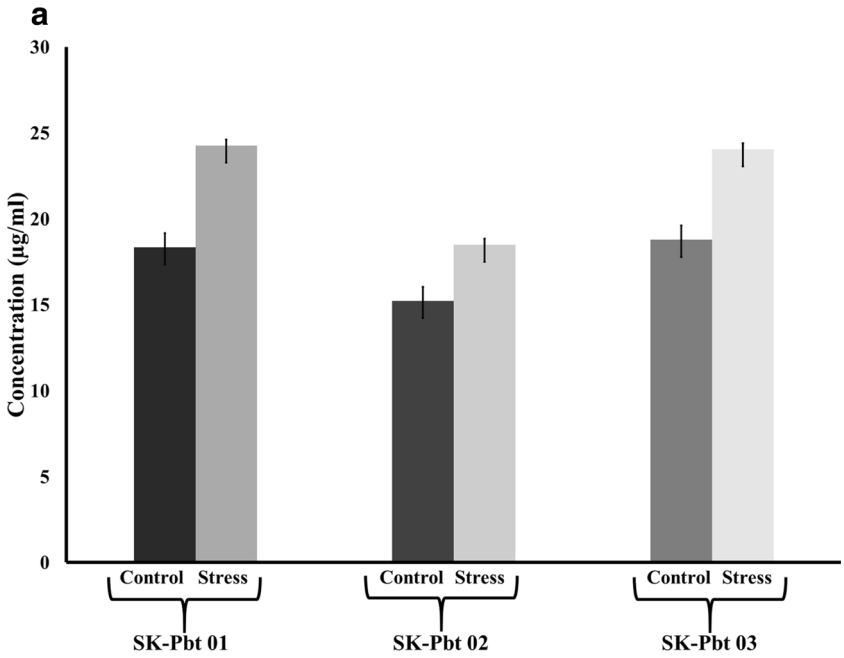

Fig. 2 **a** Concentration of IAA produced by bacterial isolates both in control (without salt) and stress (17.5% or 3000 mM of salt) condition in the presence of L-Trp. Each bar represents the mean ± SE ($n = 3$)

76% in non-inoculated plants to 84.9 and 93.8% in inoculated plants in normal soil. Likewise, the percent spikelet fertility was 44.3% in non-inoculated plants, while it was 82.6% ($P < 0.01$) and 86.78% ($P < 0.01$) in inoculated plants under salt stress. Percentage (%) of yield reduction was significantly lower in bacterial inoculated plants whereas non-inoculated plants showed higher percentage of yield reduction. SK-Pbt 02 and SK-Pbt 03 decreased the percentage of yield reduction to 8.1% ($P < 0.001$) and 7.9% ($P < 0.01$) compared to non-inoculated plants (22.8%) in saline (50 mM salt) conditions.

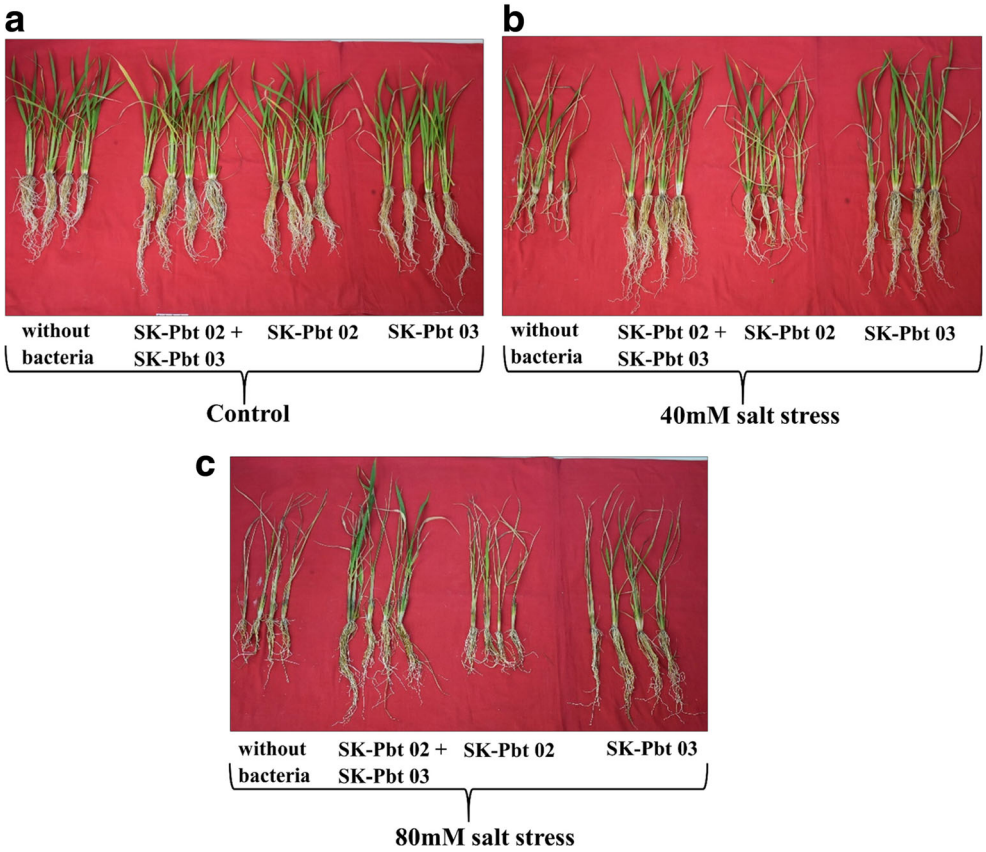

Fig. 3 Bacterial inoculated plants showed better phenotypic results both in control and salt (NaCl) stress (40 and 80 mM)

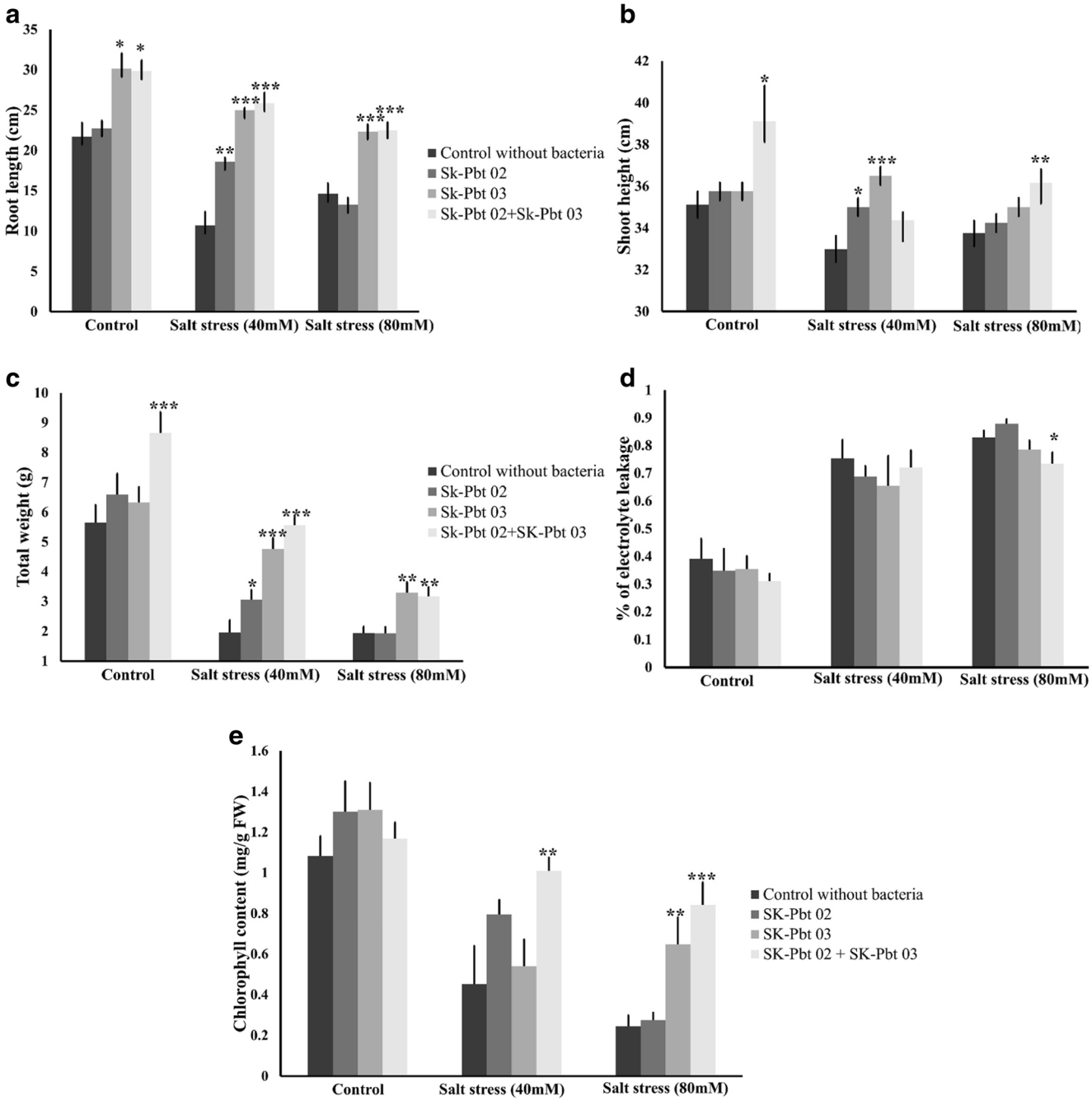

Fig. 4 Bacteria inoculated plants showed better results in case of **a** root length, **b** shoot height, **c** total weight, **d** electrolyte leakage, and **e** chlorophyll content both in control and salt (NaCl) stress (40 and 80 mM). Each bar represents the mean ± SE ($n = 4$). *Significant difference between bacterial inoculated and non-inoculated plants at $P < 0.05$. **Significant difference between bacterial inoculated and non-inoculated plants at $P < 0.01$. ***Significant difference between bacterial inoculated and non-inoculated plants at $P < 0.001$

So, it can be concluded that the bacteria had significant positive on percentage of spikelet fertility and yield reduction.

Discussion

Soil salinity is one of the major abiotic stresses in the coastal regions that restrains growth and productivity of rice plants (Shrivastava and Kumar 2015; Hoang et al. 2016). As beneficial soil bacteria have been reported to promote plant growth under salinity stress (Souza et al. 2015), bacterial inoculation could be a way to modulate or ameliorate the threat of soil salinization to agricultural productivity (Bashan et al. 2014).

Root morphology, exudates, and stage of growth of the plant influence the microbe-plant interactions in the soil and rhizosphere (Bal et al. 2013). The collected soil samples were found

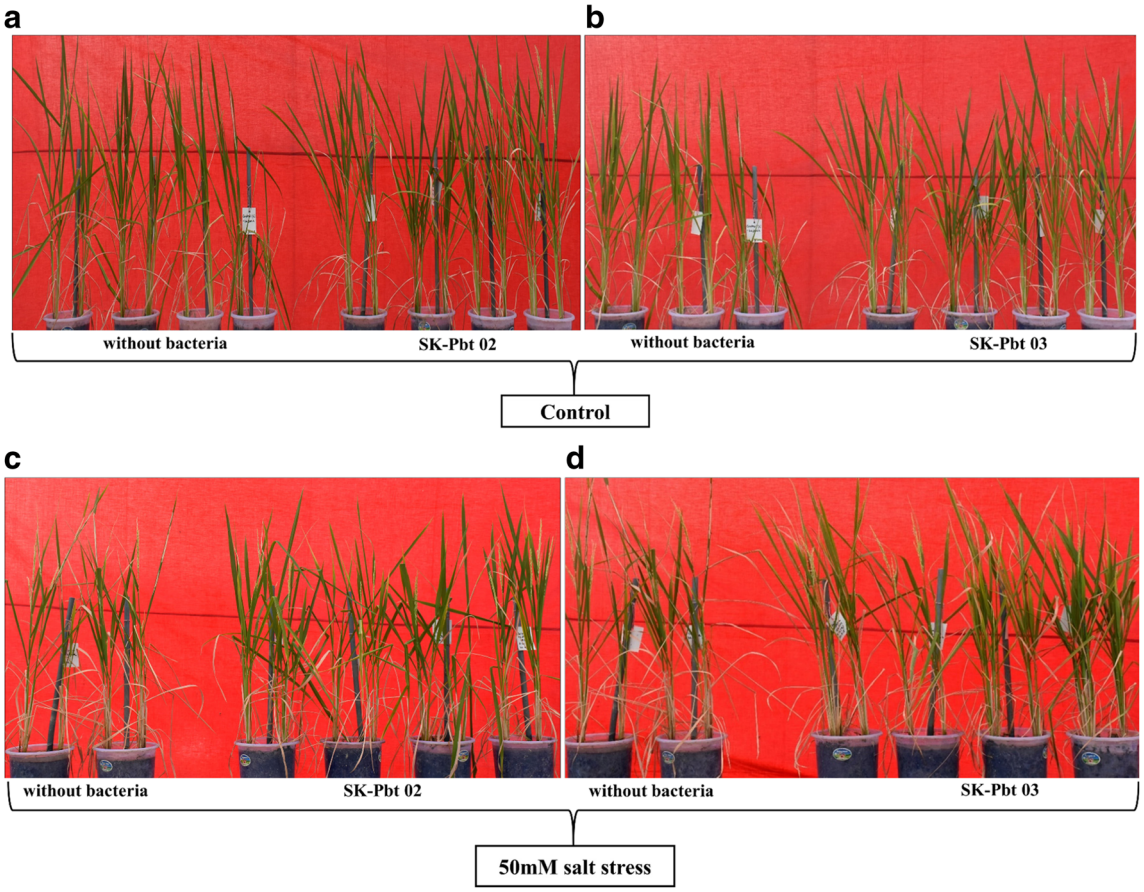

Fig. 5 Phenology of bacterial inoculated and non-inoculated plants. BRRI dhan 28 rice plants inoculated with bacteria showed better phenology at maturity both in control (0 mM NaCl) and 50 mM salt concentration than non-inoculated plants

to be calcareous and moderately saline, which hampers the bio-availability of plant nutrients and many vascular plants grow without the successful colonization of bacteria in these conditions (Ström et al. 2005). But the viable count of isolated bacteria in soil samples in the current study was 3×10^4–8.5×10^4 cfu/mL in different samples (Supplementary Fig. 3). So, it

Fig. 6 Panicles of bacteria inoculated plants showed better results than non-inoculated plants both in control and saline (50 mM NaCl) conditions

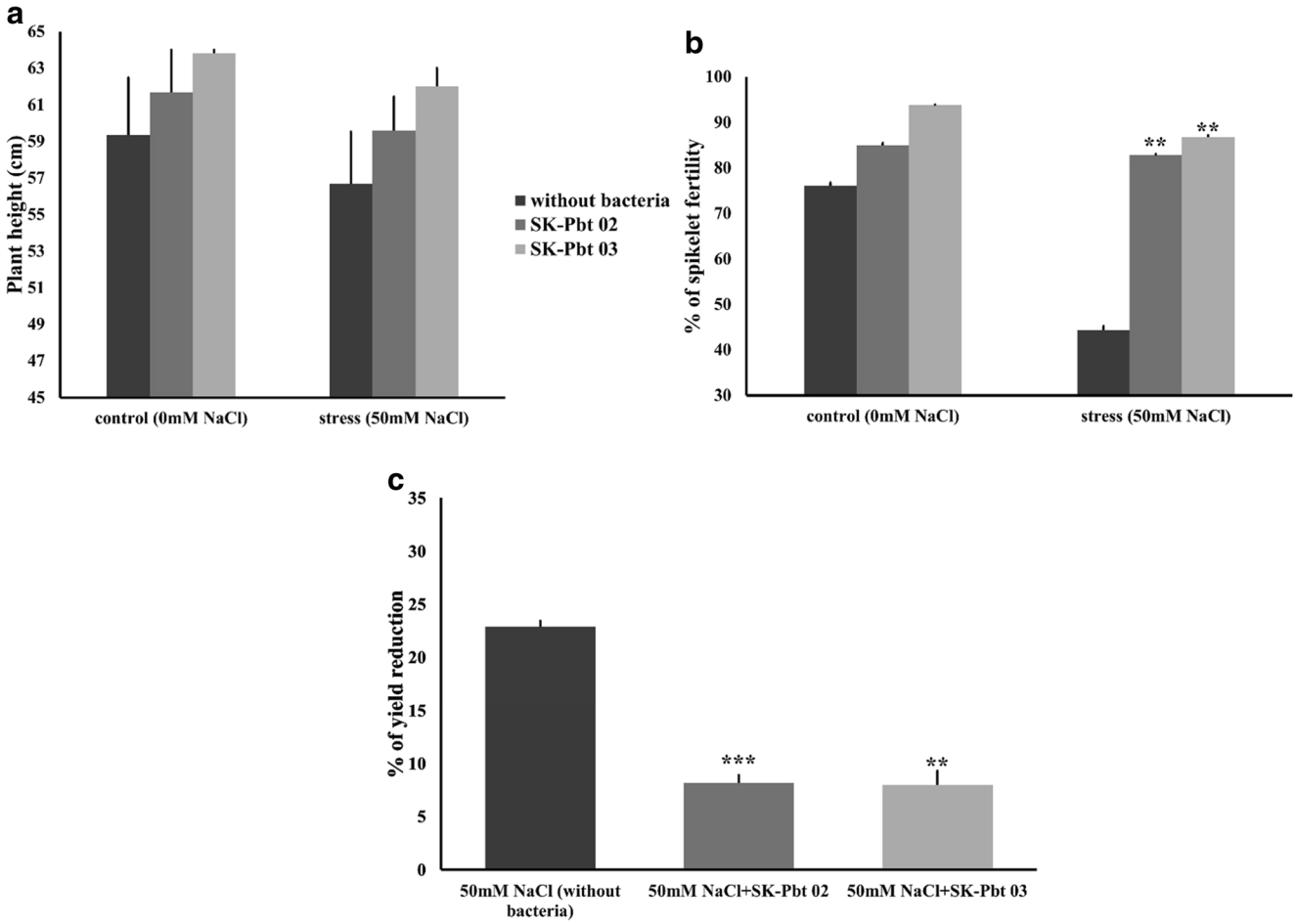

Fig. 7 Measurement of agronomic traits of bacterial inoculated and non-inoculated plants both in control and continuous 50 mM of salt stress. **a** Plant height, **b** percentage (%) of spikelet fertility, **c** percentage (%) of yield reduction. Each bar represents the mean ± SE ($n = 4$). **Significant difference between bacterial inoculated and non-inoculated plants at $P < 0.01$. ***Significant difference between bacterial inoculated and non-inoculated plants at $P < 0.001$

can be said that the C:N ratio (carbon/nitrogen) and the pH of the soil favored growth of the soil bacteria (Rousk and Bååth 2007). Three bacterial isolates (SK-Pbt 01, SK-Pbt 02, SK-Pbt 03) were selected among 20 isolates based on their highest degree of resistance to NaCl (3 M). Biochemical analysis and molecular identification of bacteria indicated that SK-Pbt 01, SK-Pbt 02, and SK-Pbt 03 belong to the *Halobacillus* species. As the other 20 bacterial isolates all belonged to the same Genus, it can be concluded that the collected soil sample from these regions was prevalent with the *Halobacillus* bacteria. Interestingly, salt-affected soils are reported to be enriched with salt-tolerant bacteria for example, *Bacillus*, *Staphylococcus*, *Halobacillus*, *Halomonas*, *Microbacterium*, *Arthrobacter*, *Oceanimonas*, *Micrococcus* (Siddikee et al. 2010; Orhan and Gulluce 2015), *Enterobacteriaceae*, *Clostridium*, *Corynebacterium*, *Vibrio* (Rahman et al. 2017) etc.

The identified bacteria were *Halobacillus dabanensis strain SB-26* and *Halobacillus GSP 34* and they are reported to withstand the high range of salt concentration by accumulating the compatible solute to adjust with the external osmotic pressure due to NaCl. So, to understand and explain the mechanism of salt tolerance, it has been reported that at 1 to 25% of salt, *Halobacillus dabanensis* bacteria accumulates ectoines and expresses 59 heat shock proteins. Ectoines serve as an osmoprotectant which is used to stabilize the enzymes of the cell though its molecular mechanism is not clear as yet. The reported 59 heat shock proteins are involved in signal transduction, energy metabolism pathways, and proteins involved in salt stress (Zhao et al. 2006; Zhang et al. 2006). On the other hand, the salt tolerance mechanism of *Halobacillus GSP34* has not been reported yet, so this is an area of future study.

In order to exploit their salt tolerance properties and be able to use them as biofertilizer, they were screened for their plant growth promotion activities. The presence of *nifH* gene indicated that these bacteria can fix the inert nitrogen to ammonia (NH_3) even under salt stress. Alongside, production of indole acetic acid (IAA) in the presence of salt revealed that these bacteria may play an important role in the growth promotion of rice plants.

As the rice plant is most sensitive to salinity stress at seedling and reproductive stages (Moradi and Ismail 2007), SK-Pbt 02 and SK-Pbt 03 were used as bioinoculant to ameliorate salt stress at both of these stages. At seedling stage, the combined action had more significant effect on plant growth because these bacteria were shown to enhance photosynthetic capacity by increasing chlorophyll retention and reducing the electrolyte leakage. The nitrogen fixation and IAA activity also exerted a positive effect on the root, shoot, and growth of the plants. Interestingly, multiple strain inoculants showed significantly superior plant growth promotion activity compared to single strain inoculants (Lippi et al. 1992) as was also found from this study. Salt stress added negative effect on the spikelet fertility, grain weight, 1000 grain weight, and yield etc. due to the increase of ROS and other inefficient antioxidant defense system in rice plants (Selote and Khanna-Chopra 2004). But the bacterial inoculated plants had significantly positive effect on each of the yield parameters both in control and stress conditions.

The positive effects on plant growth were longer root and shoot length, higher percentage of spikelet fertility, and lower reduction of yield. This clearly showed that these bacterial isolates can mitigate the effects of salt stress in rice plants. Salt stress causes nitrogen deficiency in soil by restraining nitrogen turnover which is in turn carried out by soil microorganisms (Zhang and Feng 2008). Since these bacteria possess nitrogen fixation activity, they can convert atmospheric nitrogen into ammonia under micro aerobic conditions at low nitrogen levels, through the action of the nitrogenase complex, making it accessible to plants (Seefeldt et al. 2009). In the presence of L-Trp released in plant root exudates, bacteria can synthesize IAA, some of which is taken up by plants (Glick et al. 2007). IAA promotes the development of plant root architecture including primary roots, higher order lateral roots, and root hairs. It also enhances nutrient uptake and increase of plant biomass (Khan et al. 2016). So, this property of the isolated bacteria may promote root growth directly, by stimulating cell elongation or cell division, even under salt stress.

Plant growth-promoting activities like nitrogen fixation and IAA activity of *Halobacillus dabanensis strain SB-26* and *Halobacillus GSP 34* have not been reported before. There has also not been any study on the effects of their inoculation on plant growth. Some of the salt-tolerant bacteria like *Pseudomonas*, *Bacillus*, *Enterobacter*, *Methylobacterium* have been used to for inoculation in saline soil to observe their effects on plants but these were limited to seedling stage only (Madhaiyan et al. 2007; Nakbanpote et al. 2014; Han et al. 2014b; Bhise et al. 2017). On the other hand, in this study, the two species of *Halobacillus* when inoculated in both control and saline soil showed a significant positive effect on the rice plants at both seedling and reproductive stages. The positive effect was however observed only up to 50 mM salt. It has been reported that formulated biofertilizer by mixing autoclaved charcoal powder, $CaCO_3$, gum acacia, and liquid culture have significant effect on seedling stage of rice plant both in control and 200 mM salt concentration (Sahoo et al. 2014).

Conclusion

So, our isolated bacteria can also be utilized for formulating biofertilizer in a similar manner and may result in withstanding a higher concentration of salt. These bacteria can therefore be used as an alternative to chemical fertilizers to increase the growth, productivity, and yields of crops in the saline and non-saline soils.

Acknowledgments This work was supported by the grant to Z.I.S. by the Ministry of Science and Technology. We would like to thank Raju Ahmed for taking care of rice plants in the net house at DU.

Authors' contribution All authors contributed significantly to this study. FR and SB performed isolation techniques, molecular identification, did some plant growth promotion activities, comparative physiology, compiled the data, and did statistical analyses. MRI helped in those techniques. PS helped in doing physiological screening both in seedling and reproductive stages. ZIS designed the study and helped in writing the manuscript.

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