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Influence of Bacterial Endophytes on Salinity Stress Tolerance and Growth Promotion in Rice (*Oryza sativa* L.)

Tasmiya Imtiyaz, N. Earanna and Nakul Kale
Department of Microbiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : tasmiyaaimtiyaz6760@gmail.com

Authors Contribution

Tasmiya Imtiyaz : Screening, characterisation of endophytes, pot study & stastical analysis ;
N. Earanna : Correction of manuscripts and data;
Nakul Kale : Collection of data during pot experiment

Corresponding Author :
Tasmiya Imtiyaz
Deapartment of Microbiology, College of Agriculture, UAS, GKVK, Bengaluru

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Abstract

Salt stress adversely affects the growth and development of plants. However, plants counteract such stress with different mechanisms to some extent. Symbiotic association of bacterial endophytes alleviate the abiotic stress and promote the growth of plant. In this study, 58 bacterial endophytes isolated from Himalayan cold desert plants were screened *in-vitro* at different concentrations (0.5M, 1.0M, 1.5M, 2.0M, 2.5M) of NaCl. Out of 58 isolates, 9 isolates designated as PBE 4, PBE 6, PBE 8, PBE 15, CBE 12, NBE 7, NBE 20, NBE 21, NBE 23 showed salt tolerance up to 2.0 M NaCl. Nine bacterial isolates inoculated to pre-germinated rice (*var.* IR 64) seeds and grown on paper towel treated with 150 mM NaCl (based on LC_{50}value) only two endophytes (PBE 8 and NBE 7) significantly increased the seedling length compared to unino culated seedlings. The two isolates were identified as *Enterobacter hormaechei* and *Pseudomonas fluorescens* using 16S rRNA gene sequence. These two bacterial isolates also showed production of growth promoting traits such as nitrogen fixation, phosphate solubilization, production of HCN, NH_3, siderophore, proline, IAA, gibberellic acid, abscisic acid and salicylic acid. Further, inoculation of these bacteria (*Enterobacter hormaechei* and *Pseudomonas fluorescens*) to pre germinated rice seeds significantly increased the plant growth at 4 dS m^{-1} NaCl stress under greenhouse conditions. This study suggested that endophytic isolates *Enterobacter hormaechei* and *Pseudomonas fluorescens* impart salinity stress tolerance and promote growth of rice seedlings.

Keywords : Bacterial endophytes, Salinity, Rice (var., IR 64), *Enterobacter hormaechei, Pseudomonas fluorescens*

Abiotic stress has significant impact on agricultural yield and the distribution of plant species in the environment. Severe temperatures, salt, drought and chemical toxicity all have major repercussions for agricultural productivity, resulting in more than 50 per cent yield losses worldwide (Wang and Frei, 2011). One of the biggest obstacles to crop productivity globally is the salt stress. Salinity is considered as violent problem affecting the productivity of crops. Salt-affected soils are a catastrophic ecological entity in any arid or semi-arid regions. At 25°C, saline soils have an electrical conductivity (EC) of the saturation extract (ECe) in the root zone that surpasses 4 dS m^{-1} and sodium exchangeable of 15 per cent (Shrivastava and Kumar, 2015). Salinity not only reduces agricultural productivity of most crops, but also has an impact on soil physico-chemical qualities and the area's ecological balance. Increased levels of harmful ions limit photosynthesis, protein synthesis, inactivate enzymes and damage chloroplasts and other organelles finally affects crop development (Hu and Schmidhalter, 2002).

Rice (*Oryza sativa* L.) is a major crop that feeds more than half of the world's population and serves as a model system for monocotyledonous plants such as cereals. Asia produces and consumes around 90 per cent of the world's rice. On the other hand, it is extremely vulnerable to salinity stress with a salinity stress threshold of 4 dS m^{-1} for most farmed types

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(USDA, 2020). A wide range of adaptations and mitigation methods are necessary to maintain sustainable agriculture in order to address challenge related to salinity stress. The need for salt-tolerant rice varieties that can also withstand a variety of other stresses puts a lot of pressure on breeders to better understand the physiology and genetic regulation of salt tolerance (Khan *et al.,* 2012). However, roles of endophytes to combat salt stress and boost the plant growth are studied and proved in many crops.

Endophytes are microorganisms that infiltrate into and reside in plants without causing any disease symptoms. Endophytes populate practically every part of the plant (leaves, stems, roots, flowers and fruits) and are found almost everywhere (Wilson, 1995). Endophytes may be found in a wide range of plants, from grasses to higher order plants, notably woody plants, which can harbour hundreds of species. Many of them can create vital biochemical components that aid in the defence of plants against illnesses and insect assault. The nature of endophytic partnerships might be commensal, parasitic or mutualistic. They offer a host with advantages like heat tolerance, salt resistance and resistance to plant diseases or animal feeding (Rodriguez and Redman, 2008). Therefore, in this study, we explored the bacterial endophytes isolated from the plants growing in the harsh environment for mitigating salt stress in salt-sensitive rice variety IR-64.

Material and Methods

In-vitro Screening of Bacterial Endophytes for Salt Tolerance

Fifty-eight bacterial endophytes isolated from the plants growing in the harsh environments of north Himalayan cold deserts (Pangong, Changla and Namika La regions) and maintained at the School of Ecology and Conservation (SEC) Laboratory, Department of Crop Physiology, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bengalore - 560 065. These bacteria were revived on nutrient agar medium. Nutrient broth having 0.5 M, 1.0 M, 1.5 M, 2.0 M and 2.5 M concentrations of NaCl was prepared in a test tube. The 58 bacterial isolates were individually inoculated in to the medium and incubated at 30°C for 24h. The optical density (OD_{600}) for bacterial growth was measured using spectrophotometer (Viscardi *et al.,* 2016).

Determination of NaCl Concentration for Rice

The NaCl tolerance threshold of rice seedling was determined by paper towel method. Solutions of different concentrations of NaCl (25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM, 200mM) were prepared in distilled water (Hardoim *et al.*, 2008). The seed germination paper towels were separately dipped in each concentration of NaCl. The pre-germinated rice seeds were placed on germination paper towel and kept at 30°C in a growth chamber. After 14 days, shoot-and root lengths were recorded. The lethal concentration (LC_{50}) of NaCl was calculated for seedling length using IBM SPSS statistics 2.0 (https://www.ibm.com/in-en/analytics/spss-statistics-software).

Screening of Selected Bacterial Endophytes on Rice Seedlings Against Salt Stress

Rice seeds were surface sterilised by 3 per cent Sodium hypochlorite for 5 min and 70 per cent ethanol for 1 mint then rinsing them once or twice with sterile water and germinated at ambient temperature. Thus, germinated seeds were inoculated by dipping in bacterial cultures (~$8x10^7$ CFU/ml) for 3 hours. Sterile distilled water was used as control. The endophytes inoculated seedlings were placed on germination paper towels treated with 150 mM NaCl (LC_{50} value) and kept at ambient temperature for 14 days. There were two replications of each treatment with 10 seedlings in each replication. Root and shoot lengths were recorded at 14-days after inoculation (Walitang *et al.,* 2017).

Identification of Bacterial Endophytes Using 16S rRNA gene Sequence

The genomic DNA of selected bacterial endophytes was extracted by alkaline lysis method (Sambrook and Frist so Maniatis 1989). Sigma-Aldrich custom synthesised NCBI primers for the 16S rRNA sequence

(26 bp forward primer 5' GTTAGATCTTGGCTCA GGACGAACGC 3' and 24 bp reverse primer 5' GATCCAGCCGCACCTTCCGATACG 3') and the amplified product obtained was sequenced using a gel elution kit (The Gene JETTM Gel Extraction Kit, Thermo Scientific) by Chromgene Biotech Pvt. Ltd., Bengaluru, Karnataka. Sequences were analysed for homology using NCBI GenBank.

Characterization of Bacterial Endophytes Isolates for Plant Growth Promoting Traits

Phosphate Solubilization : The qualitative solubility of tricalcium phosphate by the endophytic isolates was determined using Pikovskaya's agar. Selected bacterial endophytes were spot inoculated on the surface of Pikovskaya agar medium and incubated at 28°C. The phosphate solubilizing activity was determined after 72 hours of incubation period. Appearance of a transparent / clear zone around the bacterial colony indicated that inorganic phosphates had been dissolved (Gour, 1980).

Hydrogen Cyanide Production : Isolates were streaked in each nutrient agar plate amended with 4.4g/l glycine except one, which served as control. Under aseptic conditions, a Whatman filter paper strip coated with an alkaline picric acid solution was put in the upper lids of inoculated petri plates. At 28°C, these plates were incubated for 24 hours. The colour of Whatman filter paper soaked with alkaline picric acid changed from yellow to orange, indicating HCN production by isolates. (Bakker and Schipper, 1987)

Siderophore Production : Bacterial isolates were spot inoculated on nutrient agar supplemented with universal Chrome Azurol S (CAS) reagent for 24h at 30°C. A clear orange colour zone appeared, indicating siderophore production. (Schwyn and Neilands, 1987)

Ammonia Production : Bacterial isolates were tested for ammonia production in peptone water. Freshly developed cultures were injected into each tube separately with 5 mL peptone water and incubated for 48 to 72 hours at 28°C. Nessler's reagent was added to each test tube. The change in colour from brown to yellow was a positive indication of ammonia production. (Geetha *et al.,* 2014).

Proline Production : The amount of proline accumulated in bacterial isolates was calculated in nutrient broth with and without stress (2.0 M NaCl). Mishra *et al.* (2011) technique used to quantify inoculated broths after 48 hours of culture. The extracted proline was then separated and transferred to new tubes and the absorbance was measured at 520 nm with a spectrophotometer and the result was expressed as µg of proline ml^{-1} of bacterial culture (Ceylan *et al.,* 2012).

Quantification of IAA, Gibberellic Acid (GA), Abscisic Acid (ABA) and Salicylic Acid (SA) Using HPLC

Cultures were inoculated in 20 mL nutrient broth flasks with and without stress (2.5 M NaCl) and incubated at 30°C for 7 days. After incubation, they were centrifuged for 10 minutes at 6000 rpm and the supernatant was collected and adjusted to pH 2.8 using 1 N HCl solution. The acidified supernatant was mixed with an equivalent volume of diethyl ether in a 100 mL conical flask and incubated at 4°C for 4 hours. The solvent phase (top layer) that had been collected was allowed to evaporate. After sterilisation of the membranes, 2 to 3 mL of HPLC grade methanol was added to the evaporated samples, which were then stored at -20°C for HPLC (Patten and glick, 2002).

Evaluation of Endophytes Treated Rice plants under Greenhouse Conditions

A pot experiment was conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bengaluru - 560 065. The soil's physico-chemical properties were investigated before to the experiment. (Soil pH 7.32, Electrical conductivity 0.65 dS/m, Available K_2O 39 kg/ha, Exchangeable Na 0.35 meq/lt) The quantity of salt required to maintain the EC at 4 dS/m was calculated ($CaCl_2.2H_2O$ 1225 mg/lt, NaCl 384 mg/lt, $MgSO_4.7H_2O$ 319 mg/lt, $MgCl_4.6H_2O$ 1549 mg/lt) and added to the soil.

A 2:1:1 mixture of soil, sand and farmyard manure (FYM) was used to fill plastic pots with a capacity of 15 kg. Before they germinated, IR 64 Rice seedlings were treated with bacterial suspension. These seeds were grown for 14 days in small pots. The seedlings were then placed to the pots in a gentle manner. Using the Karnal approach (Tomer and Minhas, 2005), the plants were stressed with salt. A salt stress of 4 dS/m was maintained in the individual pots by injecting a solution of dissolved salts in sterile distilled water. In each container, two seedlings were kept alive. At 30 days after transplantation, growth parameters such as plant height, number of leaves and number of branches, as well as physiological parameters such as chlorophyll content, proline content, relative water content and electrolyte leakage as influenced by endophytes under salt stress conditions, were recorded.

Re-isolation and Confirmation of Inoculated Bacterial Endophytes

Rice plant parts (root, stem and leaf) were harvested and used to isolate endophytes on nutrient agar media. The presence of same injected isolate was verified using the 16S rRNA gene sequence.

Statistical Analysis

The WASP: 2.0 (Web Agri Stat Package 2) statistical programmes (www.icargoa.res.in/wasp2/index.php) were used to analyse data and Duncan Multiple Range Test was used to differentiate the means (DMRT).

Results and Discussion

Screening of Bacterial Endophytes for Salt Tolerance

The salt tolerance of 58 bacteria isolated from different locations of the Himalayan cold desert was tested using varying amounts of sodium chloride. At 2.0 M NaCl, nine bacterial isolates (PBE 4, PBE 6, PBE 8, PBE 15, CBE 12, NBE 7, NBE 20, NBE 21, NBE 23) showed tolerance. There are four strains from Pangong area (PBE 4, PBE 6, PBE 8, PBE15) (Table 1), one isolate from Changla (CBE 12) (Table 2) and four isolates from Namkila La (NBE 7, NBE 20, NBE 21, NBE 23) (Table 3). Remaining 49 isolates grew to 1.5 M NaCl but did not grow at 2.0 M, showing that they are vulnerable to higher concentrations. This represents the salt tolerance limit of several isolates. In general, when concentration of NaCl increased, growth of bacterial endophytes reduced. Increased ionic influxes, oxidant imbalances, cell division impairment, membrane degradation and reduced superoxide dismutase activity might all contribute to this (Munns and Tester, 2008).

Evaluation of Selected Bacterial Endophytes for Salinity Stress Tolerance in Rice.

Standardization of NaCl concentration for Rice: Rice seedlings were tested for salt stress using NaCl concentrations at 25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM and 200 mM. Increased NaCl concentrations reduced root and shoot length. Untreated rice seedlings had longest seedling length, whereas increasing concentrations had shortest seedling length. Concentration of NaCl was found to have an LC_{50} value of 150 mM. Results obtained agree with Jagadheesh, (2014) reported that fungal endophytes isolated from Pokalli rice varieties (VTL-4, VTL-6 and VTL-8) withstand NaCl stress up to 2.00 M under in-vitro condition. Fungal endophytes isolated from roots of halophytes *Phragmites australis, saueda salsa* etc., decreased their growth with increased NaCl concentration from 0-12 per cent (Qin *et al.,* 2017).

In-vitro inoculation bacterial endophytes to salt sensitive Rice (*var.,* IR-64): Out of nine bacterial endophytes, two endophytes (NBE 7 and PBE 8) inoculated seedlings recorded maximum seedling length (Fig. 1) compared to uninoculated plants showed least growth of the seedlings, Further the isolates showed significantly higher shoot length were selected for characterization. Jogawat *et al.* (2013) reported that *P. indica* association on rice seedlings can be observed during high salt stress conditions (200 and 300 mM NaCl), which enhanced root and shoot length of plants in *P. indica* inoculated rice seedlings

TABLE 1

Effect of different concentration of NaCl on growth of endophytic bacteria isolated from Pangong region plants

Bacterial isolates	Optical density at 600 nm indicating bacterial growth					
	Control (0.0 M)	0.5 M	1.0 M	1.5 M	2.0 M	2.5 M
PBE 1	0.65 (0.80^{c})	0.35 (0.58^{i})	0.15 (0.38^{jk})	0.10 (0.31^{d})	0.0 (0.71^{d})	0 (0.71)
PBE 2	0.58 (0.75^{e})	0.40 (0.63^{f})	0.23 (0.47^{g})	0.13 (0.35^{d})	0.0 (0.71^{d})	0 (0.71)
PBE 3	0.38 (0.61^{h})	0.27 (0.51^{j})	0.13 (0.35^{l})	0.09 (0.29^{def})	0.0 (0.71^{d})	0 (0.71)
PBE 4	0.58 (0.75^{e})	0.37 (0.60^{g})	0.31 (0.55^{e})	0.28 (0.52^{b})	0.22 (0.84^{b})	0 (0.71)
PBE 5	0.66 (0.80^{c})	0.54 (0.73^{c})	0.22 (0.46^{g})	0.04 (0.18^{efg})	0.0 (0.71^{d})	0 (0.71)
PBE 6	0.78 (0.88^{b})	0.58 (0.75^{b})	0.49 (0.69^{b})	0.33 (0.57^{ab})	0.24 (0.85^{ab})	0 (0.71)
PBE 7	0.30 (0.55^{j})	0.29 (0.53^{i})	0.25 (0.49^{f})	0.07 (0.25^{def})	0.0 (0.71^{d})	0 (0.71)
PBE 8	0.98 (0.98^{a})	0.78 (0.88^{a})	0.65 (0.80^{a})	0.44 (0.66^{a})	0.26 (0.86^{a})	0 (0.71)
PBE 9	0.62 (0.78^{d})	0.38 (0.61^{c})	0.22 (0.46^{g})	0.15 (0.38^{cd})	0.0 (0.71^{d})	0 (0.71)
PBE 10	0.96 (0.97^{a})	0.80 (0.89^{j})	0.40 (0.62^{c})	0.27 (0.51^{b})	0.0 (0.71^{d})	0 (0.71)
PBE 11	0.61 (0.77^{d})	0.56 (0.74^{bc})	0.16 (0.39^{ij})	0.11 (0.32^{d})	0.0 (0.71^{d})	0 (0.71)
PBE 12	0.21 (0.45^{k})	0.27 (0.51^{j})	0.19 (0.43^{h})	0.10 (0.30^{de})	0.0 (0.71^{d})	0 (0.71)
PBE 13	0.54 (0.73^{f})	0.45 (0.66^{e})	0.35 (0.58^{d})	0.25 (0.49^{bc})	0.0 (0.71^{d})	0 (0.71)
PBE 14	0.37 (0.60^{h})	0.24 (0.48^{k})	0.19 (0.43^{h})	0.13 (0.35^{d})	0.0 (0.71^{d})	0 (0.71)
PBE 15	0.66 (0.80^{c})	0.48 (0.68^{d})	0.39 (0.62^{c})	0.31 (0.55^{ab})	0.25 (0.78^{c})	0 (0.71)
PBE 16	0.54 (0.73^{f})	0.18 (0.41^{m})	0.14 (0.36^{kl})	0.02 (0.12^{g})	0.0 (0.71^{d})	0 (0.71)
PBE 17	0.45 (0.66^{g})	0.22 (0.45^{l})	0.16 (0.39^{ij})	0.12 (0.33^{d})	0.0 (0.71^{d})	0 (0.71)
PBE 18	0.35 (0.58^{i})	0.27 (0.51^{j})	0.19 (0.43^{h})	0.11 (0.35^{d})	0.0 (0.71^{d})	0 (0.71)
PBE 19	0.34 (0.58^{i})	0.23 (0.47^{k})	0.17 (0.40^{i})	0.14 (0.18^{fg})	0.0 (0.71^{d})	0 (0.71)
CD (P<0.05)	0.01	0.01	0.02	0.12	0.08	NS

Note : Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT). Transformed data presented in parenthesis; PBE = Pangong Bacterial Endophytes

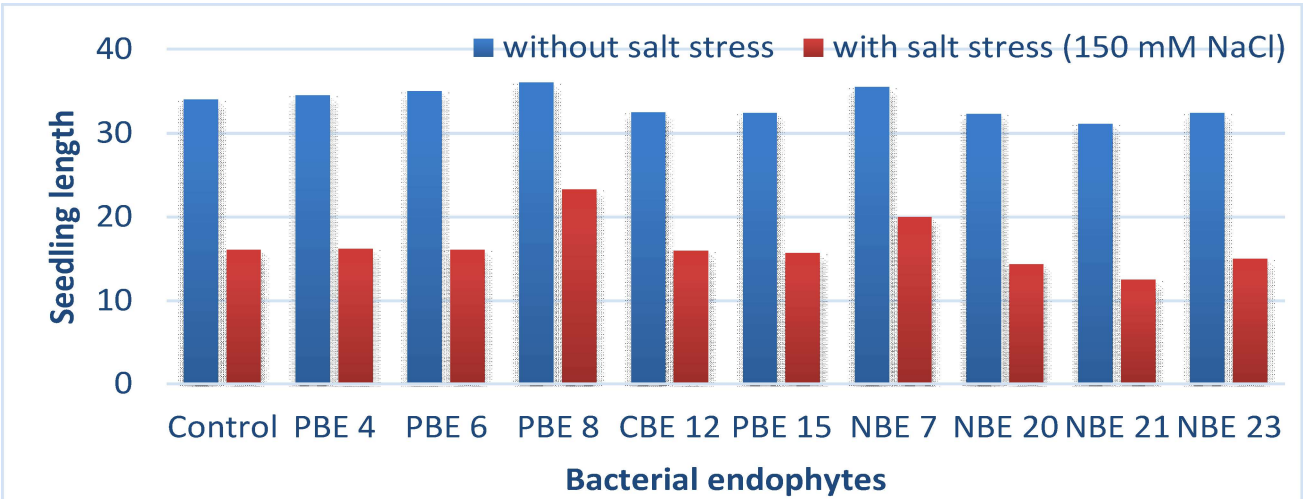

Fig. 1 : Effect of inoculation of bacterial endophytes on seedling length of rice (IR-64) with and without salinity stress (150 mM NaCl) after 14 days of germination

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TABLE 2

Effect of different concentration NaCl on growth of endophytic bacteria isolated from Chang La region plants

Bacterial isolates	Optical density at 600 nm indicating bacterial growth					
	Control (0.0 M)	0.5 M	1.0 M	1.5 M	2.0 M	2.5 M
CBE 1	0.49 (0.69^{i})	0.26 (0.50^{h})	0.20 (0.45^{i})	0.11 (0.77^{i})	0.0 (0.71^{b})	0 (0.71)
CBE 2	0.89 (0.94^{c})	0.79 (0.88^{c})	0.72 (0.84^{c})	0.36 (0.92^{d})	0.0 (0.71^{b})	0 (0.71)
CBE 3	0.95 (0.97^{a})	0.82 (0.90^{b})	0.65 (0.80^{d})	0.25 (0.86^{g})	0.0 (0.71^{b})	0 (0.71)
CBE 4	0.95 (0.97^{a})	0.86 (0.92^{a})	0.73 (0.85^{b})	0.33 (0.90^{e})	0.0 (0.71^{b})	0 (0.71)
CBE 5	0.86 (0.93^{d})	0.68 (0.82^{e})	0.16 (0.39^{k})	0.10 (0.77^{i})	0.0 (0.71^{b})	0 (0.71)
CBE 6	0.79 (0.88^{f})	0.50 (0.71^{g})	0.34 (0.57^{h})	0.25 (0.86^{g})	0.0 (0.71^{b})	0 (0.71)
CBE 7	0.85 (0.91^{e})	0.20 (0.45^{i})	0.19 (0.43^{j})	0.12 (0.78^{i})	0.0 (0.71^{b})	0 (0.71)
CBE 8	0.92 (0.95^{b})	0.85 (0.91^{ab})	0.71 (0.95^{b})	0.45 (0.97^{b})	0.0 (0.71^{b})	0 (0.71)
CBE 9	0.77 (0.87^{g})	0.68 (0.82^{e})	0.54 (0.73^{f})	0.30 (0.89^{f})	0.0 (0.71^{b})	0 (0.71)
CBE 10	0.93 (0.96^{b})	0.81 (0.89^{c})	0.65 (0.80^{d})	0.44 (0.96^{b})	0.0 (0.71^{b})	0 (0.71)
CBE 11	0.86 (0.92d^{e})	0.78 (0.88^{c})	0.74 (0.90^{a})	0.56 (1.02^{a})	0.0 (0.71^{b})	0 (0.71)
CBE 12	0.66 (0.80^{d})	0.52 (0.71^{g})	0.42 (0.64^{j})	0.39 (0.94^{c})	0.24 (0.85^{a})	0 (0.71)
CBE 13	0.87 (0.93^{d})	0.73 (0.85^{d})	0.59 (0.76^{e})	0.44 (0.96^{b})	0.0 (0.71^{b})	0 (0.71)
CBE 14	0.75 (0.86^{h})	0.61 (0.77^{f})	0.43 (0.65^{g})	0.21 (0.84^{h})	0.0 (0.71^{b})	0 (0.71)
CD (P<0.05)	0.01	0.01	0.01	0.01	0.09	NS

Note : Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT). Transformed data presented in parenthesis; CBE = Chang La Bacterial Endophytes

Molecular Identification of Bacterial Endophytes Using 16S rRNA gene Sequence

The genomic DNA of 2 best bacterial endophytes were isolated and 16S rRNA amplication was carried out and based on similarity percentage obtained from BLAST tool, phylogenetic tree was constructed using neighbor-joining method. Isolates PBE 8 and NBE 7 were identified as *Enterobacter hormaechei* and *Pseudomonas fluorescens* respectively Fig. 2 & Fig. 3.

Kumar *et al*. (2016) reported fourteen endophytic bacterial isolates from the rhizome of *Curcuma longa* L. and were characterized on the basis of

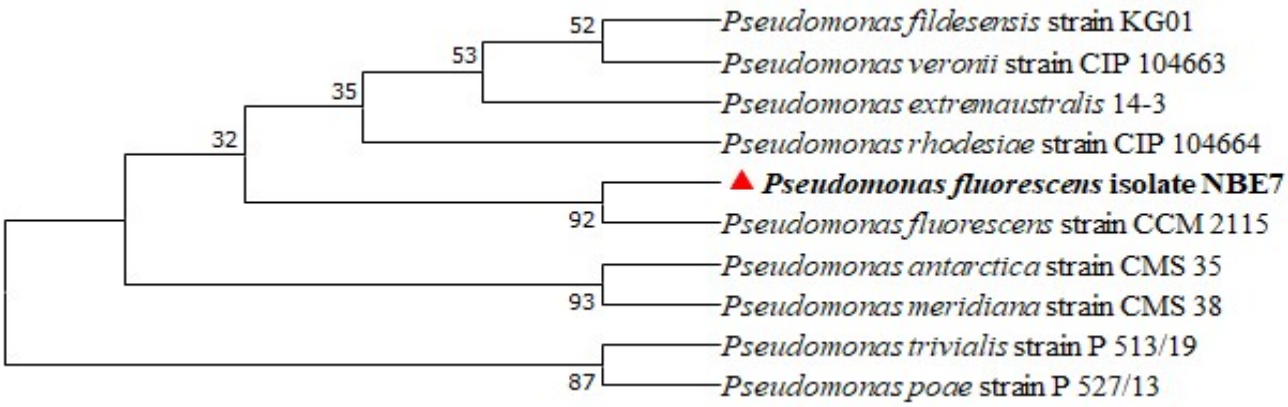


Fig. 2 : Phylogenetic tree of *Pseudomonas fluorescens* isolate NBE 7

TABLE 3

Effect of different concentration of NaCl on growth of endophytic bacteria isolated from Namika La region plants

Bacterial isolates	Optical density at 600 nm indicating bacterial growth					
	Control (0.0 M)	0.5 M	1.0 M	1.5 M	2.0 M	2.5 M
NBE 1	0.98 (0.98^{a})	0.88 (0.99^{a})	0.55 (0.73^{c})	0.39 (0.60^{e})	0.0 (0.71^{d})	0 (0.71)
NBE 2	0.66 (0.80^{f})	0.33 (0.57^{m})	0.22 (0.46^{mn})	0.13 (0.35^{k})	0.0 (0.71^{d})	0 (0.71)
NBE 3	0.32 (0.56^{opq})	0.24 (0.48^{q})	0.17 (0.40^{p})	0.11 (0.32l^{m})	0.0 (0.71^{d})	0 (0.71)
NBE 4	0.34 (0.57^{mn})	0.27 (0.51^{p})	0.22 (0.46^{mn})	0.19 (0.43^{j})	0.0 (0.71^{d})	0 (0.71)
NBE 5	0.94 (0.96^{b})	0.50 (0.71^{i})	0.35 (0.58^{h})	0.23 (0.47^{gh})	0.0 (0.71^{d})	0 (0.71)
NBE 6	0.89 (0.94^{d})	0.54 (0.73^{h})	0.33 (0.57^{i})	0.22 (0.46^{hi})	0.0 (0.71^{d})	0 (0.71)
NBE 7	0.88 (0.94^{d})	0.67 (0.81^{f})	0.50 (0.70^{d})	0.47 (0.68^{b})	0.26 (0.86^{b})	0 (0.71)
NBE 8	0.35 (0.58^{m})	0.28 (0.52^{o})	0.23 (0.47^{m})	0.18 (0.41^{j})	0.0 (0.71^{d})	0 (0.71)
NBE 9	0.45 (0.66^{k})	0.35 (0.58^{l})	0.25 (0.49^{l})	0.12 (0.33^{kl})	0.0 (0.71^{d})	0 (0.71)
NBE 10	0.38 (0.61^{l})	0.27 (0.51^{p})	0.14 (0.36^{r})	0.11 (0.32^{kl})	0.0 (0.71^{d})	0 (0.71)
NBE 11	0.33 (0.57^{nop})	0.24 (0.48^{q})	0.18 (0.41^{o})	0.10 (0.30^{lm})	0.0 (0.71^{d})	0 (0.71)
NBE 12	0.45 (0.66k)	0.30 (0.54^{n})	0.25 (0.49^{l})	0.12 (0.33^{kl})	0.0 (0.71^{d})	0 (0.71)
NBE 13	0.74 (0.86^{e})	0.60 (0.77^{g})	0.45 (0.66^{f})	0.24 (0.48^{fg})	0.0 (0.71^{d})	0 (0.71)
NBE 14	0.54 (0.73^{h})	0.37 (0.60^{k})	0.30 (0.55^{j})	0.21 (0.45^{i})	0.0 (0.71^{d})	0 (0.71)
NBE 15	0.30 (0.55^{q})	0.27 (0.51^{p})	0.20 (0.45^{n})	0.10 (0.32^{lm})	0.0 (0.71^{d})	0 (0.71)
NBE 16	0.53 (0.72^{hi})	0.33 (0.57^{m})	0.25 (0.49^{l})	0.12 (0.33^{kl})	0.0 (0.71^{d})	0 (0.71)
NBE 17	0.98 (0.98^{a})	0.78 (0.88^{e})	0.36 (0.59^{h})	0.29 (0.53^{e})	0.0 (0.71^{d})	0 (0.71)
NBE 18	0.48 (0.68^{j})	0.31 (0.55^{n})	0.27 (0.51^{k})	0.12 (0.33^{kl})	0.0 (0.71^{d})	0 (0.71)
NBE 19	0.52 (0.71^{i})	0.41 (0.63^{j})	0.30 (0.55^{j})	0.19 (0.43^{j})	0.0 (0.71^{d})	0 (0.71)
NBE 20	0.62 (0.78^{g})	0.55 (0.73^{h})	0.42 (0.64^{g})	0.35 (0.58^{d})	0.24 (0.85^{c})	0 (0.71)
NBE 21	0.91 (0.95^{c})	0.86 (0.95^{c})	0.68 (0.82^{a})	0.51 (0.71^{a})	0.26 (0.86^{b})	0 (0.71)
NBE 22	0.96 (0.98^{a})	0.78 (0.88^{e})	0.48 (0.68^{e})	0.22 (0.45^{hi})	0.0 (0.71^{d})	0 (0.71)
NBE 23	0.87 (0.93^{d})	0.69 (0.82^{f})	0.57 (0.75^{c})	0.35 (0.58^{d})	0.28 (0.88^{a})	0 (0.71)
NBE 24	0.92 (0.95^{c})	0.87 (0.97^{b})	0.55 (0.73^{c})	0.33 (0.57^{d})	0.0 (0.71^{d})	0 (0.71)
NBE 25	0.94 (0.97^{ab})	0.84 (0.91^{d})	0.65 (0.80^{bac})	0.25 (0.49^{f})	0.0 (0.71^{d})	0 (0.71)
CD (P<0.05)	0.01	0.02	0.02	0.02	0.03	NS

Note : Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT). Transformed data presented in parenthesis; NBE = Namika La Bacterial Endophytes

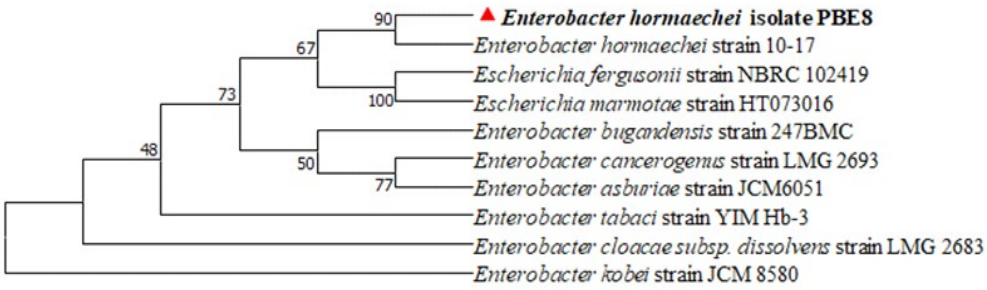


Fig. 3 : Phylogenetic tree of *Enterobacter hormaechei*isolate PBE8

morphology, biochemical characteristics and 16S rRNA gene sequence analysis. The isolates are of six strains *viz., Bacillus cereus* (ECL1), *Bacillus thuringiensis* (ECL2), *Bacillus* sp. (ECL3), *Bacillus pumilis* (ECL4), *Pseudomonas putida* (ECL5) and *Clavibacter michiganensis* (ECL6). Santhoshagowda and Earanna (2017) identified the *Gluconoacetobacter diazotrophicus* isolated from Maize using 16S rRNA gene sequence.

Characterization of Bacterial Endophytes Isolates for Plant Growth Promoting Traits

Bacterial endophytes were characterized for plant growth promotion like phosphate solubilization which is a primary element needed for plant growth and involved in normal development and maturity. Siderophores are minute, high affinity iron chelating compounds secreted by microorganism which help to chelate few elements essential for plants. Hydrogen cyanide indirectly promotes plant growth and plays an important role in pathogen suppression and ammonia production are useful for plant growth directly or indirectly. Both bacterial endophytes were able to produce HCN, siderophore productions, ammonia and phosphate solubilization. Result obtained are similar to Damodaran *et al.* (2013) isolated 16 rhizobacteria through natural selection from saline-sodic soils and characterized them using morphological and biochemical parameters. These bacteria were assessed for their plant growth-promoting rhizobacteria (PGPR) traits like indole 3-acetic acid (IAA) production, ammonia and hydrogen cyanide (HCN) production, phosphate solubilization etc.

Proline Production : Osmolytes concentration increases in plants during abioitic stress and are mainly involved in monitoring homeostasis of cellular contents in them. In the case of proline, maximum production was observed by both isolates (Fig. 4). Results obtained are like the results of Danish *et al.* (2020), where under severe salt stress the inoculation of *Pseudomonas aeruginosa* led to the proline accumulation of 15.97 µmol g^{-1} of fresh weight (F.W.) compared to the inoculation with *Enterobacter cloacae* (13.73 µmol g^{-1} of F.W.), *Achromobacter xylosoxidans* (12.95 µmol g^{-1} of F.W.) and *Leclercia adecarboxylata* (15.73 µmol g^{-1} of F.W.) in maize plants.

TABLE 4

Effect of different concentration of NaCl on seedling length of rice.

Treatments	Seedling length (cm)
Control	33.40[a]
25 mM	31.50[b]
50 mM	28.40[c]
75 mM	24.70[d]
100 mM	21.80[e]
125 mM	20.50[f]
150 mM	16.10[g]
175 mM	14.20[h]
200 mM	10.90[i]
CD (P<0.05)	0.45

Note :

1. LC_{50} value of rice seedling under salinity stress was found to be 150 mM by probit analysis.
2. Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT)

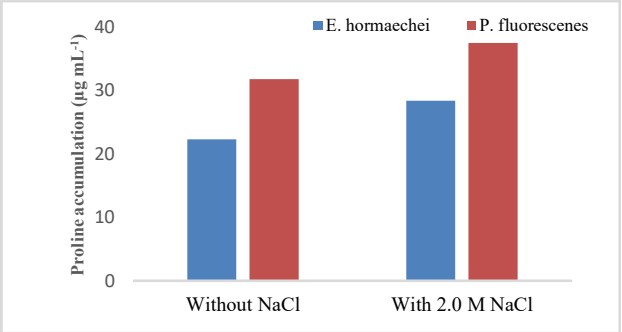


Fig. 4 : Proline accumulation of salinity tolerant bacterial endophytes without and with stress induced conditions

Quantification of IAA, Gibberellic Acid (GA), Abscisic acid (ABA) and Salicylic Acid (SA) using HPLC

Phytohormones play a vital role in development of plants. Quantification of IAA, gibberellic acid, abscisic acid and salicylic acid production by selected 2 endophytic bacterial isolates through high performance liquid chromatography (HPLC) is presented in Fig. 5

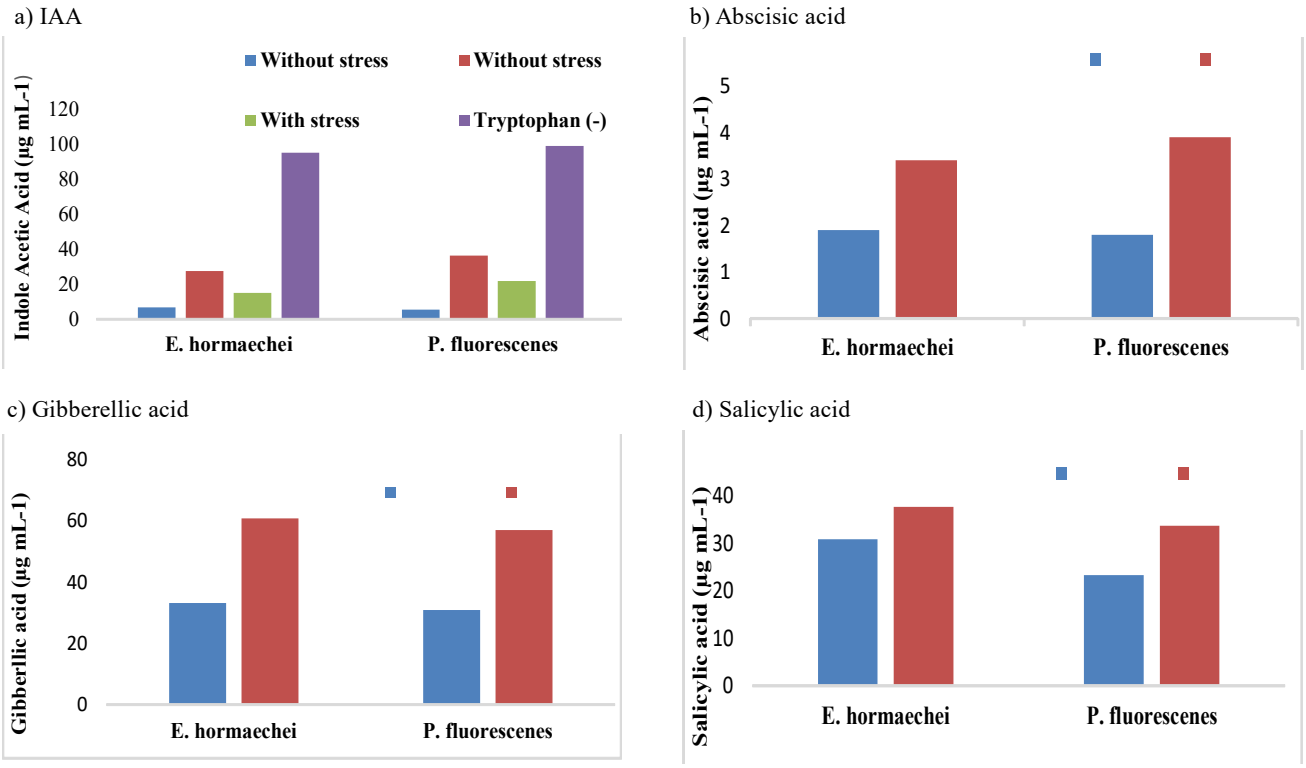
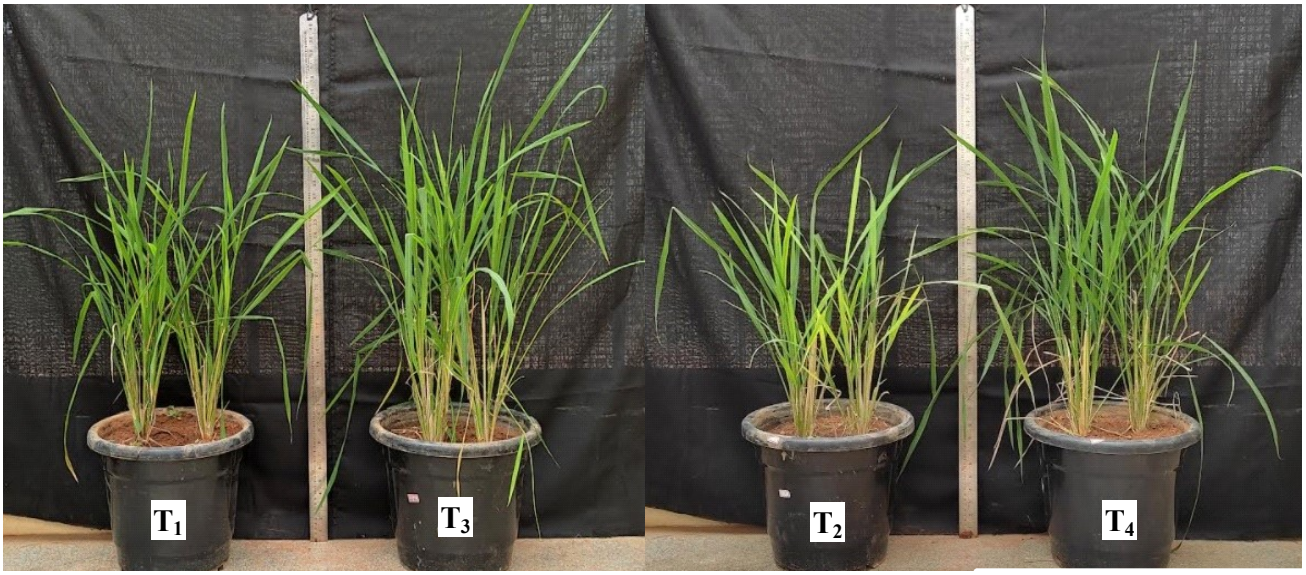


Fig. 5 : IAA, Gibberellic acid, Abscisic acid and Salicylic acid production by endophytic bacterial isolates

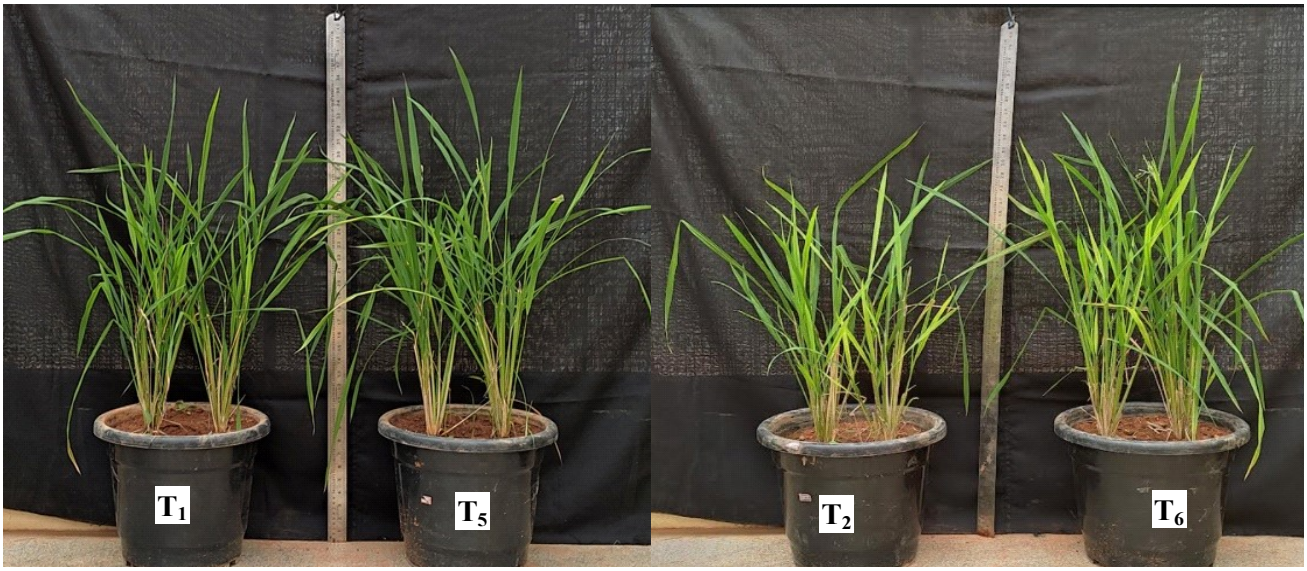
Indole-3-acetic acid being most common type of auxin, it regulates various aspects of plant development and growth. Gibberellic acid also helps plant in stimulating cell division and elongation. The plant can withstand stresses by producing glyoxalase I and II, which reduces the methylglyoxal concentration and there by plant could withstand against abiotic stresses (Moumita *et al.,* 2019). Abscisic acid and salicylic acid also regulated abiotic stress by stomatal closure and lowering transpiration water loss and inducing acquired resistance in plants against diseases. In this study, bacterial endophytes grown under abiotic (salt and drought) stress condition showed the highest production of GA, ABA and SA compared to the endophytes not exposed to abiotic stress. The results obtained are in agreement with Goswami *et al*. (2014) reported bacteria *Kocuria turfanensis* 2M4, that was found to be dependent on L-tryptophan for producing IAA and could produce 38 µg mL^{-1} of IAA in the presence of 600 µg mL^{-1} of tryptophan. Similarly with production of gibberellic acid (0.108 mg mL^{-1}) by *Bacillus siamensis* BE 76 isolated from the banana plant (*Musa* spp.) as reported by Amawade and Pathade (2015). Shahzad *et al*. (2017) reported that the production of varying concentration of ABA (0.32 ± 0.015 to 0.14 ± 0.030 ng mL^{-1}) under normal and saline condition by the bacterial endophyte *Bacillus amyloliquefaciens* RWL^{-1} was noticed.

Evaluation of Endophytes Treated Rice Plants under Greenhouse Conditions

Two bacteria *viz., E. hormaechei* and *P. fluorescens* were evaluated for growth, yield, physiological and biochemical parameters in rice at different intervals under normal as well as salt stress (4 dS/m). Plants treated with *E. hormaechei* showed significant increase in plant height and leaf numbers followed by *P. fluorescens* compared to uninoculated plants. Number of tillers at 60, 90 and 120 DAT significantly enhanced due to *E. hormaechei* inoculation followed by *P. fluorescens* (Fig. 6; Plate 1). The increased growth parameters in endophyte inoculated plants under salt stress was due to production of auxins, gibberellin, abscisic acid which helped in maintaining cell division and cell elongation in plants (Khan



Control | *E. horm aechei* | Salt stress (4ds/m) | Salt stress (4 ds/m)+ *E. horm aechei*



Control | *P. fluorescens* | Salt stress (4ds/m) | Salt stress (4 ds/m)+ *p. fluorescens*

Plate 1 : Effect of bacterial endophytes *E. hormaechei* and *P. fluorescens* on growth of rice under normal and salt stress (4 dS/m) conditions at 60 DAT

et al., 2012). The *E. hormaechei* and *P. fluorescens* inoculated plants showed significantly increased number of panicles, number of seeds and seed yield under salt stress as compared to uninoculated plants (Table 5). The photosynthetic pigments (Chl a, b, total chl and carotenoid) content significantly increased in *E. hormaechei* inoculated plants (Table 6). The endophytes under stress increased the chlorophyll synthesis by decreasing the levels of ABA and ROS (Jogawat *et al.,* 2013). Endophyte treated plants had a marginal increase in Relative Water Content (RWC) levels under normal conditions but under salt stress, higher RWC was maintained by protecting membrane integrity due to endophyte inoculation. The membrane integrity is expressed in terms of electrolyte leakage which was found to be less in endophyte treated plants

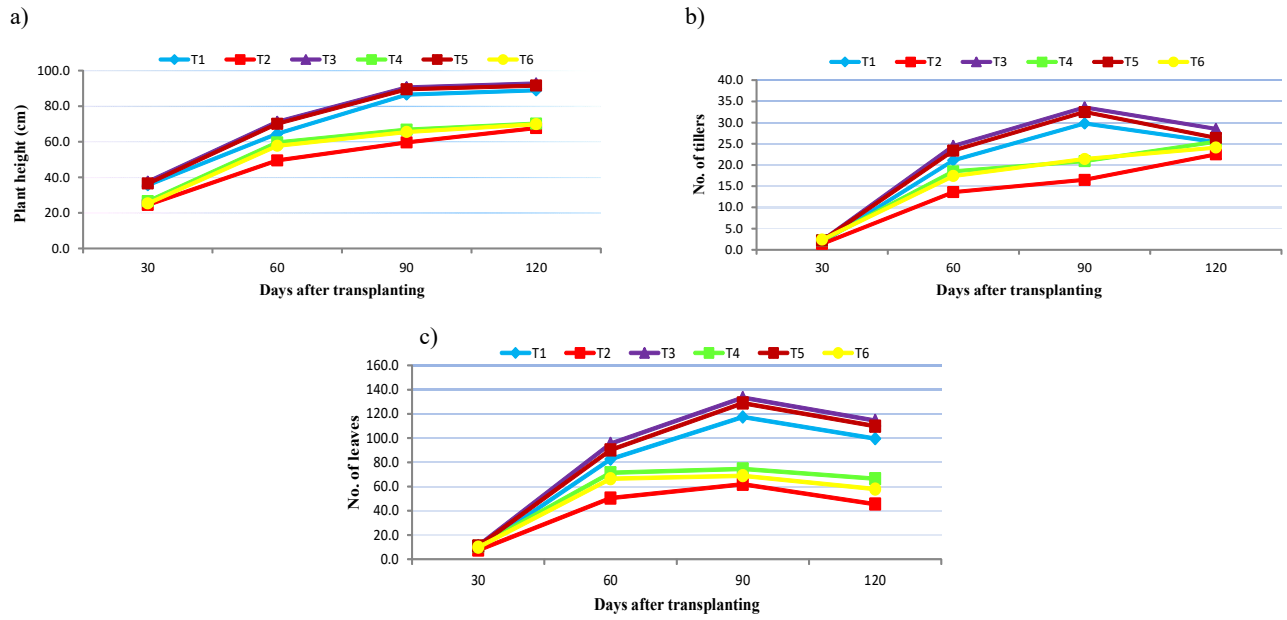


Fig. 6 : Effect of bacterial endophytes on growth parameters (a) Plant height (b) No. of tillers (c) No. of leaves of rice under salinity stress (4 dS/m).

Note : T1= Control, T2 = Salt stress (4 dS/m), T3= *E. hormaechei*, T4= Salt stress (4 dS/m) + *E. hormaechei*, T5= *P. fluorescens*, T6= Salt stress (4 dS/m)+*P. fluorescens*

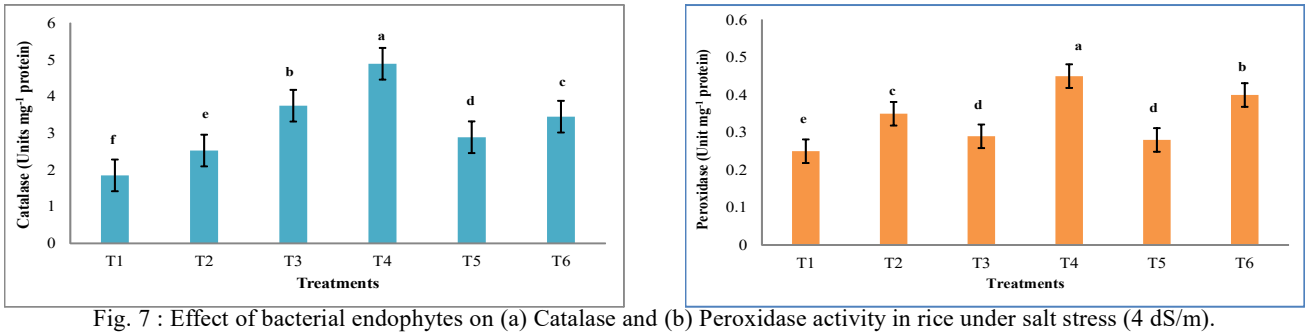


Fig. 7 : Effect of bacterial endophytes on (a) Catalase and (b) Peroxidase activity in rice under salt stress (4 dS/m).

Note : T1= Control, T2 = Salt stress (4 dS/m), T3= *E. hormaechei*, T4= Salt stress (4 dS/m) + *E. hormaechei*, T5= *P. fluorescens*, T6= Salt stress (4 dS/m)+*P. fluorescens*

TABLE 5

Effect of bacterial endophytes on yield parameters of rice under salinity stress (4 dS/m).

Treatments	No. of panicles	No. of seeds/ plant	Seed yield (g)/ plant
T_1= Control	10.40^{c}	575.50^{c}	13.60^{b}
T_2 = Salt stress (4 dS/m)	6.60^{e}	272.20^{f}	5.64^{d}
T_3= *E. hormaechei*	12.20^{a}	796.60^{a}	17.50^{a}
T_4= Salt stress (4 dS/m) + *E. hormaechei*	8.70^{d}	415.20^{d}	8.55^{c}
T_5= *P. fluorescens*	11.60^{b}	734.50^{b}	17.40^{a}
T_6= Salt stress (4 dS/m) + *P. fluorescens*	8.60^{d}	400.50^{e}	8.26^{c}
CD (P<0.05)	0.33	12.74	0.31

Note : Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT)

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TABLE 6

Effect of bacterial endophytes on physiological parameters of rice under salinity stress (4 dS/m).

Treatments	Chl a (mg/ g FW)	Chl b (mg/gFW)	Total chl (mg/g FW)	Carotenoid content (mg/g FW)	RWC (%)	Proline content (µmol/gFW)	Electrolyte leakage (%)
T_1 = Control	0.76 ^{b}	0.34 ^{a}	0.93 ^{a}	0.35 ^{a}	88.55 ^{b}	7.30 ^{c}	34.52 ^{d}
T_2 = Salt stress (4 dS/m)	0.56 ^{d}	0.26 ^{b}	0.74 ^{c}	0.28 ^{b}	70.50 ^{d}	11.20 ^{b}	55.55 ^{a}
T_3 = *E. hormaechei*	0.82 ^{a}	0.35 ^{a}	0.94 ^{a}	0.35 ^{a}	89.40 ^{a}	7.38 ^{c}	32.15 ^{f}
T_4 = Salt stress (4 dS/m) + *E. hormaechei*	0.65 ^{c}	0.33 ^{a}	0.83 ^{b}	0.34 ^{a}	83.90 ^{c}	11.94 ^{a}	38.31 ^{c}
T_5 = *P. fluorescens*	0.75 ^{b}	0.35 ^{a}	0.94 ^{a}	0.33 ^{a}	88.57 ^{b}	7.33 ^{c}	34.11 ^{e}
T_6 = Salt stress (4 dS/m) + *P. fluorescens*	0.64 ^{c}	0.35 ^{a}	0.75 ^{c}	0.33 ^{a}	83.65 ^{c}	11.95 ^{a}	39.47 ^{b}
CD (P<0.05)	0.04	0.04	0.04	0.04	0.35	0.22	0.25

Note : Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT)

compared to control under stress (Asaf *et al.,* 2018). Among the bacterial endophytes, the *E. hormaechei* treated plants had less electrolyte leakage. The proline is known to scavenge the reactive oxygen species thereby preventing cell damage (Khan *et al.,* 2012). The proline content was increased in endophytes treated plants. The antioxidant enzymes like catalase and peroxidase activity were higher in *E. hormaechei* treated plants followed by P. fluorescens under salt stress as compared to control (Fig. 7). The increased enzymatic activity indicates effective scavenging of H_2O_2 by endophyte under salt stress (Sewelam *et al.,* 2016). The above results are in agreement with Nautiyal *et al.* (2013) who reported similar activities of *Bacillus amyloliquefaciens* in rice.

Therefore, presence of inoculated bacterial endophytes within inoculated plants were confirmed through re-isolation. Hence, this study suggests the inoculation of endophytes is necessary to confer salinity tolerance. Further more, it can be conferred that out of two bacterial endophytes, *Enterobacter hormaechei* collected from harsh condition presented superior salinity tolerance to Rice plant under salt stress and these findings can be explored in other agricultural crops.

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