BIOPROSPECTING OF BACTERIAL ENDOPHYTES ISOLATED FROM HIMALAYAN COLD DESERT FOR ABIOTIC STRESS TOLERANCE IN PLANTS

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Affectionately Dedicated To

My Parents,

Dr. Imthyaz Ahmed Khan

And

Nusrath Jabeen Khanum

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
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BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "**BIOPROSPECTING OF BACTERIAL ENDOPHYTES ISOLATED FROM HIMALAYAN COLD DESERT FOR ABIOTIC STRESS TOLERANCE IN PLANTS**" submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in AGRICULTURAL MICROBIOLOGY** to the University of Agricultural Sciences, Bangalore is a record of *bona fide* research work carried out by **Ms. TASMIYA IMTIYAZ, PALB 8039** during the period of her study in this University, under my guidance and supervision. This thesis has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar titles.

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BIOPROSPECTING OF BACTERIAL ENDOPHYTES ISOLATED FROM HIMALAYAN COLD DESERT FOR ABIOTIC STRESS TOLERANCE IN PLANTS

TASMIYA IMTIYAZ

ABSTRACT

Symbiotically conferred abiotic stress tolerance by endophytes can provide fitness to plants. In this study, 58 bacterial endophytes isolated from plants growing at Himalayan cold desert were explored for induction of drought and salt stress tolerance in rice (IR-64) and tomato (Arka Saurabh) using NaCl and Polyethylene glycol PEG-8000) at different concentrations. Of which, nine bacteria showed growth up to 2.0 M NaCl and eight bacteria up to 20% PEG. As per probit analysis, the LC_{50} value of NaCl (Salinity) for rice was 150 mM and 117mM for tomato. The LC_{50} value of PEG for drought was 14.3 % for rice and 15.3% for tomato. These bacteria when inoculated to pre-germinated rice and tomato seeds and grown in paper towel treated with 150 Mm and 117 mM NaCl, the isolates NBE 20 and NBE 23 showed increased growth of tomato seedlings and PBE 8 and NBE 7 showed increased growth of rice seedlings. Under drought (20% PEG) the isolates PBE 2, PBE 4 and CBE 11 showed better growth of rice seedlings and PBE 6, CBE 11 and NBE 5 showed better growth of tomato seedlings. These bacteria were identified as *Bacillus cereus, Pseudomonas chlororaphis, Lysinibacillus macroides, Enterobacter hormaechei, Stenotrophomonas maltophilia, Acinetobacter lwoffii, Pseudomonas fluorescenes, Enterobacter cloacae* and *Enterobacter asburiae* by 16S rRNA sequences and further evaluated under greenhouse conditions. The endophytes inoculated rice and tomato plants showed increased growth, yield and physiological traits. Among the 9 selected bacteria, the *E. hormaechei* and *E. asburiae* were efficient in mitigating salt stress (4 dS/m) and *S. maltophilia* and *A. lwoffii* were efficient in mitigating drought stress (50% FC) in rice and tomato crops respectively. This study revealed that the selected endophytes can mitigate salinity and drought stress in rice and tomato.

May 2022

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Major Advisor

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ಸಸ್ಯಗಳಲ್ಲಿ ಅಜೀವಿಕ ಒತ್ತಡಗಳ ಸಹಿಷ್ಣುತೆ ನೀಡುವ ಹಿಮಾಲಯ ಶೀತ ಮರುಭೂಮಿ ಸಸ್ಯಗಳಿಂದ ಬೇರ್ಪಡಿಸಿದ ಅಂತರ್ಜೀವಿ ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳ ಪರಿಶೋಧನೆ

ತಸ್ಮಿಯಾ ಇಮ್ತಿಯಾಜ್

ಸಾರಾಂಶ

ಸಸ್ಯಕೋಶ ಅಥವಾ ಅಂಗಾಂಶಗಳಲ್ಲಿ ಸಾಂಘಿಕವಾಗಿ ಬದುಕುವ ಸೂಕ್ಷ್ಮಜೀವಿಗಳಿಗೆ ಸಸ್ಯಂತರ್ಜೀವಿಗಳೆಂದು ಕರೆಯಲಾಗುತ್ತದೆ. ಸಸ್ಯಗಳು ಅಂತರ್ಜೀವಿಗಳ ಮುಂಖಾಂತರ ಅನೇಕ ಅಜೀವಿಕ ಒತ್ತಡಗಳನ್ನು ನಿಯಂತ್ರಿಸಬಲ್ಲವು. ಈ ಅಧ್ಯಯನದಲ್ಲಿ, ಭತ್ತ ಮತ್ತು ಟೊಮ್ಯಾಟೋ ಸಸ್ಯಗಳು ಅಂತರ್ಜೀವಿ ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳಿಂದ ಅಜೀವಿಕ ಒತ್ತಡಗಳಾದ ಅಧಿಕ ಲವಣ ಮತ್ತು ಶುಷ್ಕ ಪರಿಸರದ ಒತ್ತಡಗಳನ್ನು ಹೇಗೆ ನಿಯಂತ್ರಸಬಲ್ಲವೆಂದು ತಿಳಿಯಲಾಯಿತು. ವಿವಿಧ ಸಾಂದ್ರತೆಯ ಲವಣ (ಉಷ್ಣ) ಮತ್ತು ಪಾಲಿಇಥಿಲಿನ್ ದ್ರಾವಣಗಳುಳ್ಳ ಮಾಧ್ಯಮದಲ್ಲಿ 58 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳ ಬೆಳವಣಿಗೆಯನ್ನು ಪರೀಕ್ಷಿಸಲಾಯಿತು. ಅವುಗಳಲ್ಲಿ 9 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳು 2 M ಸಾಂದ್ರತೆಯ ಲವಣ (NaCl) ದಲ್ಲಿ ಹಾಗೂ 8 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳು ಶೇ 20ರ ಶುಷ್ಕತೆಯಲ್ಲಿ ಬೆಳೆದಿದ್ದು ಕಂಡು ಬಂದಿತು. ಹಾಗೆಯೇ, ಭತ್ತ ಮತ್ತು ಟೊಮ್ಯಾಟೋ ಸಸಿಗಳ ಲವಣ ಮತ್ತು ಶುಷ್ಕ ಸಹಿಷ್ಣುತೆಯ ಮೌಲ್ಯಗಳನ್ನು ಪ್ರೋಬಿಟ್ ವಿಶ್ಲೇಷಣೆಯಿಂದ ಪರೀಕ್ಷಿಸಿದಾಗ, ಭತ್ತಕ್ಕೆ 150 mM ಹಾಗೂ (LC_{50} value) ಟೊಮ್ಯಾಟೋ ಸಸಿಗೆ 117 mM (LC_{50} value) ಎಂದು ತಿಳಿಯಲಾಯಿತು. ಇದರ ಆಧಾರದ ಮೇಲೆ 9 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳನ್ನು ಭತ್ತ ಮತ್ತು ಟೊಮ್ಯಾಟೋ ಸಸಿಗೆ ಉಪಚರಿಸಿ ಕ್ರಮವಾಗಿ 150 mM ಹಾಗೂ 117 mM ಸಾಂದ್ರತೆಯಲ್ಲಿ ಬೆಳೆಸಿದಾಗ, ಅವುಗಳಲ್ಲಿ (NBE-20 ಮತ್ತು NBE-23) ಮಾತ್ರ ಭತ್ತಕ್ಕೆ ಲವಣ ಒತ್ತಡ ಸಹಿಷ್ಣುತೆ ನೀಡಬಲ್ಲವೆಂದು ಹಾಗೂ PBE-8 ಮತ್ತು NBE–7 ಗಳು ಟೊಮ್ಯೋಟೋ ಸಸಿಗಳಿಗೆ ಲವಣ ಒತ್ತಡ ಸಹಿಷ್ಣುತೆ ನೀಡಬಲ್ಲವೆಂದು ಕಂಡುಬಂದಿತು. ಅದೇ ರೀತಿ ಶುಷ್ಕ ಪರಿಸ್ಥಿತಿಯಲ್ಲಿ PBE-2, PBE-4 ಮತ್ತು CBE-11 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳು ಭತ್ತದ ಸಸಿಗಳ ಬೆಳವಣಿಗೆಯನ್ನು ಹೆಚ್ಚಿಸಿರುತ್ತವೆ. ಹಾಗೆಯೇ, PBE-6, CBE-11 ಮತ್ತು NBE–5 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳು ಟೊಮ್ಯಾಟೋ ಸಸಿಗಳ ಬೆಳವಣಿಗೆಯನ್ನು ಹೆಚ್ಚಿಸಿರುವುದು ಕಂಡು ಬಂದಿತು. ಈ 9 ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳು ಯಾವುವೆಂದು 16 S rRNA ಅನುಕ್ರಮದ ಆಧಾರದ ಮೇಲೆ ಕಂಡು ಹಿಡಿಯಲಾಯಿತು. ನಂತರ ಈ ಅಂತರ್ಜೀವಿಗಳನ್ನು ಭತ್ತ ಮತ್ತು ಟೊಮ್ಯೋಟೋ ಮೊಳಕೆ ಬೀಜಗಳಿಗೆ ಉಪಚರಿಸಿ, ಪರಿಶೋಧಿಸಿದಾಗ, ಅಂತರ್ಜೀವಿಗಳನ್ನು ಉಪಚರಿಸಿದ ಸಸ್ಯಗಳಲ್ಲಿ ಬೆಳವಣಿಗೆ, ಇಳುವರಿ ಮತ್ತು ಸಸ್ಯ ಶರೀರದ ಕ್ರಿಯಾ ಗುಣಗಳು ಉತ್ತಮವಾಗಿದ್ದು ಅವುಗಳಲ್ಲಿ *ಎಂಟಿರೋ ಬ್ಯಾಕ್ಟರ್ ಹಾರ್ಮೋಣಿ* ಮತ್ತು *ಎಂಟಿರೋ ಬ್ಯಾಕ್ಟರ್ ಅಬ್ಸುರಿ* ಗಳು ಅಧಿಕ ಲವಣ ಒತ್ತಡ ನಿರೋಧಿಸುವಲ್ಲಿ ಹೆಚ್ಚು ಪರಿಣಾಮಕಾರಿಗಳೆಂದು ಹಾಗೂ ಶುಷ್ಕ ಪರಿಸ್ಥಿತಿಯನ್ನು ನಿರೋಧಿಸಲು *ಸ್ಟೀನೋಸ್ಟೋಫೋಮೋನಾಸ್ ಮಾಲ್ಟೋಫಿಲಿಯಾ* ಮತ್ತು *ಅಸಿನಿಟೋಬ್ಯಾಕ್ಟರ್ ಲ್ವೋಫಿ* ಹೆಚ್ಚು ಪರಿಣಾಮಕಾರಿಯೆಂದು ತಿಳಿದು ಬಂದಿದೆ. ಈ ಅಧ್ಯಯನವು, ಅಂತರ್ಜೀವಿ ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳನ್ನು ಬಳಸಿಕೊಂಡು ಭತ್ತ ಮತ್ತು ಟೊಮ್ಯಾಟೋ ಬೆಳೆಗಳಲ್ಲಿ ಅಧಿಕ ಲವಣ ಮತ್ತು ಶುಷ್ಕ ಪರಿಸ್ಥಿತಿಯನ್ನು ಪ್ರತಿರೋಧಿಸಬಹುದೆಂದು ಸಾದರಪಡಿಸಿದೆ.

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I INTRODUCTION

Abiotic stress refers to the negative pressure created by non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond the normal range which adversely affect the population performance or individual physiology of the organism in a significant way. Abiotic stresses have significant impact on agricultural yield and plant species dispersion in the environment. Drought, increased temperature, salt and chemical toxicity have negative impact on crop productivity, resulting in more than 50% yield loss (Wang and Frei, 2011). These stressors are predicted to be more common in future decades owing to global climate change (Battisti and Naylor, 2009). The abiotic stresses often cause a series of morphological, physiological, biochemical, and molecular changes that adversely affect the growth and yield of crops.

Among environmental stresses, drought and salinity are the key abiotic stresses drastically affect the agricultural productivity. According to the FAO (Food and Agriculture Organization), salinity affects more than 500-800 million hectares (ha), or around 6% of the world's arable land. Out of 1500 million hectares planted in arid regions, 2% of farmland is damaged by salt (Munns, 2005). Soil salinity changes the physical qualities of the soil, affecting its health and rendering it unfit for cultivation. Soil salinity is primarily caused by the effects of primary and secondary salinization. Primary salinization occurs naturally in soils and water resulting due to prolonged crop cultivation, poor cultural practises, low-quality irrigation water, increased evaporation rate, low precipitation, and decreased soil water infiltration (Manchanda and Garg, 2008). Human activities, such as land development, cause secondary salinization (Nimir *et al*., 2015). It also disables photosynthetic and respiratory electron transfer. Increased ion stress has a physiological and biochemical impact on cellular processes, leading to cell death.

Drought is one of the most severe and emerging abiotic stresses that affects growth and productivity of plants *via* affecting several physiological and metabolic processes in crop plants. It has drastic impact on root physiology, leaf structure, nutrient uptake, photosynthetic activity and seedling germination, resulting in overall decreased growth of

agricultural crops. Drought stress hampers the seed germination and seedling establishment significantly owing to limited water intake, insufficient energy supply, and hampered enzyme functioning. Plant growth initiating from vegetative stage to reproductive stage is shifted prematurely, which affect agricultural phenology and shortening crop growth cycles. Furthermore, drought has a considerable impact on leaf relative water content (RWC), water potential, osmotic potential, pressure potential and transpiration rate, resulting in reduced agricultural throughput. In addition, the drought stress causes generation of reactive oxygen species such as superoxide (O_2^-), singlet oxygen, and hydroxyl radicals (OH). Increased production of reactive oxygen species (ROS) impairs electron transport pathways in mitochondria and chloroplasts. Plants fight against these detrimental impacts by activating biochemical and metabolic pathways such as osmolyte accumulation, hormone control, ion homeostasis maintenance, and enhanced ROS (Reactive Oxygen Species) activity scavengers (Golldack *et al.,* 2011).

Rice is a major cereal crop in India and stands first in its cultivation area of about 43.86 million hectares and more than 50 % of world's population depends on rice as staple food. Similarly, Tomato (*Solanum lycopersicum*) is an extensively cultivated vegetable crop standing second among the largest cultivated crops in India, covering 53.40 million ha. Both the crops are sensitive to drought and salinity stresses (Negrao *et al.*, 2011).

The investigations on imparting abiotic stress tolerance in crop plants assumes a greater challenges in agriculture. The development of resistant varieties to cope up with environmental stresses through breeding methods and molecular tools is often uncertain, time consuming and costly affair. Therefore, the endophyte mediated stress tolerance in crop plants can assure sustainable agriculture against environmental stresses. Endophytes have developed two mechanisms to confront abiotic stresses (1) by activating response system of the host on exposure to stress, in order to circumvent impact caused by the stress (2) by producing anti-stress metabolites (Meena *et al.,* 2017). Therefore, to ease the encounters of abiotic stress and their influence on growth, yield and throughput of plants, application of beneficial microbial strains is the utmost viable, consistent, and sustainable option

Endophytes are microorganisms (bacteria as well as fungi) which infiltrate plants without creating disease symptoms. They inhabit practically every part of the plant, including the leaves, stems, roots, flowers, and fruits. They are found in a broad variety of plants, from grasses to higher order plants. They live symbiotically inside plant tissue and could impart stress tolerance by various mechanisms. Many of them create vital biochemical components that aid in the defence of plants against illnesses and insect assault (Abutana *et al.,* 2015). Microorganisms live inside the host and play a crucial role in plant growth, development and protect their hosts from various biotic and abiotic stresses. The nature of endophytic partnerships might be commensal, parasitic, or mutualistic. Endophytes are extensively exploited for plant growth promotion and imparting stress tolerance when plants are exposed to a variety of abiotic stresses. Bacterial endophytes can confer benefit to plant fitness including increased biomass (root and shoot), yield and tolerance to abiotic stresses such as heat, drought and salt. The most recently issued reports emphasize application of endophytic strains in the management of abiotic stress. Several endophytic bacterial strains isolated from various crops in which most of the strains conferred drought tolerance up to (−1.02) matric potential also had growth promotion potential (Sandhya *et al.,* 2017). Chen *et al*. (2017) reported endophytic strain *Pantoea alhagi* isolated from *Alhagi sparsifolia* enhanced the growth of wheat seedlings under drought conditions after inoculation. Additionally, the endophyte-treated plant showed enhanced accumulation of soluble sugars and decreased malondialdehyde concentrations.

In this context, microbial endophytes clearly give an impression to be a suitable substitute for drought as well as salinity stress management. Moreover, plants growing in harsh environment like Himalayan cold deserts might have the possibility of inhabiting stress tolerant endophytes as Western Himalayan cold deserts have extremes of hot and cold climate combined with excessive dryness. Soil is light grey, poor in fertility and has less water holding capacity and bacteria associated with the plants growing in such harsh environment have habitat adapted symbiosis which can be exploited appropriately for mitigating abiotic stresses in crop plants. Therefore, the present study was intended to explore the drought and salinity tolerant bacterial endophytes for mitigating abiotic stress

tolerance in rice (IR-64) and tomato (Arka Saurabh). The objectives of the study were as follows.

1) Screening of bacterial endophytes for abiotic stress tolerance under *in-vitro*.
2) Identification/Characterization of efficient bacterial endophytes.
3) Evaluation of selected bacterial endophytes for their ability to impart abiotic stress tolerance in rice and tomato under greenhouse condition.

II REVIEW OF LITERATURE

The abiotic stresses include salinity, drought, extreme temperatures (cold and heat), flooding and freezing, excess light, UV radiation and heavy metal toxicity. These stresses often cause a series of morphological, physiological, biochemical and molecular changes that adversely affect the yield of crops. The endophytes (bacteria and fungi) live inside plant tissue could impart stress tolerance by various mechanisms. Literature pertaining to endophyte mediated abiotic stress tolerance is reviewed and presented in this chapter.

2.1 Endophytes

Anton de Bary in 1866 coined the term "endophyte" referring to the microorganism that live symbiotically inside the plant. Vogl in 1898 observed a thread like structure (mycelium) in the grass *Lolium temulentum.* In 1904, Freeman found an endophytic fungus in Persian darnel (annual grass). Perotti (1926) described advanced stage of endophyte infection as mutualistic symbiosis. Endophyte is a microorganism that infiltrate into the plant without producing disease symptoms (Wilson, 1995).

Endophytes colonize the interior parts of the plant by entering the host plant through horizontal (from the environment) or vertical transmission (*via* seeds). On the basis of evolutionary relatedness, taxonomy, host plant and ecological niche, endophytes are divided into two major groups: (i) Clavicipitaceous or class I endophytes which inhabit some grasses and (ii) Non clavicipitaceous or class II endophytes which are associated in the asymptomatic tissues of non-vascular plants such as ferns, allies, conifers and angiosperms (Harman, 2000).

In endophyte-host interaction the minimum contribution of host is providing nutrition. However, the plant may also provide compounds critical for the completion of their life cycle (Metz *et al*., 2000). In return endophytes have been shown to play a significant role in survival of plants at high stressful environments (Rodriguez *et al*., 2004). Endophytes seem to enhance crop yield, inhibit pathogens, remove contaminant, and produce novel substances (Rosenbleuth and Romero, 2006).

Aravind *et al.* (2009) isolated and evaluated endophytic bacteria against the plant pathogen *Phytophthora capsici* in black pepper. Three isolates, IISRBP 35, IISRBP 25 and IISRBP 17 were found to be more effective against *Phytophthora* suppression in multilevel screening assays which recorded over 70 % disease suppression in greenhouse trials. These endophytic bacteria were identified as effective antagonistic endophytes for biological control of *Phytophthora* foot rot disease in black pepper. The biocontrol efficiency of Rhizosphere and endophytic bacterial isolates (roots and corms of banana) against Banana bunchy top virus (BBTV) was explained by Harish *et al.* (2009). Bio-formulations of mixtures of the rhizobacterial isolate *Pseudomonas fluorescens* (Pf1) and endophytic *Bacillus* sp. (EPB22) were effective in reducing the incidence of BBTV under green-house (80%) as well as in field conditions (52%).

Pereira *et al*. (2011) investigated bacterial diversity associated with the roots of maize using culture-dependent and culture independent methods and showed that the γ-Proteobacteria comprising the genera *Enterobacter*, *Erwinia*, *Klebsiella*, *Pseudomonas*, and *Stenotrophomonas* as predominant groups.

Kaul *et al*. (2013) isolated endophytes from the symptomless leaves and stem of the angiosperm, *Digitalis lanata* (Foxglove), an important medicinal plant known for the production of glycoside, digoxin which has medicinal importance. Results showed that these microbial endophytes, mimic the bioactive compounds produced by the plant itself and making them a promising source of novel compounds.

Dawwam *et al*. (2013) studied the beneficial effects of plant growth promoting bacteria *Bacillus cereus* and *Achromobacter xylosoxidans* isolated from roots of potato. The results of inoculated plants showed significant differences in vegetative growth parameters, photosynthetic pigments and N, P, and K concentrations compared to control. Hassan (2017) characterized two bacterial endophytes (*Bacillus cereus* and *Bacillus subtilis*) and two fungal endophytes (*Penicillium chrysogenum* and *Penicillium crustosum*) from *Teucrium polium* for plant growth-promotion.

Bacterial endophytes ubiquitously colonize internal tissues of plants and found nearly in every plant worldwide. Some endophytes are able to promote the growth of plants similar to the rhizospheric plant growth promoting bacteria. These endophytic plant growth-promoting bacteria can facilitate plant growth in agriculture, horticulture and silviculture crops beside cleaning up the environment (Santoyo *et al.,* 2016).

Sandhu *et al*. (2017) studied the endophytic microorganisms like bacteria, actinomycetes, and fungi which play an important role in production of novel secondary metabolites for defence of host which can be utilized for treatment of a number of ailments. The bacterial endophytes are the manufacturer of bioactive compounds which can be widely used in agricultural, medical and industrial application.

Riduot *et al*. (2019) reported that the seed endophytes, as primary symbionts, influenced the formation of secondary symbioses in maturing hosts. They hypothesized that such priority effects are a function of the presence and the identity of the primary symbionts. They sampled primary symbionts in winter wheat using culture-based techniques and next-generation sequencing and concluded that the wheat seed, primary symbionts have a strong, natural priority effect dependent on their presence and identity.

Zhu and She (2020) isolated endophytic bacteria from *Ammodendron bifolium* through physiological characteristics detection and identified eleven isolates, belonging to the genera, *Bacillus, Staphylococcus* and *Kocuria.* These isolates were capable of promoting seed germination, radicle growth besides providing beneficial effects to host plants at different growth stages.

2.2 Salinity stress and its effect on plants

Salinity is one of the major threats to plant growth and development in irrigated agriculture, which affects 45 m ha of irrigated land and it substantially reduces the food production. Salinity causes both hyper-ionic and hyperosmotic stress which affects the plant at physiological, biochemical and molecular levels (Zeng and Shannon, 2000). Salinity stress leads to membrane damage, accumulation of metabolites, reduced cell expansion, cell division, programmed cell death and ultimately leads to plant death (Tuteja,

2007). Plants basically counteract these negative effects of salinity by the activation of various biochemical and metabolic pathways including osmolyte accumulation, maintaining ion homeostasis and ROS scavenging (Munns and Tester, 2008)

Salt stress interferes with the vegetative growth by reducing leaf area, number of tillers, inhibition of leaf emergence and decreased root growth due to ion toxicity (Jugale, 2008; Ghosh *et al*., 2016). Osmotic stress severely affected various stages in rice (IR-64) and tomato (Arka Saurabh) plants (Golldack *et al*., 2011). Reclaimation measures such as chemical treatment, agro technical treatment, biological method, hydro technical methods, etc. are recommended for salt affected soils (Castillo *et al*., 2015).

Crop responses to salinity are influenced by the electrical conductivity of saturated soil extract (Ghosh *et al*., 2016).

EC (dS m^{-1} at 25°C)	Crop response
0-2	Salinity effect is practically nil
2-4	Reduction in yield of very sensitive crops
4-8	Reduction in yield of most crops
8-16	Only tolerant crops produce satisfactory yield
>16	Few highly tolerant crops produce satisfactory

The effects of salinity stress on plants (Munn and Tester, 2008)

Effect of stress	Osmotic stress	Stress due to high leaf Na^+ (ionic stress)
Speed of onset	Rapid	Slow
Primary site of visible effect	Decreased new shoot growth	Increased senescence of older leaves

2.3 Drought stress and its effect on plants.

Drought refers to the condition in which inadequate soil moisture leads to acute decrease in agricultural productivity. Drought is one of the most serious world-wide problems in agriculture. According to the Drought Early Warning System (DEWS), a real-time drought monitoring platform indicated that about 42 % of India's land area was drought affected. Water deficit resulted in reduced crop yield (Kerepesi and Galiba, 2000).

Effect of drought on plant differs with the stage at which plants experience drought. The foremost effect of drought is reduced germination and poor establishment (Mohamed *et al*., 2002). At the onset of water stress, inhibition of cell growth occurs leading to a reduction in leaf development. Stress at vegetative stage inhibits photosynthesis in plants by closing stomata, damaging chlorophyll content and photosynthetic apparatus (Zhu, 2002). Drought increases the accumulation of reactive oxygen species (ROS) that in turn induces oxidative stress to membrane lipids, proteins, and other cellular components.

Plants response to drought stress is by complex mechanisms varied from genetic expression to biochemical metabolism. The accumulation of the phytohormone abscisic acid (ABA) is noticed in plants during drought stress (Karl *et al*., 2009). Plants also adapted to extreme drought by developing a close association with microorganisms such as endophytic fungal and bacterial symbionts and arbuscular mycorrhiza (AM).

2.4 Endophytes mediated salinity stress tolerances

Bacterial endophyte interaction with plants promotes plant growth, productivity, and biotic and abiotic stress tolerance in the host plant. Drought, salt, heat, cold, oxidative stress, and heavy metal toxicity are some of the most frequent abiotic stressors that impact on plant development and agricultural yield across the world. The endophytic microorganisms (bacteria and fungi) provide an ideal model for studying stress tolerance, adaptation, and response mechanisms, which can be designed into agricultural plants to cope up with climate change-induced stresses (Grover *et al.*, 2011).

Sadrnia *et al.* (2011) reported the resistance of tomato plants to NaCl in pot and greenhouse experiments by treating the tomato plant with cloned *P. mendocina* (with

plasmid) and *P. mendocina* (without plasmid) and control group. Results from the pot experiment revealed that the plants treated by cloned *P. mendocina* showed increased growth than those treated with *P. mendocina* (without plasmid) after five weeks of tomato crop grown under NaCl stress. Recombinant *P. mendocina* resulted in improvement of plant resistance against salinity. Jha *et al*. (2011) studied the effect of endophytic bacterium *P. pseudoalcaligenes* in rice variety GJ-17 under salt stress. Plants inoculated with *P. pseudoalcaligenes* showed significantly higher concentration of glycine betaine-like quaternary compounds and higher shoot biomass under salinity stress.

An endophytic bacterium *Achromobacter xylosoxidans* AUM54 isolated from *Catharanthus roseus* was evaluated for their salinity stress tolerance in Wheat. *A. xylosoxidans* AUM54 treated plants recorded the maximum germination percentage, vigor index, plant height, root dry weight compared to control. This isolate decreased plant ethylene levels and increased the antioxidative enzyme content like ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) under salt affected conditions (Karthikeyan *et al*., 2012).

Zarea *et al*. (2012) conducted an experiment to investigate the salt stress tolerance abilities of the endophytic fungi, *P. indica* and *Azospirillum* strains, isolated from non-saline and saline soil, at five NaCl levels (0, 0.1, 0.2, 0.3, 0.4, 0.5 mol L^{-1}). The mechanisms for protecting plants from the detrimental effects of salinity by *P. indica* fungus and *Azospirillum* strains differed in their salinity tolerance, and this influenced the uptake of water, photosynthetic pigment content and proline accumulation in wheat seedlings.

The beneficial effects of *Piriformospora indica* on rice seedlings during high salt stress conditions (200 and 300 mM NaCl) on growth parameters of rice seedlings such as root and shoot length or fresh and dry weights and photosynthetic pigment content were found to be increased in inoculated rice seedlings compared to uninoculated seedlings (Jogawat *et al*., 2013). Proline accumulation was also observed during *P. indica* colonization which helped inoculated plants to become salt tolerant.

Damodaran *et al*. (2013) isolated 16 rhizobacteria through natural selection from saline-sodic soils and characterized by using morphological and biochemical parameters. These bacteria were assessed for their plant growth-promoting (PGP) traits like indole 3-acetic acid (IAA) production, ammonia and hydrogen cyanide (HCN) production, phosphate solubilization, etc. These isolates also elicited significantly higher vigour index in tomato seedlings grown in pot experiment under saline sodic soils of pH 9.35 and EC 4.2.

Ramadoss *et al*. (2013) screened eighty-four halotolerant bacterial strains at different NaCl concentrations. The 16S rRNA gene sequence confirmed that the isolates belonged to *Bacillus* and *Halobacillus* species. Out of 84 isolates, only five isolates (SL3, SL32, SL35, J8W, and PU62) had shown growth at 20 percent NaCl but all grew well at 5 per cent NaCl. The 5 halotolerant bacterial strains showed to ameliorate salt stress (80 mM, 160 mM and 320 mM) in wheat seedlings by increasing the root length compared to uninoculated control. *Halobacillus sp* SL3 and *Bacillus halodenitrificans* PU62 showed more than 90 percent increase in root elongation and 17.4 per cent increase in dry weight compared to uninoculated wheat seedlings at 320 mM NaCl concentration.

The plant growth and productivity are negatively affected by soil salinity, and it is predicted that the plant growth-promoting bacterial (PGPB) endophytes that contain 1-aminocyclopropane-1-carboxylate (ACC) deaminase can facilitate plant growth and development in the presence of different stresses. The use of PGPB with ACC deaminase activity has potential influence on plant growth in saline soils (Trevor *et al*., 2014).

Patel *et al*. (2014) screened for salt stress tolerance by growing in medium supplemented with 5 percent and 18 percent NaCl and confirmed that, two strains (BR5 and BN7) could grow at 18 percent NaCl concentration. These strains were identified by 16S rRNA gene sequencing and BLAST analysis as *Bacillus subtilis* and *Bacillus megaterium*. These bacteria showed increased germination percentage, root and shoot length of *Vigna radiata L.* compared to uninoculated plants.

Yaish *et al.* (2015) isolated endophytic bacteria from date palm (*Phoenix dactylifera* L.) roots and assessed for salt tolerance. Some of these strains produced the enzyme 1- aminocyclopropane-1-carboxylic acid (ACC) deaminase as well as the plant growth hormone indole-3-acetic acid (IAA). Shrivastava *et al*., (2015) suggested that the endophytes can play a significant role in mitigating salinity problems by their unique properties like tolerance to saline conditions, genetic diversity, synthesis of compatible solutes, production of plant growth hormones, bio-control potential, and their interaction with crop plants.

Mondala and Borromeob (2016) conducted an experiment at International Rice Research Institute (IRRI) to assess the response of rice hybrid seeds with their parental checks FL478 and NSIC Rc222 for salt stress tolerance (12 dS/m) in the dry season at seedling stage using IRRI screening techniques. Out of two hundred thirty-one seeds, only 1.73% populations were identified as tolerant, 18.18% moderately tolerant, 37.26% sensitive and 46.86% were highly sensitive. The tolerant genotypes also showed resistance against Brown Plant Hopper (BPH) infested during experimentation.

Amjad *et al.* (2017) identified eight bacterial endophytes isolated from *Avicennia marina* as *Paenibacillus*, *Bacillus*, *Microbacterium*, *Citrobacter*, *Lysinibacillus, Halomonas, Virgibacillus, Exiguobacterium,* and *Vibrio* through molecular analysis using 16Sr RNA. The *Bacillus pumilus* AM11 and *Exiguobacterium sp*. AM25, grew considerably faster in saline media and the tomato plants treated with AM11 and AM25 considerably increased biomass, photosynthetic rate, and pigment accumulation in response to salinity stress.

Twenty-two endophytic bacteria isolated from the roots of *Salicornia brachiata* L. were characterized on the basis of morphology and biochemical characteristics by Abbas *et al*. (2018). Out of 22, only five endophytes were selected based on their PGPR activity under salt stress. These bacteria were identified by 16S rRNA gene sequence analysis as *Bacillus aereus* SA1, *Serratia nematodiphila* SG1, *Pantoea agglomerans* SG2, *Enterobacter sp.* SL and *Enterobacter sp.* SRh. All the five bacterial strains produced IAA. Siderophore production was observed in *Serratia nematodiphila* SG1, *Pantoea*

agglomerans SG2 and *Enterobacter sp.* SL. All the five tolerated high concentration of NaCl.

Increased soil salinity is often associated with accelerated ethylene production in plants, leading to overall growth reduction. The salt-tolerant 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing PGPR may alleviate salt stress by reducing the production of ethylene stress. A salt-tolerant ACC deaminase-producing strain named P50 was isolated from a coastal rice field in Odisha, India, which enhanced the growth of rice seedlings under salt stress. The P50 strain was identified as *Burkholderia* sp. (Sarkar *et al.,* 2018).

Khan *et al.* (2020) isolated 59 bacteria from plants inhabiting sand dunes at Pohang beach and screened for different concentration of NaCl. Only six isolates (*Curtobacterium oceanosedimentum* SAK1, *Curtobacterium luteum* SAK2, *Enterobacter ludwigii* SAK5, *Bacillus cereus* SA1, *Micrococcus yunnanensis* SA2, *Enterobacter tabaci* SA3) showed significant increase in growth of Waito-C rice and higher salt concentratilons. The culture filtrates (CFs) of bacterial endophytes produced indole-3-acetic acid, gibberellins and organic acid and reduced the endogenous ABA content upon inoculation in rice under NaCl stress.

Saryanah *et al.* (2021) identified 150 bacterial endophytes isolated from *Curcuma xanthorrhiza* (Javanese turmeric) and tested for salt stress tolerance and plant growth promoting characteristics. Only ten out of 150 bacterial endophytes showed tolerance to NaCl and PGP activity. These isolates dramatically increased the growth of rice seedlings. Sharma *et al.* (2021) isolated 18 bacteria from the soil samples collected from Haryana state were screened for salt-tolerance. Only thirteen isolates were found to be salt tolerant at 10% salt

2.5 Endophytes mediated drought stress tolerance

Sun *et al.* (2010) studied effect of *Piriformospora indica* colonized plants when exposed to polyethylene glycol to mimic drought stress, the activities of peroxidases, catalases and superoxide dismutases in the leaves were upregulated within 24 hours. The

fungus retarded the drought stress by protecting the degradation of chlorophylls and thylakoid proteins. The endophytes inoculated plants showed increased plant biomass, relative water content, leaf water potential, root adhering soil/root tissue ratio, aggregate stability, decreased leaf water loss at minimum water potential (Vardharajula *et al*., 2011). Yandigeri *et al*. (2012) isolated drought tolerant endophytic actinobacteria *Streptomyces coelicolor* DE07, *S. olivaceus* DE10 and *S. geysiriensis* DE27 from cultivated plants of arid and drought affected regions of Rajasthan, India. These isolates induced plant growth promotion traits and intrinsic water stress tolerance from - 0.05 to -0.73 M Pa.

Salicylic acid (SA) application to endophyte (*Penicillium resedanum* LK6) infected plants not only increased the shoot length and chlorophyll content but also improved the biomass recovery of bell pepper (*Capsicum annuum*) under polyethylene glycol (15 %) induced osmotic stress. SA application enhanced the colonization rate of endophyte in host-plant roots. Endophyte and SA in combination reduced the production of ROS by increasing the total polyphenol, reduced glutathione, catalase, peroxidase, and polyphenol oxidase compared to control plants (Khan *et al*., 2013).

Kavamura *et al*. (2013) isolated cacti-associated bacteria from Brazilian semi-arid based on their ability to grow in medium with reduced water availability and great proportion of the isolates belonged to the genus *Bacillus*. Out of forty-eight bacteria, 65 per cent were able to grow in medium with reduced water availability (0.919 aW) and isolates of two strains of *Bacillus* achieved the growth promotion of *Zea mays* L. at 30 percent field capacity.

The endophytic fungus, *Neotyphodium coenophialum*, enhanced drought tolerance of its host grass, *Tall fescue*. The comprehensive profiling of plant metabolite level of both shoots and root tissues of genetically identical clone pairs of *Tall fescue* with endophyte (E+) and without endophyte (E-) showed survival and tillering under direct water stress (Nagabhyru *et al*., 2013). Naveeda *et al*. (2013) studied the effect of inoculation of two bacterial endophytes *Burkholderia phytofirmans* strain PsJN and *Enterobacter sp*. FD17 on growth, water status and photosynthetic activity of two maize cultivars under drought

stress conditions. The inoculated plants showed significantly increased shoot and root biomass, leaf area, and chlorophyll content.

The *Bacillus subtilis* strain B26 increased root and shoot weight, accelerated growth rate and seed yield of *Brachypodium distachyon* Bd2l grass compared to control plants exposed to acute and chronic drought stress (Gagne-Bourque *et al.,* 2015). *Brachypodium* seedlings and mature plants minimized the phenotypic effect of drought compared to the plants not harbouring the bacterium *Bacillus subtilis* strain B26. The bacterium was recovered from roots, stem and blades as well as from seeds. Protection from drought by the bacterium was linked to upregulation of drought-response gene like, DREB2B, DHN3 and LEA-14A and modulation of DNA methylation genes, MET1B, CMT3 and DRM2.

Molina-Montenegro *et al*. (2016) isolated bacterial endophytes from Antarctic plants and evaluated in a lettuce cultivar, grown under different water availability regimes. The presence of endophytes showed higher water use efficiency in drought conditions and resulted in greater fresh and dry biomass production. In addition, presence of bacterial endophytes in lettuce was correlated with a higher proline concentration, lower peroxidation of lipids and up-/down-regulation of ion homeostasis.

Gagne-Bourque *et al*. (2016) investigated the effect of bacterial endophyte *Bacillus subtilis* strain B26 on inoculation with Timothy (*Phleum pratense L*.) for growth, water status, photosynthetic activity and metabolism when exposed to drought stress. Exposure of inoculated plant to 8-week drought-stress led to significant increase in shoot and root biomass by 26.6 and 63.8 per cent. The strain B26 colonized the internal tissues of Timothy successfully and improved Timothy growth through modification of osmolyte accumulation in roots and shoots.

Influence of arbuscular mycorrhiza *Rhizophagus intraradices* (Ri) and endophyte-*Piriformospora indica* (Pi) on drought tolerance was studied in finger millet. The finger millet seedlings grown with or without symbiotic association under three water regimes showed enhanced seedling growth under drought. The finger millet seedlings under

drought showed stronger antioxidant defence system, higher chlorophyll content and stable osmoregulatory network (Tyagi *et al*., 2017).

Zhang *et al.* (2018) reported that the maize seedlings profited from the presence of the endophytic fungus *Piriformospora indica* under drought stress. Drought tolerance conferred by *P. indica*, from the root transcriptome of colonized and uncolonized seedlings analyzed at 0, 6 and 12 h after drought stress and concluded that the number of *P. indica*-responsive genes increased from 464 (no stress at 0 h) to 1337 (6 h drought) and 2037 (12 h drought). They also reported that, the fungus improved the oxidative potential of the roots, and stimulated genes for hormone functions, including those which respond to abscisic acid, auxin, salicylic acid and cytokinins under drought stress.

Li *et al*. (2019) reported that the application of *Streptomyces pactum* Act12 enhanced drought resistance in drought-sensitive wheat (*Triticum aestivum L*.) cultivar Xinong 979. Act12 seedling treatment significantly increased total soluble sugars in wheat leaves while decreasing their malondialdehyde content by 20.5 per cent. The endophyte inoculated plants showed accumulation of more abscisic acid and upregulated the expression levels of several drought resistance-related genes, such as EXPA2, EXPA6, P5CS, and SnRK2 under drought conditions. Vigani *et al.,* (2019) tested the role of endophytes in inducing drought resistance in pepper (*Capsicuum annuum* L.) and the expression of the vacuolar proton pumps, H+ -ATPase (V-ATPase) and H+ -PPase (V-PPase) which confer drought resistance and found that the bacterial colonization enhanced vacuolar H+ -pumping pyrophosphatase.

Devarajan *et al.* (2020) identified 44 bacteria from the leaf surface of drought-tolerant rice cultivars Mattaikar, Nootripattu, Anna (4), and PMK3 and tested their abiotic stress tolerance by subjecting to high temperature, salt and osmotic stress. Under various abiotic stress conditions, only eight isolates survived. The stress tolerance imparting ability of the endophytes of sorghum was demonstrated *in vivo* using ½- MS medium and ½- MS medium Plus 15 percent PEG 8000. Seed bacterization with these isolates improved plant growth in sterile soil-rite mix based experiment (Govindasamy *et al.,* 2020).

Gowtham *et al.* (2020) studied ten ACC deaminase producing PGPR isolates selected and evaluated for the induction of drought stress tolerance in tomato. The tomato plants grown upon treatment with *Bacillus subtilis* Rhizo SF 48 significantly enhanced plant growth even after exposing to different levels of drought stress. Inoculated plants increased 0.76, 0.23 and 0.78 fold in proline, superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity respectively and decreased 0.3 fold malondialdehyde (MDA) content.

Dubey *et al.* (2021) studied 3 endophytes isolated from root tissue of soybean. Of which AKAD A1-16 performed better than AKAD A1-2 and AKAD A1-1, which was further validated for ACC deaminase in the following order: AKAD A1-16 > AKAD A1-2 > AKAD A1-1. Scanning electron microscopy images showed presence of bacterium inside the roots of soybean seedlings. These bacteria were identified as *Bacillus cereus (*AKAD A1-1), *Pseudomonas otitidis* (AKAD A1-2) and *Pseudomonas* sp. (AKAD A1-16).

2.6 Molecular identification of bacterial endophytes

Phylogeny is the evolutionary history of organisms. The genes encoding for 16S rRNA in prokaryotes and 18S rRNA/ITS in eukaryotes are most widely used in molecular phylogenetics. These small subunit 30S ribosomal RNA (SSU rRNA) genes have been used extensively for sequence based evolutionary analysis. Identification of bacteria by amplification of coding region of the 16S rRNA gene was performed by PCR with forward and reverse primers FD1 (5’ AGAGTTTGAT CCTGGCTCAG 3’) and RD1 (5’ AAGGAGGTGATC CAGCC 3’). The 16S rRNA gene is the primary gene target for identification of bacteria as the gene sequences contain conversed, variable and hyper variable regions. The reasons behind the use of 16S rRNA gene to be utilized for identification purpose include, i) occurrence of the gene in all organisms performing the same function, ii) the gene sequence is conserved sufficiently and iii) around 1500 bp of sequence size, which is relatively easy to sequence and large enough for identification and phylogeny analysis (Madigan *et al*., 2009).

Endophytic bacteria isolated from root, stem, and leaves of *Plectranthus tenuiflorus* plant were identified by partial sequencing of their 16S rRNA gene as *Bacillus sp., Bacillus megaterium, Bacillus pumilus, Bacillus licheniformis, Micrococcus luteus, Paenibacillus sp., Pseudomonas sp*. and *Acinetobacter calcoaceticus* (Deeba *et al.,* 2013). Praveena and Bhore (2013) isolated fifty bacteria from *Tridax procumbens* and identified by 16S rRNA gene nucleotide sequence analysis using the BLAST as *Bacillus* spp., *Cronobacter sakazakii, Enterobacter* spp., *Lysinibacillus sphaericus, Pantoea* spp., *Pseudomonas* spp. and *Terribacillus saccharophilus.* Jasim *et al*. (2013) isolated and identified the presence of four different endophytic bacterial strains from ginger rhizome using 16S rRNA gene sequence.

A total of 50 endophytic bacteria from the roots of *Amaranthus hybridus, Solanum lycopersicum and Cucurbita maxima* were isolated by Ngoma *et al*. (2013) and identified by partial 16S-rDNA gene sequencing using polymerase chain reaction (PCR) for phylogenetic analysis. These bacteria were identified as *Stenotrophomonas maltophilia* KC010525, *Pseudomonas putida* KC010526, *P. putida* KC010527, *P. putida* KC010528, *S. maltophilia* KC010529, *Achromobacter xylosoxidans* KC010530, *A. xylosoxidans* KC010531 and *Achromobacter* sp. KC010532.

Ji *et al*. (2014) isolated endophytic bacteria from leaves, stems and roots of 10 rice cultivars and identified 12 of them as diazotrophic bacteria using 16S rDNA sequence, two as *Penibacillus*, three species as *Microbacterium*, three as *Bacillus* species and four species as *Klebsiella*. Rice seeds treated with these bacteria showed improved plant growth, increased height and dry weight and antagonistic effects against fungal pathogens.

Yaish *et al*. (2015) isolated endophytic bacteria from date palm (*Phoenix dactylifera L*.) and carried out molecular characterization which showed that majority of these strains belonged to the genera *Bacillus* and *Enterobacter*. The results suggested that the characterized endophytic bacteria could alter ethylene, IAA levels, facilitate nutrient uptake in roots and had potential role to promote the growth and development of date palm trees growing under salinity stress. Two endophytes isolated from *Prosopis cineraria* were identified on the basis of morphological and biochemical characterization of the

endophytes as well as 16S rRNA sequencing technique they were identified as *Bacillus subtilis* and *Stenotrophomonas maltophilia* (Gupta *et al.*, 2015).

Zoulikha *et al.* (2016) isolated 73 bacterial endophytes from native plants grown in a fallow land and 17 strains of them were identified using 16S rRNA gene sequence. The phylogenetic analyses revealed that the presence of Gram-positive *Bacillus amyloliquefaciens, B. cereus, B. methylotrophicus, B. pumilus, Curtobacterium flaccumfaciens* and Gram-negative *Pseudomonas brassicacearum.*

Bacterial endophytes isolated from root crown, stem and leaf tissues were identified by 16S rRNA region sequence represented six taxonomic groups of which *Pseudomonas* were the most predominant bacteria isolated, making up 20 to 30 per cent of the total isolates, followed by *Curtobacterium* and *Microbacterium.* (Dombrowski *et al.*, 2017). Etminani and Harighi (2018) isolated 61 endophytic bacteria from the leaves and stems of healthy wild Pistachio trees (*Pistacia atlantica L.*) from various locations of Baneh and Marivan regions of Iran and grouped according to phenotypic properties. Ten selected isolates from each group were further identified by partial sequencing of the 16S rRNA gene as *Pseudomonas, Stenotrophomonas, Bacillus, Pantoea* and *Serratia* genera.

Bind and Nema (2019) reported molecular characterization of 18 selected endophytic bacteria isolated from root, stem and leaves of pigeon pea through 16S rRNA gene. The bacteria belonged to *Chryseobacterium endophyticum* (SS1), *Paenibacillus castaneae* (SR1), *Streptomyces* sp. (SR2), *Lactobacillus plantarum* (DR1), *Bacillus proteolyticus* (DS1), *Pseudomonas* sp. (DS2), *Serratia rubidaea* (CL1), *Klebsiella aerogenes* (CS1), *Paraburkholderia* sp. (CS2), *Burkholderia* sp. (KR1), *Bacillus cereus* (KR2), *Bacillus subtilis* (KS1) and *Enterobacter cloacae* (JL1).

Significant research was carried out by Ponpandian *et al.* (2019) and 1,622 bacterial endophytes were isolated from the needle, stem and root tissues of *Pinus densiflora, P. rigida, P. thunbergii* and *P. koraiensis* across 18 sampling sites in Korea which were screened for biocontrol activity of these against the pine wilt disease (caused by

Bursaphelenchus xylophilus). These were classified into 389 members based on 16S rDNA gene analysis. Of which 215 operational taxonomic units (OTUs) were determined.

Bacterial isolates ACP1, ACP2, ACP4, and ACP6 obtained from Noni fruits (*Morinda citrifolia L.*) were reported by Sogandi and Nilasari (2019). Of which the isolate ACP6 showed the highest clear zone formation that could inhibit four pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Shigella dysenteriae* and *Streptococcus mutans*). Molecular identification through PCR amplification by 16S rRNA gene revealed the potential isolate ACP6 (Acc. No. MH915674.1) as *Enterobacter cloacae* with 99 % sequence similarities. Patel *et al.* (2020) isolated three (PR1, JB3 and JB6) diazotrophic endophytic bacteria based on their growth on nitrogen free medium. Molecular characterization of all three isolates showed affiliation to the genera *Agrobacterium, Pseudomonas* and *Brevundimonas*.

Belincanta *et al.* (2021) isolated 32 endophytic bacteria from *Dendrocalamus asper* and *Bambusa oldhamii* and identified using 16S rRNA gene as *Bacillus*, *Brevibacillus*, *Serratia*, and *Atlantibacter*. Claudia *et al.* 2021 isolated 315 bacterial isolates from tomato plants (108 roots, 102 stems, and 105 leaves) and identified them using 16S ribosomal gene sequencing. *Bacillus, Microbacterium, Pseudomonas* and *Stenotrophomonas* were the most common genera.

2.7 Influence of endophytes on plant growth promotion

Bacterial endophytes have been shown to boost plant growth and development in a variety of ways. Phosphate solubilization, phytohormone production, siderophore synthesis, ammonia production, and giving critical nutrients to the host plant are some of the benefits (Vacheron *et al.*, 2004).

The capacity of various bacterial endophytes to support plant growth and development is due to direct or indirect association with plants. When a bacterium aids the uptake of vital nutrients or regulates the hormones within a plant, it promotes directly plant growth and development.

Pandey *et al*. (2005) isolated *Burkholderia sp*. MSSP from the surface sterilized root nodules of *Mimosa pudica* which showed positive for the plant growth promoting characters like nitrogen fixation, phosphate solubilization, production of IAA, ACC deaminase, siderophore, HCN and antagonistic activity against different phytopathogens representing the role of endophytic bacteria within the colonized plant.

Bacterial endophytes isolated from field grown *Solanum nigrum* were characterized for PGP traits (ACC deaminase activity, IAA production, phosphate solubilization and seedling colonization) and determined their effects on *Solanum nigrum* and *Nicotiana attenuate.* Majority of the isolates that promoted the root growth of *S. nigrum* were associated with ACC deaminase activity and IAA production (Long *et al*., 2008).

Jha and Kumar (2009) characterised plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat variety Malviya 234. This isolate showed increased level of nitrogenase activity, IAA production, and P solubilization resulting in increased plant growth. The gibberellin production by the newly isolated fungus *Gliomastix murorum* was reported to be higher than wild-type fungus *Gibberella fujikuroi*. Gibberellin analysis of *G. murorum* culture filtrate showed presence of bioactive gibberellins GA1, GA3, GA4, and GA7 along with physiologically inactive GA5, GA9, GA20 and GA24 (Khan *et al*., 2009).

Khan and Doty (2009) characterised 11 endophytic bacteria associated with sweet potato plant (*Ipomoea batata L. Lam*.) for their ability to fix nitrogen, produce IAA and stress tolerance. These bacteria belonged to the genera, *Enterobacter*, *Rahnella*, *Rhodanobacter, Pseudomonas, Stenotrophomonas, Xanthomonas* and *Phyllobacterium*. Four strains were shown to produce IAA and one strain showed the ability to grow in nitrogen free medium and had the nitrogenase subunit gene, nif H.

Population of endophytic bacteria from root, stem and leaf of different leguminous plants were determined by Umamaheshwari *et al*. (2013). The population ranged from 2.1 $\times 10^3$ to 8.6 $\times 10^3$, 1.5 $\times 10^3$ to 5.0 $\times 10^3$ and 0.3 $\times 10^3$ to 2.3 $\times 10^3$ respectively. Based on the polyphasic characterization, 25 distinct isolates obtained were identified as *Bacillus* sp,

Micrococcus sp, *Pseudomonas* sp, *Flavobacterium* sp *and Serratia* sp. All the isolates were evaluated for the production of phytohormones *viz.*, gibberellic acid (GA), indole-3-acetic acid (IAA) and cytokinin.

Sixteen rhizobacteria through natural selection from saline-sodic soils were isolated and characterized using morphological and biochemical characteristics. These bacteria were assessed for their plant growth-promotion traits like indole 3-acetic acid (IAA) production, ammonia and hydrogen cyanide (HCN) production, phosphate solubilization, etc. The isolates elicited significantly higher vigour index in tomato seedlings grown in pot culture under saline sodic soils having pH 9.35 and EC 4.2 (Damodaran *et al.*, 2013).

Oteino *et al.* (2015) identified three endophytic bacteria responsible for phosphate solubilization. Many of the endophytic bacteria produced GA (14 - 169 mM) and had a moderate to high phosphate solubilizing capacity (400 - 1300 mg/L). When these endophytes were inoculated into *Pisum sativum* L. cultivated in phosphate-limiting soils, the endophytes produced high amount of GA which was favourable for plant growth. Raweekul *et al.* (2016) examined 126 bacteria within rice roots and stems. All isolates were evaluated for their ability to promote rice seedling development. The endophytes inoculated seedlings showed increased fresh weight ranging from 2.30 to 3.18 times.

A potential of rice seed-borne bacteria *Micrococcus yunnanensis* RWL-2, *Micrococcus luteus* RWL-3, *Enterobacter soli* RWL-4, *Leclercia adecarboxylata* RWL-5, *Pantoea dispersa* RWL-6, and *Staphylococcus epidermidis* RWL-7 were isolated from rice seed to explore the growth promotion. These endophytes showed IAA production when analysed by GC-MS (Shahzad *et al.*, 2017). Adhikari *et al.* (2018) evaluated three sweet potato endophytic strains to produce IAA and fix nitrogen. The IAA production activity peaked at 15 - 60 mg NH_4NO_3 L^{-1}. Similarly, acetylene reduction activity was peaked at 0 - 6.25 mg NH_4NO_3 L^{-1}, and both strains colonised plants, enhanced tomato growth.

Etminani and Harighi *et al.* (2018) isolated 61 endophytic bacteria for phytohormone synthesis, siderophore production, phosphate solubilization, atmospheric nitrogen fixation, protease and hydrogen cyanide generation. The plant growth hormones

auxin and gibberellin were produced in varied levels by all the strains. Phosphate was solubilized by majority of the strains. The capacity to fix nitrogen from the air and generation of protease and siderophore, differed amongst strains.

Four endophytic *Bacillus* strains isolated for understanding solubilization of iron phosphate (Fe-P), production of siderophore, and IAA *in-vitro*. These isolates were evaluated for plant growth promotion under greenhouse conditions by inoculating endophytes into pearl millet grown in P-deficient soils supplemented with rock phosphate or soluble triple superphosphate. In the presence of tryptophan, all strains solubilized Fe-P, and three of them formed carboxylate type siderophores and large quantities of IAA (Ribeiro *et al.,* 2018). Singh *et al.* (2018) studied a group of 201 bacteria isolated from 13 distinct wheat types. When these endophytes were screened for growth promotion, 11 bacteria have shown zinc solubilization, 10 bacteria were potential siderophore producers. The plant growth and output increased by 14 to 20% in field trial and Fe or Zn accumulation increased by 75% compared to RDF in wheat grains.

Eighty-four endophytes were isolated from *Cicer arietinum* and *Pisum sativum* plant nodules and analysed for growth promoting traits. Quantitative analysis was performed for 14 isolates out of 84 for siderophore production, majority of them produced hydroxamate and carboxylate siderophores. Besides, all the isolates produced ammonia and indole-3-acetic acid (Maheshwari *et al.,* 2019).

Jayakumar *et al.* (2020) discovered bacterial endophytes from *Ananas comusus*, that improved agricultural output by boosting plant growth, yield, and disease resistance with antifungal properties. Under drought conditions, these organisms exhibited plant-beneficial processes such as IAA, ACC deaminase synthesis, and nitrogen fixation. The selected organisms were identified as *Bacillus sp.* (Acb9), *Providencia sp.* (Acb11), *Staphylococcus sp.* (Acb12), *Staphylococcus sp.* (Acb13) and *Staphylococcus sp.*

Husseiny *et al.* (2021) isolated 106 endophytic bacteria from different parts of the medicinal plant *Artemisia annua L.* and characterized for their PGPR activity. Out of 106 isolates, 95% of them were able to produce IAA. Similarly, five bacterial endophytes

isolated from seeds of three different Pakistani wheat varieties (Ghaneemat-e-IBGE, Atta-Habib, and Siren) showed plant growth promotion and showed phylogenetic similarity with *Bacillus altitudinis, B. aryabhattai, B. wiedmannii, Pseudomonas aeruginosa* and *Burkholderia gladioli*, respectively (Shah *et al.*, 2022). This study on bioprospecting of bacterial endophytes was undertaken with the background of the above literature.

III MATERIAL AND METHODS

Experiments were conducted on the role of bacterial endophytes in imparting salinity and drought stress tolerance in rice and tomato. The materials used and methods followed during experimentation are described in this chapter.

3.1 Collection of bacterial endophytes

Fifty-eight bacterial endophytes were isolated from the plants of North Western Himalayan (India) cold deserts and were maintained in the School of Ecology and Conservation (SEC) Laboratory, Department of Crop Physiology, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bengaluru – 560 065. These bacteria were rejuvenated on fresh Nutrient agar medium. They were isolated from variety of plants growing in different regions of North Western Himalaya (India) cold deserts namely, Pangong, Changla and Namika La which are located in Ladakh, Jammu and Kashmir of India. The geographical information on Himalayan cold desert regions are as follows.

Sl. No.	Location	Latitude (° N)	Longitude (° E)	Altitude (msl)	No. of endophytes isolated
1	Pangong	33°43′2.74″	78°53′29.08″	4250	19
2	Chang La	34°30″	77°55°	5216	14
3	Namika La	34°22′	76° 35′	3700	25

3.2 Screening of bacterial endophytes for salt and drought tolerance

Fiftyeight bacterial isolates were tested for their salt and drought tolerance in liquid medium with three replications. Nutrient broth (NB) was prepared with different concentrations of NaCl (0, 0.5, 1.0, 1.5, 2.0, 2.5 M) and inoculated with each bacterium in separate tubes (Viscardi *et al*., 2016). The standard NB was used as control (0=no extra NaCl was added). Similarly, for inducing drought different concentration of PEG-8000 (0%, 5%, 10%, 15%, 20%, 25%) was added and inoculated with each bacterium in separate tubes along with NB as control (0=no PEG) (Bandeppa *et al*., 2017). The tubes were

incubated at 30 °C for 48 h. After incubation, the turbidity due to cell growth was measured by reading optical density (OD) at 600 nm using spectrophotometer.

3.3 Determination of LC_{50} value of NaCl concentration for rice and tomato seedlings

Seeds of rice (*Oryza sativa*) *var.* IR 64 and tomato (*Solanum lycopersicum*) *var.* Arka Saurabh were selected. The selected seeds were surface sterilized by treating with 2% sodium hypochlorite solution for 1 minute, followed by 70% alcohol for 1 minute, and then washed with 3-4 times using sterile distilled water to eliminate residual chemical (Shaik *et al.,* 2019). The surface sterilized seeds were then soaked for 48 hours in sterile distilled water and incubated for germination. The paper towel method was used to standardize the Lethal Concentration 50 (LC_{50}) of NaCl (50 % reduction in plant growth) for rice and tomato. The germination paper was soaked in different concentrations of NaCl (25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM and 175 mM) for tomato. For rice, NaCl at 25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM and 200 mM concentrations were used. Then the pre-germinated seeds of rice and tomato respectively were placed on soaked germination paper and incubated at 25 °C for 14 days for rice and 7 days for tomato in growth chamber. For control sterile water was used. After incubation, the shoot and root length were recorded. The LC_{50} value of NaCl concentration for each seedling was determined by Probit analysis (Sangamesh *et al*., 2018).

3.4 Determination of LC_{50} value of PEG (MW 8000) for rice and tomato seedlings

For determination of LC_{50} value of PEG-8000, the paper towel method was used (Sangamesh *et al*., 2018). The germination paper was soaked in Poly ethylene glycol solutions having 5, 10, 15, 20 and 25 per cent concentrations which corresponded to the osmotic potential -0.04 MPa, -0.15 MPa, -0.30 MPa, -0.51 MPa and -0.77 MPa respectively (Muddarsu and Manivannan, 2017). The pre-germinated seeds (aseptically) were placed on soaked germination paper at different concentrations of PEG and incubated at 25 °C in growth chamber for 14 days for rice and 7 days for tomato. Sterilized water was used as control. Four replications were maintained for each treatment. The shoot and root length were recorded after 14 and 7 days of incubation respectively and LC_{50} value of PEG (8000) for each seedlings was determined by Probit analysis.

3.5 Screening of bacterial endophytes imparting salinity stress tolerance in rice and tomato

The pure culture of selected bacterial isolates were inoculated into nutrient broth and incubated at 30 ºC for 48 h. Then the 48 h old bacterial cultures were centrifuged at 7,200 rpm for 5 min and the bacterial suspension (~ 6×10^7 cells mL^{-1}) was prepared with sterile water (Zhang *et al.,* 2014). The surface sterilized seeds of rice (IR-64) and tomato (Arka Saurabh) were pre-germinated as described previously and incubated in 10 ml bacterial suspension for 3 h for effective colonization (Sangamesh *et al*., 2018).

The bacterial endophytes inoculated seeds of rice and tomato were subjected to salinity stress using NaCl at 150 mM for rice and 117 mM for tomato (LC_{50} value) by placing the nine endophytes treated pre-germinated seeds on germination paper and incubated at 25 °C. There were four replications for each treatment and un-inoculated pre-germinated seeds were maintained as control. Observations for shoot and root length were recorded after 14 days for rice and 7 days for tomato. The treatment details are as follows.

Treatment details	
Rice	Tomato
T_1 : Control (Sterile water)	T_1 : Control (Sterile water)
T_2 : Bacterial endophyte	T_2 : Bacterial endophyte
T_3 : NaCl (150 mM)	T_3 : NaCl (117 mM)
T_4 : NaCl (150 mM)+ Bacterial endophyte	T_4 : NaCl (117 mM)+ Bacterial endophyte

3.6 Screening of bacterial endophytes imparting drought tolerance in rice and tomato

Bacterial suspension was prepared as described earlier (Zhang *et al.,* 2014) and inoculated to pre-germinated seeds of rice and tomato. The pre-germinated seeds inoculated with eight endophytes were subjected to drought stress (PEG 8000) by placing the seeds on germination papers soaked with 14.3% PEG for rice and 15.3% PEG for tomato as per LC_{50} values. The control was maintained using sterile distilled water. Thus treated seeds were incubated at 25º C for 14 days for rice and 7 days for tomato

respectively. The observations were recorded for shoot and root length after incubation. The treatment details are given below.

Treatment details	
Rice	Tomato
T_1 : Control (Sterile water)	T_1 : Control (Sterile water)
T_2 : Bacterial endophyte	T_2 : Bacterial endophyte
T_3 : PEG-8000 (14.3%)	T_3 : PEG-8000 (15.3%)
T_4 : PEG-8000 (14.3%)+ Bacterial endophyte	T_4 : PEG-8000 (15.3%)+ Bacterial endophyte

3.7 Confirmation of inoculated bacterial endophytes in the seedlings of rice and tomato under salinity and drought stress by re-isolation

The endophytes colonised in the rice and tomato seedlings were re-isolated on NA medium. The root, shoot and leaves were cut into bits using sterile blades. The segments were surface sterilized by treating with 70 per cent ethanol for 1 minute followed by Sodium hypochlorite solution (1 %) for 1 minute and repeatedly washed with sterile water (Shaik *et al.,* 2019). Then the surface sterilized segments were inoculated to NA and incubated at 30ºC for 24 h. The re-isolated bacteria was confirmed with mother culture by examining their morphological characteristics and microscopic observation.

3.8 Identification of salinity and drought tolerant bacterial isolates

The morphological characteristics of bacterial isolates were studied by observing their colony characteristics (colour, margin, elevation and translucency), Gram reaction and cell shape as described by Kalaivanan and Mohan, (2017). Then the nine isolates which showed tolerance to salt and drought stresses were identified using 16S rRNA gene sequencing.

3.8.1 Isolation of Genomic DNA of endophytic bacteria

The genomic DNA of the nine endophytic bacteria were extracted by alkaline lysis method (Sambrook *et al.,* 1989). The bacteria were grown individually in LB (Luria-

Bertani) broth for 24 hours at 30 °C. The cells were pelleted by centrifuging at 12,000 rpm for 2 minutes. Then the pellet was re-suspended in 650 µL of extraction buffer (100 mM Tris HCl, pH 8.2, 100 mM EDTA and 250 mM NaCl) and incubated at 65 °C for 30 minutes. To the extract, 100 µL of 5 M potassium acetate solution was added and placed on ice for 10 minutes for precipitation of protein and carbohydrates. The supernatant was collected by centrifuging at 12000 rpm for 8 min. The DNA was precipitated by adding equal volume of chilled Isopropanol for 2 hrs at -20 °C and then it was centrifuged at 12,000 rpm for 5 min. The pellet obtained was washed twice with 70 per cent chilled ethanol, air dried and dissolved in 20 µL of sterile distilled water. The RNA present in the sample was removed by treating the sample with 5 µL of RNase A at 37 ^{0}C for 1h.

Agarose gel electrophoresis

Agarose gel (0.8 percent) was prepared by dissolving 0.34 g of agarose in 40 mL of 1X TAE buffer and melted. The intercalating dye ethidium bromide (2.5 µl) was added and poured into the gel casting tray placing single comb at one side and allowed for solidification. Then the comb was removed, and the gel was transferred to gel tank containing 1X TAE buffer. The gel loading dye was added to the known quantity of DNA and the electrophoresis was carried out at 100 V for 30 minutes. The DNA band in the gel was visualized under UV transilluminator and documented using gel documentation unit. The concentration and purity of DNA was measured using UV spectrophotometer.

3.8.2 PCR (Polymerase Chain Reaction)

The two primers (26 bp forward primer 5’ GTTAGATCTTGGCTCAGG ACGAACGC 3’ and 24 bp reverse primer 5’ GATCCAGCCGCACCTTCCGATACG 3’) already reported for 16S rRNA sequences from the NCBI (http://www.ncbi.nlm.nih.gov) were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly for the PCR reactions. PCR was performed in 20 µL volume reaction mixture containing the following PCR components.

PCR components (20 µl)

✓ 2.0 µL of 1X PCR Taq. Buffer with $MgCl_2$ (1.5 mM)

✓ 2.0 µL of 10 mM dNTP's mix (200 µM)

✓ 0.5 µL of each forward and reverse 16S rRNA primers

✓ 0.3 µL of Taq DNA Polymerase (1U Genei Bengaluru)

✓ 1.0 µL of Template DNA (~50 ng /µL)

✓ 13.7 µL of Sterile distilled water

PCR amplification was carried out with an initial denaturation at 96 ℃ for 4 minutes followed by 35 amplification cycles consisting of 94 ℃ for 1 minute, 55 ℃ for 30 seconds and 72 ℃ for 1 minute and a final extension at 72 ℃ for 12 minutes. Controls for PCR reaction were carried out with the same primers without providing template DNA. Then the amplified product of DNA was electrophoresed using 1 percent agarose gel as described earlier. The DNA band was visualized under UV light and documented using gel documentation unit. Amplified DNA was eluted by using gel elution kit (The Gene JET™ Gel Extraction Kit, Thermo Scientific) and sequenced by Chromgene Biotech Pvt. Ltd., Bengaluru, Karnataka. The sequence data received from the company was analysed for homology using NCBI GenBank.

3.8.3 Sequence analysis and homology search

Sequence results were analysed with online software of National Centre for Biotechnology Information (NCBI), USA. The Basic Local Alignment Search Tool (BLAST) search gave the partial length sequence homology with NCBI data (http//www. Ncbi.nlm.nih.gov/BLAST/) (Altschul *et al.,* 1997).

3.8.4 Phylogenetic analysis

Phylogenetic analyses were performed to know the relationship between identified species with the species deposited in the NCBI GenBank. Preliminary pairwise and multiple alignments were performed using ClustalW for all nine endophytes sequenced

data independently. Phylogenetic analyses were performed using MEGA7 software and phylogenetic trees were generated using the neighbour-joining algorithm.

3.9 Characterization of selected bacterial endophytes for plant growth promotion and stress tolerance traits

3.9.1 Phosphate solubilization

The qualitative evaluation of tricalcium phosphate solubilization in the isolated strains was done using Pikovskaya's agar. Selected bacterial isolates were spot inoculated on the surface of Pikovskaya agar medium and incubated at 28 °C for 72 h. The phosphate solubilizing activity was estimated after incubation. The formation of a clear zone around the colony indicated inorganic phosphate (Gour, 1980).

3.9.2 Hydrogen cyanide production

Isolates were streaked individually on nutrient agar dispensed plate amended with 4.4g/L glycine except control. Under aseptic conditions, Whatman filter paper strip coated with an alkaline picric acid solution (2.5 g of picric acid and 12.5 g of Na_2CO_3 in 100 mL of sterile distilled water) was put in the upper lids of inoculated petri plates. The plates were incubated at 28 °C, for 24 hours. The colour of Whatman filter paper soaked with alkaline picric acid changed from yellow to orange, indicating the HCN production by the isolates (Bakker and Schipper, 1987).

3.9.3 Siderophore production

The bacterial isolates were spot inoculated on nutrient agar supplemented with universal Chrome Azurol S (CAS) reagent for 24h at 30 ^{o}C. The appearance of a clear orange colour indicated siderophore production (Schwyn and Neilands, 1987).

3.9.4 Ammonia production

The bacterial isolates were tested for ammonia production by the method described by Cappuccino and Sherman, (1992). The bacterial cultures were inoculated into 10 mL peptone water and incubated at 30 ± 0.1 °C for 72 h. After incubation 0.5 mL Nessler's

reagent was added into each tube. The development of brown to yellow colour is considered as positive for ammonia production.

3.9.5 Proline production

Proline content accumulated in bacterial isolates was estimated in nutrient broth with stress (2.0 M NaCl and 20 % PEG-8000) and without stress. Inoculated broth was incubated for 48 h and quantified following the method described by Mishra *et al.* (2011). Two mL of culture was centrifuged at 10,000 rpm for 10 minutes. Cell pellets were kept in water bath with 80 % ethanol at 60 °C for 45 minutes. Further, ethanol added suspension was centrifuged at 8000 rpm for 15 min and 1 mL of supernatant was collected. The supernatant was mixed with 1 mL of acid ninhydrin and 1 mL of glacial acetic acid. The reaction mixture tube was kept in boiling water for 1 h and transferred to ice bath for cooling. Extraction of proline from the reaction mixture was done by adding 2 mL of toluene. Then the extracted proline appears pinkish to red colour, which was separated and transferred to new tubes and the absorbance was measured using the spectrophotometer, BioMate 3S, USA at 520 nm. The results were expressed as µg of proline per mL (µg mL^{-1}) of bacterial culture (Ceylan *et al.*, 2012).

3.9.6 Quantification of Indole acetic acid, Gibberellic acid, Abscisic acid and Salicylic acid production using HPLC

The 24 h old cultures were inoculated in 20 mL nutrient broth containing with stress (2.0 M NaCl and 20 % PEG-8000) and without stress. For IAA, tryptophan was amended in the broth and incubated at 30 °C for 7 days. After incubation they were centrifuged at 6000 rpm for 10 minutes and the supernatant was collected, which was adjusted to a pH of 2.8 using 1 N HCl solution. The acidified supernatant was taken in 100 mL conical flask and equal volume of diethyl ether was added and incubated for 4 h at 4 °C. The solvent phase (upper layer) formed was collected and allowed to evaporate. To the evaporated samples 2 - 3 mL of HPLC grade methanol was added and stored at -20 °C after membrane filtration to perform high performance liquid chromatography (Patten and Glick, 2002).

3.10 Evaluation of salt and drought tolerant bacterial endophytes for their ability to impart salinity and drought stress tolerance in rice and tomato under greenhouse conditions

A pot experiment was carried out under greenhouse conditions in the Department of Agricultural Microbiology, UAS, GKVK campus, Bengaluru-560 065, to evaluate the selected salinity and drought stress tolerant bacterial endophytes to study their ability to impart salinity and drought stress tolerance in rice and tomato.

a. Analysis of soil physico-chemical properties

The soil physico-chemical properties were analysed prior to the experiment. Salt requirement to maintain the 4 dS/m EC was calculated and salinity stress was imposed in the pot experiment.

Physico-chemical properties of the soil

Parameters	Value	Method
Soil pH	7.32	Potentiometry (Jackson, 1973)
Electrical conductivity (dS/m)	0.65	Conductometry (Jackson, 1973)
Available K_2O (kg /ha)	39	Flame photometry method (Page *et al.,* 1982)
Exchangeable Na (meq/lt)	0.35	Flame photometry method (Page *et al.,* 1982)

b. Determination of field capacity of potting mixture

Field capacity is the amount of soil moisture or water content held in the soil after excess water has drained away. There are different models and/ or approaches to calculate the soil available water (SAW) for plants. The field capacity of potting mixture was determined by Gravimetric method as described by Earl, (2003).

Ten empty plastic pots were weighed using weighing balance and the weight was used as an empty pot value (W). All the pots were filled with the potting mixture containing red sandy loam soil: sand: FYM (2:1:1) and their weight was used as a dry weight value (WD). These pots were saturated with water during evening hours to minimize the

evaporation loss of water and the excess was allowed to drain. The weight of saturated soil was taken in the early morning and this weight was used as wet weight value (WW). The field capacity was calculated as follows.

Weight of an empty plastic pot (15 Kg capacity) = W

Weight of plastic pot + potting mixture (red sandy loam soil, sand and FYM) = W_D

Weight of plastic pot + saturated soil = W_W

Field capacity (100 %) = $(W_W - W) - (W_D - W)$

Similarly, 50 % Field capacity value were calculated.

Weight of an empty plastic pot (Capacity – 15kg) (W) = 0.512 kg.

Weight of Plastic pot + potting mixture (sand, soil, FYM) (W_D)= 14.66kg.

Weight of Plastic pot + saturated soil (W_W)= 16.71 kg.

Field capacity (100 %) = $(W_W – W) – (W_D - W)$ = 2.05 kg = 2050 mL.

Similarly, 50 % FC = 1025 mL.

Therefore, for 100 % FC 2050 mL, 50 % FC 1025 mL of water was added initially and maintained daily by weighing the pot.

c. Pot experiment for salinity and drought stress in rice and tomato

Fifteen kg capacity plastic pots were filled with the mixture of soil, sand and Farm yard manure (FYM) in the ratio of 2:1:1. Pre-germinated, rice (IR 64) and tomato (Arka Saurabh) seedlings were treated with bacterial suspension as mentioned earlier. These seeds were transferred to pro-trays containing autoclaved coir pith and allowed to grow for 15 and 25 days for Rice and Tomato plants. Then the seedlings were transferred carefully to the pots. The plants were subjected to salt stress according to Karnal method (Tomer and Minhas, 2005) and drought stress was imposed at 50 % field capacity by Gravimetric method (Earl, 2003). Two seedlings in each pot were maintained and growth, yield, physiological parameters and enzymatic activity under salinity and drought stress conditions were recorded for rice plants (30, 60, 90 and120 DAT) and for tomato plants (30, 60, 90 and 140 DAT).

Treatment details for rice and tomato under salinity stress

Treatment details	
Rice	Tomato
T_1- Control	T_1- Control
T_2- Salt stress (4 dS/m)	T_2- Salt stress (4 dS/m)
T_3- *Enterobacter hormaechei* PBE 8	T_3- *Enterobacter cloacae* NBE 20
T_4- Salt stress (4 dS/m) + *Enterobacter hormaechei* PBE 8	T_4- Salt stress (4 dS/m) + *Enterobacter cloacae* NBE 20
T_5- *Pseudomonas fluorescens* NBE 7	T_5- *Enterobacter asburiae* NBE 23
T_6- Salt stress (4 dS/m) + *Pseudomonas fluorescens* NBE 7	T_6- Salt stress (4 dS/m) + *Enterobacter asburiae* NBE 23

Treatment details for rice and tomato under drought stress

Treatment details	
Rice	Tomato
T_1- Control (100 % FC)	T_1- Control (100 % FC)
T_2- 50 % FC	T_2- 50 % FC
T_3- 100 % FC+*Bacillus cereus* PBE 2	T_3- 100 % FC+ *Lysinibacillus macrolides* PBE 6
T_4- 50 % FC+ *Bacillus cereus* PBE 2	T_4- 50 % FC+ *Lysinibacillus macroides* PBE 6
T_5- 100 % FC+ *Pseudomonas chlororaphis* PBE 4	T_5- 100 % FC+ *Stenotrophomonas maltophilia* CBE 11
T_6- 50 % FC+ *Pseudomonas chlororaphis* PBE 4	T_6- 50 % FC+ *Stenotrophomonas maltophilia* CBE 11
T_7- 100 % FC+*Stenotrophomonas maltophilia* CBE 11	T_7- 100 % FC+ *Acinetobacter lwoffii* NBE 5
T_8- 50 % FC+ *Stenotrophomonas maltophilia* CBE 11	T_8- 50 % FC+ *Acinetobacter lwoffii* NBE 5

d. Salinity stress imposition by Karnal method

The plants were subjected to salt stress at 24 days for tomato and rice after transplanting according to Karnal method (Tomer and Minhas, 2004). Salinity stress of 4 dS/m was imposed and maintained for 20 days in the respective pots by adding solution of dissolved salts in sterile distilled water. The composition of salts used is given below.

Composition of salts used for imposing salinity stress (4 dS/m)

Salts	mg/L
$CaCl_2.2H_2O$	1225
NaCl	384
$MgSO_4.7H_2O$	319
$MgCl_2.6H_2O$	1549

e. Drought stress imposition by gravimetric approach.

Drought stress was imposed at 24 days for tomato and rice after transplanting by withholding water until the required soil field capacity (50 %) was reached and the required level of FC was maintained by gravimetric approach. All pots were weighed daily in the morning to know the water loss through transpiration and the value was noted. The amount of water to be added to maintain the field capacity was calculated and added to their respective pots using measuring cylinder. The exposed soil surface was covered with the mulch to minimize soil evaporation (Earl, 2003).

f. Observations recorded for growth and yield of rice and tomato

The growth, yield and physiological parameters under salt and drought stress condition were recorded for rice plants (30, 60, 90 and 120 DAT) and tomato plants (30, 60, 90 and 140 DAT) at different intervals.

Growth parameters recorded in rice and tomato plants

Plant height: The plant height was measured from the soil surface to the tip of the growing point in rice and tomato plants and expressed in centimetre (cm).

Number of leaves per plant: Number of fully opened leaves were recorded in both rice and tomato plants.

Number of tillers per plant: Number of tillers were recorded in rice plants.

Number of branches per plant: Number of branches were recorded in tomato plant.

Yield parameters recorded in rice and tomato plants

Yield parameters for rice plant (No. of panicles, No. of seed/ plant and Seed yield g/ plant) and for tomato plant (No. of fruits/ plant, Fruit weight-g/plant were recorded.

Physiological parameters recorded in rice and tomato plants

Physiological parameters like Relative water content (RWC), photosynthetic pigments, proline content and electrolyte leakage were measured at 45 DAT for tomato and rice by following methods.

Relative water content (RWC)

Relative water content was calculated by measuring the fresh weight, turgid weight and dry weight of the known number of leaf discs from treatment plants. Fully expanded leaves were excised and fresh weight was recorded. Later, the leaf discs were soaked in distilled water for 3 hours and then the turgid weight was taken. The samples were kept in an oven at 80º C for 4 days. The dry weight of the sample was documented (Ali and Ashraf, 2011). The RWC of leaves from different plant treatment was calculated using the following formula and expressed as a percentage.

$$RWC = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

Chlorophyll estimation

The leaf bits (0.5 g) of 3rd fully opened leaf were taken and incubated in acetone and dimethyl sulphoxide (DMSO) at 1: 1 proportion for 8 hours in dark. After incubation, the optical density of the extract was measured at 645 nm, 663 nm and 470 using UV-

visible spectrophotometer. Total chlorophyll content and carotenoid content was derived according to the method described by Mafakheri *et al.* (2010).

Chlorophyll a (mg/g of fresh weight) = $(12.7\ A_{663} - 2.69\ A_{645}) \times V/1000 \times 1/$ Fresh weight

Chlorophyll b (mg/g of fresh weight) = $(22.9\ A_{645} - 4.68\ A_{663}) \times V/1000 \times 1/$ Fresh weight

Total Chlorophyll (mg/g of fresh weight) = $(20.2A_{645} - 4.68\ A_{663}) \times V/1000 \times 1/$ Fresh weight

Total Carotenoid content (mg/g fresh weight) = $[1000A_{470}$- (3.27 Chl a+104 Chl b)/22.

Extraction and Estimation of Proline

Proline was estimated using the method described by Sankar *et al.* (2016). tomato and rice leaf bits (250 mg) were extracted with 5 mL of 3% sulfosalicylic acid. The extractant was centrifuged at 10,000 rpm for 10 minutes. The supernatant collected (2 mL) was treated with 2 mL of acid-ninhydrin reagent and glacial acetic acid and boiled for 1 hour at 100º C. Chromophore was extracted using toluene (4 mL) and absorbance was read at 520 nm. Amount of proline was calculated using the following formula.

$$\text{Proline } (\mu\text{moles/g tissue}) = \frac{\mu\text{gm of proline/ml} \times \text{ml of toluene} \times 5}{115.5} \times \text{g of sample.}$$

Assay of electrolyte leakage

To quantify electrolyte leakage in plant leaves subjected to stress, procedure of Mishra *et al.* (2011) was followed. Fully grown leaf was collected from each treatment, and was rinsed and placed in conical flask containing 15 mL of Milli Q water for 24 h at 25±2 °C. After incubation at 25°C for 24 h, the conductivity (E_1) was measured with conductivity meter. Subsequently, the tissue was placed in a 100 °C water bath for 30 min, and then cooled to 25°C. A second conductivity measurement was made (E_2). The electrical conductivity of Milli Q water was also measured (E_0). The relative electrolyte leakage (REL) was calculated as follows.

$$\text{Relative electrolyte leakage (in \%)} = \frac{E_1 - E_2}{E_2 - E_0} \times 100$$

Estimation of enzymatic activity

a) Catalase activity

Five hundred milligram of fresh leaf sample was ground with liquid nitrogen to obtain a homogenized mixture in 3 mL of 100 mM potassium phosphate buffer and used as enzyme source. Catalase activity was assayed spectrophotometrically as described by (Chaparro-Giraldo *et al.,* 2000) using 3 Ml assay mixture containing 100 mM potassium phosphate buffer (pH 7.5), 100 µl enzyme extract and 2.5 mM H_2O_2 (prepared fresh before use). The activity was measured by monitoring the degradation of H_2O_2 using UV-visible Spectrophotometer at 240 nm over one and two minutes against a plant extract-free blank. The decrease in H_2O_2 was followed as the decline in optical density at 240 nm. Catalase activity was calculated using the extinction coefficient ($\varepsilon 240nm = 40\ mM^{-1}\ cm^{-1}$) for H_2O_2 and expressed in mol $min^{-1}\ g^{-1}$ of leaf tissue.

b) Peroxidase activity (POD)

The POD activity was measured at 450 nm according to the procedure followed by Subhas (1990). The rate formation of O-polyphenol Dehydrogenation product is a measure of POD activity. The 3 mL assay mixture contained 50 mM potassium phosphate buffer (pH 6.4), 1% of o-phenyl diamine (OPD), 0.3% H_2O_2 and enzyme extract. The mixture was spectrophotometrically measured for absorbance at 450 nm for 3 min at every minute interval; substrate blank was maintained for every sample run. The POD activity was expressed in unit/mg^{-1} protein.

3.11 Re-isolation of salinity and drought tolerant bacterial endophytes from inoculated rice and tomato plants

The endophyte treated plants from each treatment were taken for re-isolation of endophytes. The root, shoot, leaves were cut into segments of approximately 0.5 cm randomly using sterile blades. The segments were surface sterilized as mentioned in section

3.7. Then they were dried on sterilized tissue paper and small sections of these explants were placed on the surface of Nutrient agar plates (3 explants/plate) at 30 ºC for 24 h. The re-isolated bacteria were further identified by 16S rRNA gene sequence to confirm whether or not they were the ones inoculated to seeds.

3.12 Statistical analysis

The data obtained from pot experimentation were statistically analyzed using Web Agri Stat Package 2 statistical tool (www.icargoa.res.in/wasp2/index.php) and means were separated by Duncan Multiple Range Test (DMRT). The original data (with zero values) were transformed using logarithmic transformation and the transformed data were analyzed.

IV RESULTS AND DISCUSSION

Experiments on screening of bacterial endophytes against abiotic stress and evaluation of selected bacteria imparting abiotic stress tolerance in rice and tomato were carried out in the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore-65. The results of experiments are discussed in this chapter.

4.1. Screening of bacterial endophytes for salinity and drought tolerance

Endophytes are capable of enhancing plant growth by improving stress tolerance in plants through the production of various metabolites or modulation of host plant gene expression (Hardoim *et al.,* 2008). In the present study fifty-eight bacteria isolated from plants growing in different regions (Pangong, Chang La and Namkila La) of Himalayan cold desert were screened for salt tolerance using different concentrations of NaCl (0 M, 0.5 M, 1.0 M, 1.5 M, 2.0 M, 2.5 M). Of which only 9 bacterial isolates which are designated as PBE 4, PBE 6, PBE 8, PBE 15, CBE 12, NBE 7, NBE 20, NBE 21 and NBE 23 showed tolerance to NaCl at 2.0 M (Table-1, 2 ,3; Fig.1). Remaining 49 isolates grew up to 1.5 M NaCl concentration. This indicated their susceptibility to increased concentration of NaCl as salinity imbalances the normal physiological process in the cell (Munns and Tester, 2008). Based on the above observations, these 9 bacterial endophytes were selected for further studies.

To determine the drought tolerance *in-vitro*, fifty-eight bacterial isolates were screened against different concentrations of PEG-8000 (0%, 5%, 10%, 15%, 20%, 25 %). Of which eight bacterial isolates (PBE 2, PBE 4, PBE 6, PBE 8, PBE 14, CBE 11, CBE 13 and NBE 5) showed drought tolerance at 20 per cent PEG (-0.51 Mpa) (Table-4, 5, 6. Fig. 2). Other 50 isolates grew growth up to 15 per cent of PEG and did not show any growth at 20 per cent. This indicated that they were susceptible to increased concentration as the drought stress might have caused membrane instability and reduce the activity of superoxide dismutase which reduced drought stress in endophytes (Sun *et al.,* 2010). Therefore, these eight bacterial endophytes were selected for further studies on drought stress. Aswathy *et al.* (2020) reported that the bacterial endophytes isolated from leaves of *Ananas comosus* tolerated water potential up to -1.5 MPa. Similarly, Sandhya *et al*. (2017)

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

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)	0.5M	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

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^{c})	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

Table 4: Effect of different concentrations of PEG MW- 8000 on growth of endophytic bacteria isolated from Pangong region plants.

Bacterial isolates	Optical density at 600 nm indicating bacterial growth					
	Control (0%)	5 %	10 %	15 %	20 %	25 %
PBE 1	0.70 (1.10^{k})	0.38 (0.62^{l})	0.23 (0.85^{lm})	0.10 (0.81^{gh})	0 (0.71^{f})	0 (0.71)
PBE 2	1.78 (1.51^{c})	1.34 (1.16^{b})	0.91 (1.19^{a})	0.61(1.05^{a})	0.45 (0.98^{a})	0 (0.71)
PBE 3	0.65 (1.07^{n})	0.58 (0.76^{f})	0.32 (0.91^{j})	0.18 (0.83^{fg})	0 (0.71^{f})	0 (0.71)
PBE 4	1.84 (1.53^{b})	1.45 (1.20^{a})	0.82 (1.15^{b})	0.45 (0.97^{c})	0.26 (0.87^{c})	0 (0.71)
PBE 5	0.86 (1.17^{i})	0.68 (0.82^{e})	0.55 (1.02^{e})	0.24 (0.86^{e})	0 (0.71^{f})	0 (0.71)
PBE 6	1.92 (1.56^{a})	1.22 (1.11^{c})	0.71 (1.10^{c})	0.51(1.00^{b})	0.45 (0.92^{b})	0 (0.71)
PBE 7	1.08 (1.26^{f})	0.34 (0.58^{m})	0.25 (0.86^{k})	0.16 (0.82^{g})	0 (0.71^{f})	0 (0.71)
PBE 8	1.71 (1.48^{d})	1.31 (1.15^{b})	0.61(1.05^{d})	0.34 (0.91^{d})	0.18 (0.86^{d})	0 (0.71)
PBE 9	0.65 (1.07^{n})	0.48 (0.69$_{j}$)	0.24 (0.86^{kl})	0.12 (0.79$^{h}_{i}$)	0 (0.71^{f})	0 (0.71)
PBE 10	0.66 (1.08^{m})	0.54 (0.74^{g})	0.32 (0.91^{j})	0.21 (0.84^{ef})	0 (0.71^{f})	0 (0.71)
PBE 11	0.89 (1.18^{h})	0.52 (0.72^{h})	0.46 (0.98^{f})	0.18 (0.82^{fg})	0 (0.71^{f})	0 (0.71)
PBE 12	0.98 (1.22^{g})	0.55 (0.74^{g})	0.42 (0.96^{h})	0.12 (0.79^{hi})	0 (0.71^{f})	0 (0.71)
PBE 13	0.58 (1.04^{op})	0.44 (0.66^{k})	0.38 (0.94^{i})	0.18 (0.83^{fg})	0 (0.71^{f})	0 (0.71)
PBE 14	1.52 (1.42^{e})	1.11 (1.06^{d})	0.45 (0.92^{g})	0.21 (0.84^{ef})	0.12 (0.79^{e})	0 (0.71)
PBE 15	0.65 (1.07^{n})	0.48 (0.69^{i})	0.32 (0.90^{j})	0.17 (0.82^{g})	0 (0.71^{f})	0 (0.71)
PBE 16	0.64(1.07^{n})	0.45 (0.67^{j})	0.23 (0.85^{lm})	0.12 (0.79^{hi})	0 (0.71^{f})	0 (0.71)
PBE 17	0.68 (1.09^{l})	0.44 (0.66^{k})	0.22 (0.45^{m})	0.1 (0.78^{i})	0 (0.71^{f})	0 (0.71)
PBE 18	0.74 (1.12^{j})	0.33 (0.57^{no})	0.16 (0.81^{n})	0.1 (0.78^{i})	0 (0.71$^{f)}$	0 (0.71)
PBE 19	0.89 (1.18^{h})	0.58 (0.76^{f})	0.24 (0.86^{k})	0.12 (0.79^{hi})	0 (0.71^{f})	0 (0.71)
CD (P<0.05)	0.01	0.01	0.01	0.02	0.02	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

Table 5: Effect of different concentrations of PEG MW- 8000 on growth of endophytic bacteria isolated from Chang La region plants.

Bacterial isolates	Optical density at 600 nm indicating bacterial growth					
	Control (0 %)	5 %	10 %	15 %	20 %	25 %
CBE 1	1.14 (1.28^{c})	0.39 (0.94^{j})	0.38 (0.62^{h})	0.02 (0.73^{hi})	0 (0.71^{c})	0 (0.71)
CBE 2	0.71 (1.10^{g})	0.55 (1.03^{f})	0.35 (0.59^{j})	0.08 (0.76^{efg})	0 (0.71^{c})	0 (0.71)
CBE 3	1.18 (1.29$_{c}$)	0.53 (1.01^{g})	0.37 (0.60^{i})	0.07 (0.76^{fg})	0 (0.71^{c})	0 (0.71)
CBE 4	0.95 (1.20^{e})	0.41 (0.95^{i})	0.42 (0.65^{f})	0.01 (0.71^{i})	0 (0.71^{c})	0 (0.71)
CBE 5	0.68 (1.09$_{g}$)	0.66 (1.08^{d})	0.69 (0.83^{b})	0.01 (0.71^{i})	0 (0.71^{c})	0 (0.71)
CBE 6	0.79 (1.13^{f})	0.58 (1.04^{e})	0.66 (0.81^{c})	0.01 (0.71^{i})	0 (0.71^{c})	0 (0.71)
CBE 7	0.58 (1.04^{h})	0.35 (0.92^{k})	0.15 (0.38^{n})	0.01 (0.71^{i})	0 (0.71^{c})	0 (0.71)
CBE 8	0.78 (1.13^{f})	0.51 (1.01^{h})	0.44 (0.66^{e})	0.10 (0.74^{gh})	0 (0.71^{c})	0 (0.71)
CBE 9	1.10 (1.25^{d})	0.53 (1.02^{g})	0.31 (0.56^{k})	0.10 (0.78^{def})	0 (0.71^{c})	0 (0.71)
CBE 10	1.01 (1.23^{d})	0.80 (1.14^{c})	0.40 (0.64^{g})	0.18 (0.82^{c})	0 (0.71^{c})	0 (0.71)
CBE 11	1.93 (1.56^{a})	1.41 (1.38^{a})	0.91 (0.95^{a})	0.65 (1.07^{a})	0.52 (1.01^{a})	0 (0.71)
CBE 12	0.50 (1.00^{i})	0.17 (0.82^{m})	0.16 (0.40^{m})	0.11 (0.78^{de})	0 (0.71^{c})	0 (0.71)
CBE 13	1.71 (1.49^{b})	1.12 (1.27^{b})	0.52 (0.72^{d})	0.33 (0.91^{b})	0.22 (0.84^{b})	0 (0.71)
CBE 14	0.35 (0.92^{j})	0.29 (0.89^{l})	0.18 (0.42^{l})	0.12 (0.78^{d})	0 (0.71^{c})	0 (0.71)
CD (P<0.05)	0.02	0.05	0.01	0.02	0.05	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

Table 6: Effect of different concentrations of PEG MW- 8000 on growth of endophytic bacteria isolated from Namika La region plants.

Bacterial Isolates	Optical density at 600 nm indicating bacterial growth					
	Control (0%)	5 %	10 %	15 %	20 %	25 %
NBE 1	0.55 (1.02^{k})	0.25 (0.87^{n})	0.12 (0.79^{m})	0.02 (0.72^{op})	0 (0.71^{b})	0 (0.71)
NBE 2	0.45 (0.97^{m})	0.24 (0.86^{p})	0.11 (0.78^{n})	0.02 (0.72^{p})	0 (0.71^{b})	0 (0.71)
NBE 3	0.55 (1.02^{k})	0.45 (0.98^{h})	0.12 (0.78^{mn})	0.05 (0.74^{lm})	0 (0.71^{b})	0 (0.71)
NBE 4	1.05 (1.24^{b})	0.98 (1.21^{b})	0.50 (1.00^{d})	0.04 (0.74^{m})	0 (0.71^{b})	0 (0.71)
NBE 5	1.55 (1.43^{a})	1.21 (1.31^{a})	0.61 (1.05^{a})	0.41 (0.95^{a})	0.18 (0.82^{a})	0 (0.71)
NBE 6	1.02 (1.23^{c})	0.65 (1.07^{e})	0.45 (0.98^{e})	0.24 (0.86^{c})	0 (0.71^{b})	0 (0.71)
NBE 7	0.56 (1.03^{k})	0.32 (0.90^{m})	0.21 (0.84^{j})	0.14 (0.80^{e})	0 (0.71^{b})	0 (0.71)
NBE 8	0.23 (0.85^{r})	0.10 (0.78^{t})	0.08 (0.76^{op})	0.05 (0.74^{kl})	0 (0.71^{b})	0 (0.71)
NBE 9	0.45 (0.97^{mn})	0.32 (0.91^{m})	0.22 (0.85^{j})	0.12 (0.79^{f})	0 (0.71^{b})	0 (0.71)
NBE 10	0.89 (1.18^{e})	0.40 (0.95^{j})	0.32 (0.90^{h})	0.10 (0.78^{hi})	0 (0.71^{b})	0 (0.71)
NBE 11	0.44 (0.97^{n})	0.35 (0.92^{k})	0.25 (0.87^{i})	0.01 (0.71^{p})	0 (0.71^{b})	0 (0.71)
NBE 12	0.65 (1.07^{j})	0.52 (1.01^{g})	0.44 (0.97^{f})	0.04 (0.74^{m})	0 (0.71^{b})	0 (0.71)
NBE 13	0.78 (1.13^{g})	0.69 (1.09^{d})	0.58 (1.04^{b})	0.35 (0.92^{b})	0 (0.71^{b})	0 (0.71)
NBE 14	0.77 (1.12^{h})	0.58 (1.04^{f})	0.44 (0.97^{f})	0.21 (0.84^{d})	0 (0.71^{b})	0 (0.71)
NBE 15	0.74 (1.11^{i})	0.35 (0.92^{k})	0.25 (0.87$_{i}$)	0.10 (0.78^{i})	0 (0.71^{b})	0 (0.71)
NBE 16	0.56 (1.03^{k})	0.24 (0.86^{nop})	0.12 (0.78^{mn})	0.04 (0.74^{m})	0 (0.71^{b})	0 (0.71)
NBE 17	0.47 (0.99^{l})	0.21 (0.84^{r})	0.11 (0.78^{n})	0.06 (0.75^{k})	0 (0.71^{b})	0 (0.71)
NBE 18	0.85 (1.16^{f})	0.78 (1.13^{c})	0.54 (1.02^{c})	0.07 (0.75^{j})	0 (0.71^{b})	0 (0.71)
NBE 19	0.96 (1.21^{d})	0.44 (0.97^{i})	0.24 (0.86^{i})	0.24 (0.86^{c})	0 (0.71^{b})	0 (0.71)
NBE 20	0.74 (1.11^{i})	0.33 (0.91^{lm})	0.22 (0.85^{j})	0.12 (0.78^{fg})	0 (0.71^{b})	0 (0.71)
NBE 21	0.32 (0.91^{op})	0.24 (0.86^{nop})	0.18 (0.83^{k})	0.05(0.74^{lm})	0 (0.71^{b})	0 (0.71)
NBE 22	0.25 (0.86^{q})	0.12 (0.78^{s})	0.08 (0.76^{q})	0.02(0.72^{op})	0 (0.71^{b})	0 (0.71)
NBE 23	0.96 (1.21^{d})	0.69 (1.09^{d})	0.34 (0.92^{g})	0.11(0.78^{gh})	0 (0.71^{b})	0 (0.71)
NBE 24	0.66 (1.07^{j})	0.34 (0.91^{kl})	0.07 (0.75^{r})	0.03(0.72^{n})	0 (0.71^{b})	0 (0.71)
NBE 25	0.55 (1.02^{k})	0.24 (0.86^{op})	0.14 (0.80^{l})	0.06(0.75^{k})	0 (0.71^{b})	0 (0.71)
CD (P<0.05)	0.01	0.01	0.01	0.01	0.10	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 Endophyte

screened 81 isolates *in-vitro* for drought tolerance in trypticase soy broth supplemented with polyethylene glycol (PEG 6000). Out of 81 isolates, 26 showed maximum tolerance at −0.73 Mpa PEG**.**

4.2 Determination of LC_{50} value of NaCl concentration for rice and tomato seedlings

Rice and Tomato seedlings were tested against salt stress using NaCl concentrations at 25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM and 200 mM. Seedling length of rice decreased from 33.4 cm to 10.9 cm upon increased salt concentration at 200 mM NaCl (Table 7; Plate-1a). The LC_{50} value of NaCl concentration was found 150 mM for Rice (Fig 3). Similarly, tomato seedling length was reduced from 18.7 cm to 10.0 cm at 175 mM NaCl concentration (Table 7; Plate-1b) indicating that the LC_{50} value of NaCl concentration as 117 mM (Fig 3). This suggested that increased NaCl concentrations reduced root and shoot length due to imbalance in ion homeostasis and toxicity which is known as salt-specific or ion-excess effect of salinity (Parida and Das, 2005).

4.3 Determination of LC_{50} value of PEG (MW 8000) concentration for rice and tomato seedlings

For drought stress induction, one of the most popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG) (Landjeva *et al.*, 2008). PEG molecule is inert, non-ionic, virtually impermeable chains and had been used frequently to induce water stress artificially in crop plants (Kaur *et al.*, 1998). In the present study, rice and tomato seedlings were screened for drought tolerance using PEG (MW 8000) at 5, 10, 15, 20 and 25 per cent concentrations and these concentrations correspond to osmotic potential -0.04 MPa, -0.15 MPa, -0.30 MPa, -0.51 MPa and -0.77 MPa respectively. The length of rice seedling decreased from 24.5 cm to 10.0 cm upon increased PEG concentration at 25 % (Table 8; Plate-2a). The LC_{50} value of PEG concentration was found to be 14.3 per cent for rice (Fig 4). Similarly, tomato seedling length was reduced from 19.5 cm to 10.0 cm at 25 % (Table 8; Plate-2b). The LC_{50} value of PEG was found to be 15.3 per cent for tomato (Fig 4). The increased PEG concentration reduced the root and shoot length as the PEG decreased water potential and relative water content in seedlings resulting in decreased growth of plants (Violita and Azhari, 2020). Similar findings were

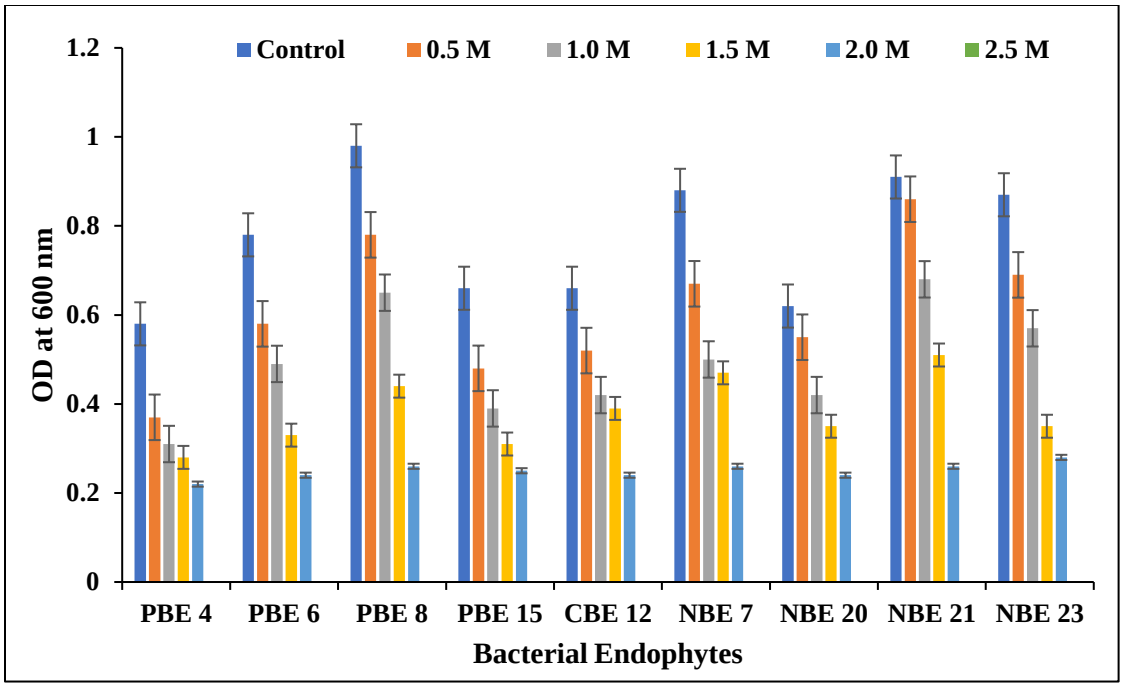


Fig. 1. Growth of salinity tolerant bacterial endophytes at different NaCl concentrations. Lines over bars indicate standard error mean ± SE (n=3).

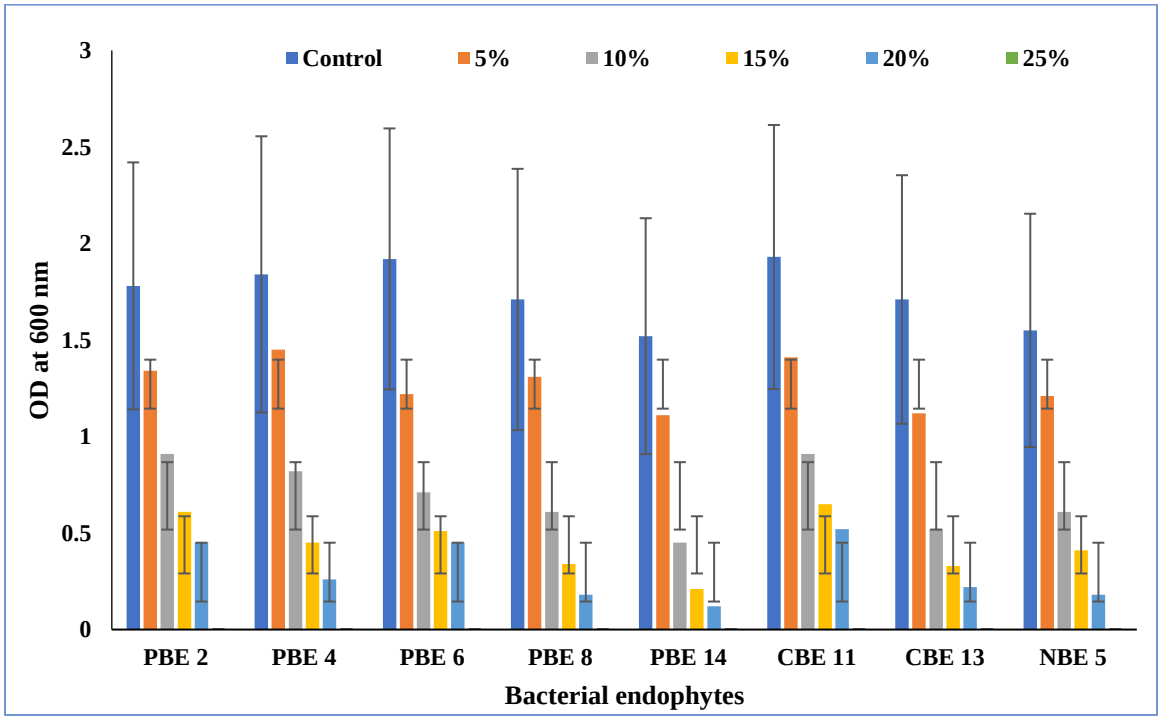


Fig. 2. Growth of drought tolerant bacterial endophytes at different concentrations of PEG-8000. Lines over bars indicate standard error mean ± SE(n=3).

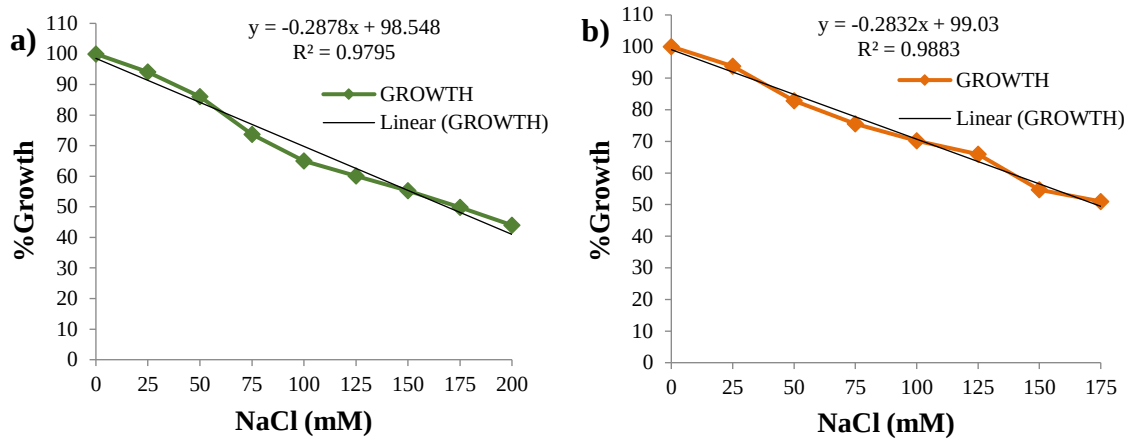


Fig. 3: LC_{50} value of NaCl concentration for (a) rice and (b) tomato seedlings.

Plate 1: Effect of different concentrations of NaCl on seedling growth of a) rice and (b) tomato at 14 and 7 days after germination.

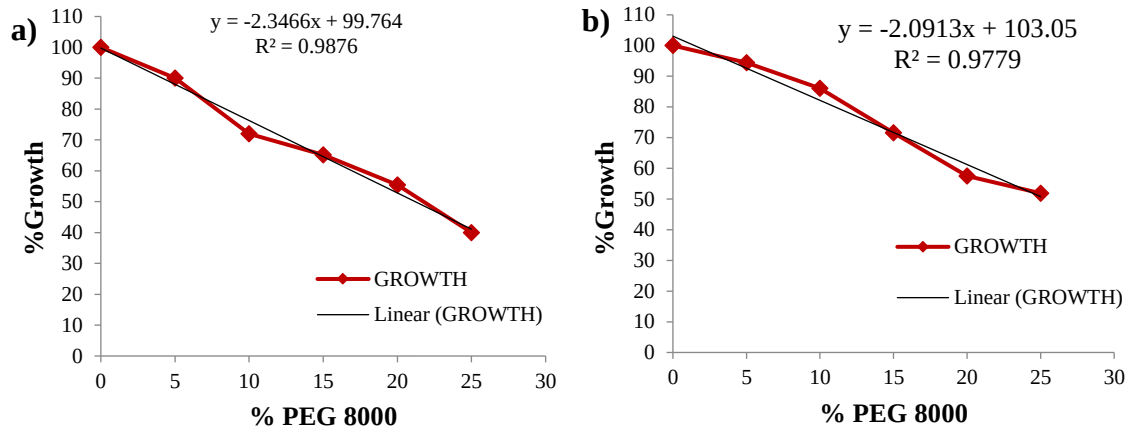


Fig. 4: LC_{50} value of PEG-8000 concentration for (a) rice and (b) tomato seedlings.

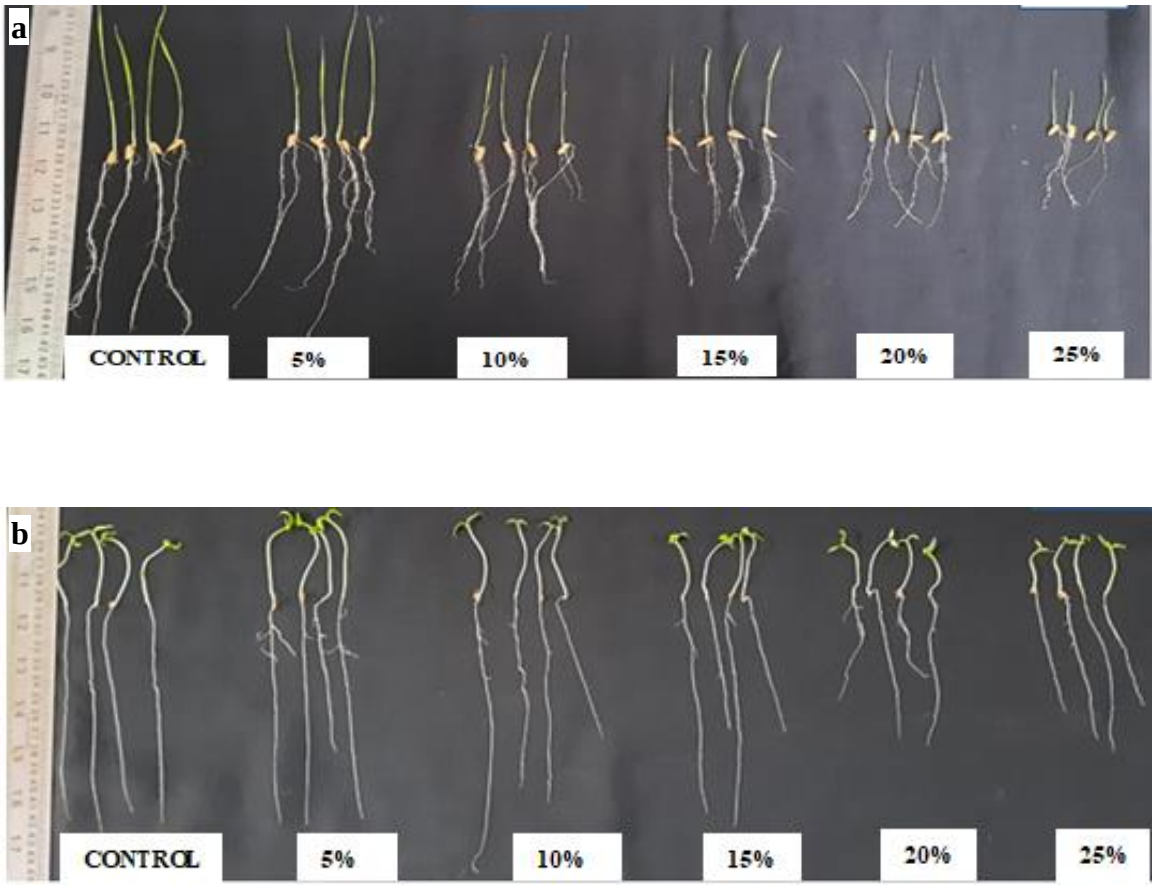


Plate 2: Effect of different concentrations of PEG on seedling growth of a) rice and (b) tomato at 14 and 7 days after germination.

Table 7: Effect of different concentration of NaCl on seedling length of rice and tomato.

Treatments	Seedling length (cm)	
	Rice	Tomato
Control	33.40[a]	18.70[a]
25 mM	31.50[b]	17.20[b]
50 mM	28.40[c]	15.50[c]
75 mM	24.70[d]	14.00[d]
100 mM	21.80[e]	13.50[e]
125 mM	20.50[f]	12.50[f]
150 mM	16.10[g]	11.50[g]
175 mM	14.20[h]	10.00[h]
200 mM	10.90[i]	
CD (P<0.05)	0.45	0.49

Note:

1. LC_{50} value of rice and Tomato seedling under salinity stress was found to be 150 mM and 117 mM by probit analysis.
2. Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT)

Table 8: Effect of different concentrations of PEG (MW-8000) on seedling length of rice and tomato.

Treatments	Seedling length (cm)	
	Rice	Tomato
Control	24.50[a]	19.50[a]
5 % (-0.04Mpa)	21.50[b]	18.20[b]
10 % (-0.14Mpa)	17.50[c]	16.80[c]
15 % (-0.30Mpa)	15.80[d]	13.80[d]
20 % (-0.51Mpa)	13.60[e]	11.00[e]
25 % (-0.77Mpa)	10.00[f]	10.00[f]
CD (P<0.05)	0.40	0.41

Note: LC_{50} value of rice and tomato seedling under drought stress was found to be 14.3 % (-0.27 Mpa) and 15.3 % (-0.33 Mpa) by probit analysis. Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT). Concentration of PEG 8000 is expressed in terms of per cent and corresponding water potential.

reported by Khodarahmpour, (2011) and Muddarsu and Manivannan, (2017) for Corn hybrids and Chilli cultivars respectively.

4.4 Screening of bacterial endophytes against salinity stress in rice and tomato by paper towel method

The pre-germinated seeds of salt sensitive rice and tomato were treated with the nine bacterial endophytes (PBE 4, PBE 6, PBE 8, PBE 15, CBE 12, NBE 7, NBE 20, NBE 21, NBE 23) and subjected to salt stress at 150 mM NaCl for rice and 117 mM NaCl for tomato as per their LC_{50} values. Among the nine bacterial endophytes, the two endophytes (NBE 7 and PBE 8) significantly increased the growth of rice seedlings (shoot and root) at 150 mM NaCl stress (Table 9; Plate 3). Similarly, the root and shoot length of tomato seedlings increased in NBE 20 and NBE 23 bacterial endophytes inoculated plants at 117 mM NaCl stress (Table 10; Plate 4). The uninoculated plants showed the least growth of the seedlings. This suggested that these bacterial endophytes imparted salt tolerance ability to rice as well as tomato seedlings. The stress tolerance ability was due to increased production of superoxide dismutase (SOD), peroxidase (POD) and proline by endophytes in Rice and Tomato plants (Jagadheesh, 2014). Nautiyal *et al*. (2013) reported that the inoculation of *Bacillus amyloliquefaciens* SN13 increased the shoot and root length of tomato at 200 mM NaCl stress. The *Piriformospora indica* inoculated rice seedlings exhibited increased shoot and root length at 200-300 mM NaCl concentrations (Jogawat *et al.*, 2013).

4.5 Screening of bacterial endophytes against drought in rice and tomato by paper towel method

The pre-germinated seeds of rice and tomato were treated with eight selected drought tolerant bacterial endophytes (PBE 2, PBE 4, PBE 6, PBE 8, PBE 14, CBE 11, CBE 13 and NBE 5) and subjected to 14.3 and 15.3 per cent PEG stress (LC_{50} value) using paper towel method. Among the eight bacterial endophytes treated, three endophytes viz., PBE 2, PBE 4 and CBE 11 enhanced the growth of rice seedlings under drought stress (Table 11; Plate 5). This indicated that the endophytes could impart drought tolerance in rice. In tomato, out of eight bacterial endophytes inoculated, three endophytes *viz.*, PBE

Table 9: 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.

Endophytes	Without Salt Stress		With Salt Stress at 150 mM NaCl Concentration	
	Root Length (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Length (cm)
Control	18.00^{b}	16.00^{c}	9.70^{d}	6.50^{c}
PBE 4	19.00^{a}	15.00^{e}	11.30^{c}	3.20^{g}
PBE 6	19.00^{a}	15.00^{e}	11.40^{bc}	4.70^{f}
PBE 8	19.00^{a}	17.00^{a}	14.30^{a}	9.00^{a}
CBE 12	17.80^{cb}	15.50^{d}	11.00^{c}	6.00^{d}
PBE 15	17.90^{cb}	16.50^{b}	9.60^{d}	6.00^{d}
NBE 7	19.00^{a}	16.50^{b}	11.50^{bc}	7.00^{b}
NBE 20	17.30^{d}	15.00^{e}	9.80^{d}	5.40^{e}
NBE 21	16.50^{f}	14.80^{f}	7.50^{f}	5.50^{e}
NBE 23	17.00^{de}	15.40^{d}	8.50^{e}	6.00^{d}
CD (P<0.05)	0.13	0.16	0.30	0.21

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

Table 10: Effect of inoculation of bacterial endophytes on seedling length of tomato with and without salinity stress (117 mM NaCl) after 7 days of germination.

Endophytes	Without Salt Stress		With Salt Stress at 117 mM NaCl Concentration	
	Root Length (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Length (cm)
Control	11.66^{c}	9.60^{c}	7.50^{c}	5.20^{cd}
PBE 4	8.53^{f}	6.10^{e}	4.80^{g}	4.00^{f}
PBE 6	9.46^{e}	6.10^{e}	6.30^{e}	4.00^{f}
PBE 8	6.53^{g}	5.70^{f}	5.40^{f}	4.90^{e}
CBE 12	9.16^{e}	5.70^{f}	7.60^{c}	5.00^{d}
PBE 15	9.50^{e}	6.60^{e}	5.40^{f}	2.80^{g}
NBE 7	9.30^{e}	5.00^{g}	5.50^{f}	5.00^{d}
NBE 20	12.20^{b}	10.10^{b}	8.00^{b}	6.20^{a}
NBE 21	11.20^{d}	7.60^{d}	7.00^{d}	5.70^{b}
NBE 23	13.20^{a}	11.00^{a}	9.40^{a}	5.50^{bc}
CD (P<0.05)	0.38	0.34	0.35	0.21

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

Table 11: Effect of inoculation of bacterial endophytes on seedling length of rice (IR-64) with and without drought stress (PEG at 14.3 %) at 14 days after germination.

Endophytes	Without Drought Stress		With Drought Stress at 14.3 % PEG MW-8000 Concentration	
	Root Length (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Length (cm)
Control	13.50^{d}	12.00^{c}	10.50^{d}	9.20^{b}
PBE 2	15.00^{b}	12.10^{c}	13.00^{b}	9.00^{c}
PBE 4	14.50^{c}	13.00^{b}	12.50^{c}	9.60^{a}
PBE 6	13.10^{e}	10.00^{d}	10.50^{d}	9.10^{c}
PBE 8	15.00^{b}	9.50^{e}	10.00^{e}	6.00^{d}
PBE 14	13.50^{d}	9.50^{e}	10.00^{e}	5.60^{e}
CBE 11	18.50^{a}	14.00^{a}	16.00^{a}	9.00^{c}
CBE 13	10.50^{f}	9.20^{f}	10.00^{e}	5.70^{e}
NBE 5	9.00^{g}	10.00^{d}	10.50^{d}	6.00^{d}
CD (P<0.05)	0.13	0.12	0.24	0.14

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

Table 12: Effect of inoculation of bacterial endophytes on seedling length of tomato (Arka Saurabh) with and without drought stress (PEG at 15.3 %) at 7 days after germination.

Endophytes	Without Drought Stress		With Drought Stress at 15.3 % PEG MW-8000 Concentration	
	Root Length (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Length (cm)
Control	15.80^{d}	6.00^{b}	8.20^{c}	5.00^{b}
PBE 2	4.30^{i}	3.40^{h}	3.10^{f}	3.00^{f}
PBE 4	4.50^{h}	4.00^{g}	4.00^{e}	3.60^{e}
PBE 6	17.50^{c}	5.80^{d}	9.00^{b}	6.50^{a}
PBE 8	14.00^{e}	5.60^{e}	9.10^{b}	4.20^{c}
PBE 14	5.10^{g}	4.50^{f}	1.50^{g}	2.00^{g}
CBE 11	18.00^{b}	5.90^{c}	14.00^{a}	4.10^{c}
CBE 13	5.40^{f}	4.00^{g}	4.20^{d}	3.80^{d}
NBE 5	19.00^{a}	8.50^{a}	14.00^{a}	6.60^{a}
CD (P<0.05)	0.15	0.13	0.17	0.14

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

Plate 3: Effect of bacterial endophytes on growth of rice (IR-64) at 14th day after germination under salinity stress (150 mM NaCl).

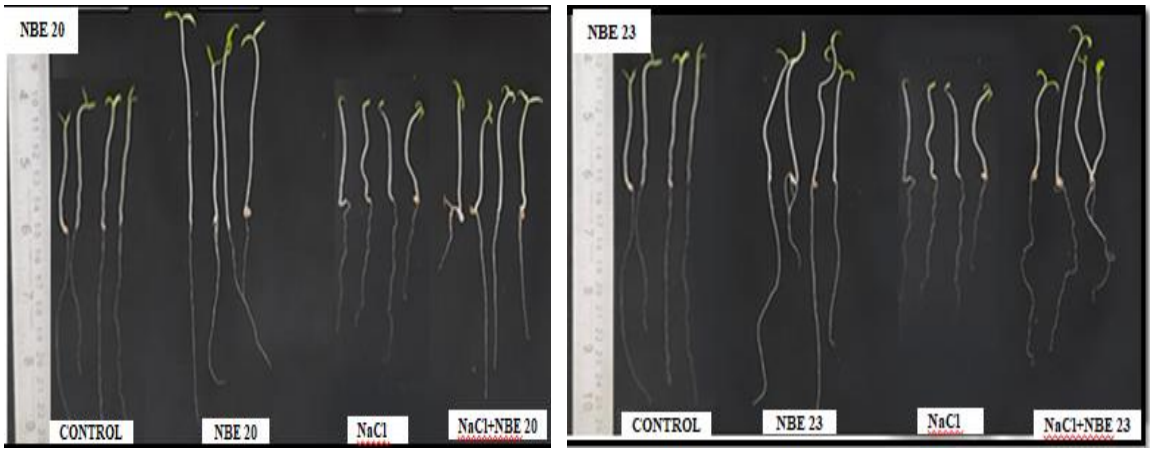


Plate 4: Effect of bacterial endophytes on growth of tomato (Arka Saurabh) at 7th day after germination under salinity stress (117 mM NaCl).

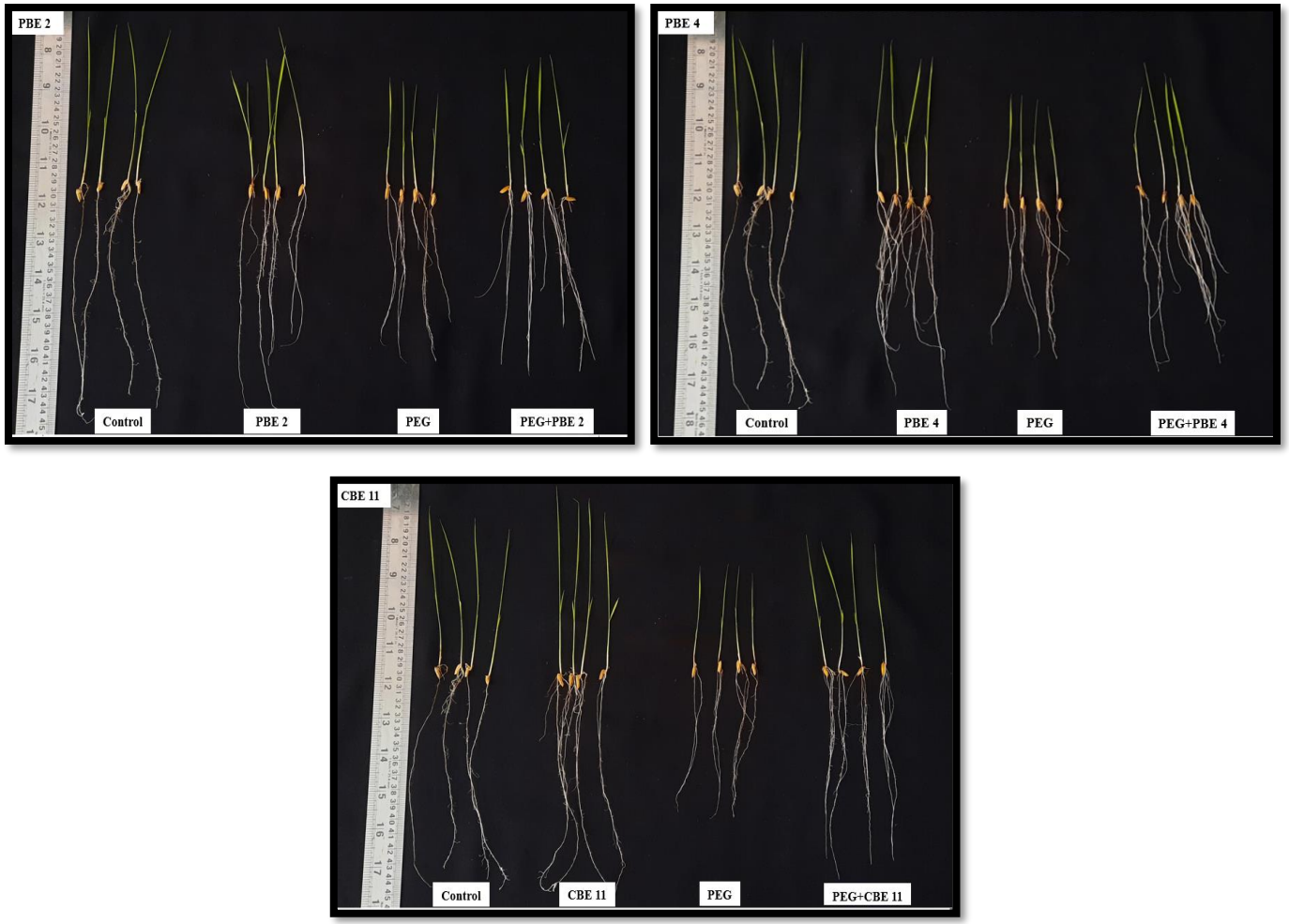


Plate 5: Effect of bacterial endophytes on growth of rice (IR-64) at 14th day after germination under drought stress (14.3 % PEG 8000)

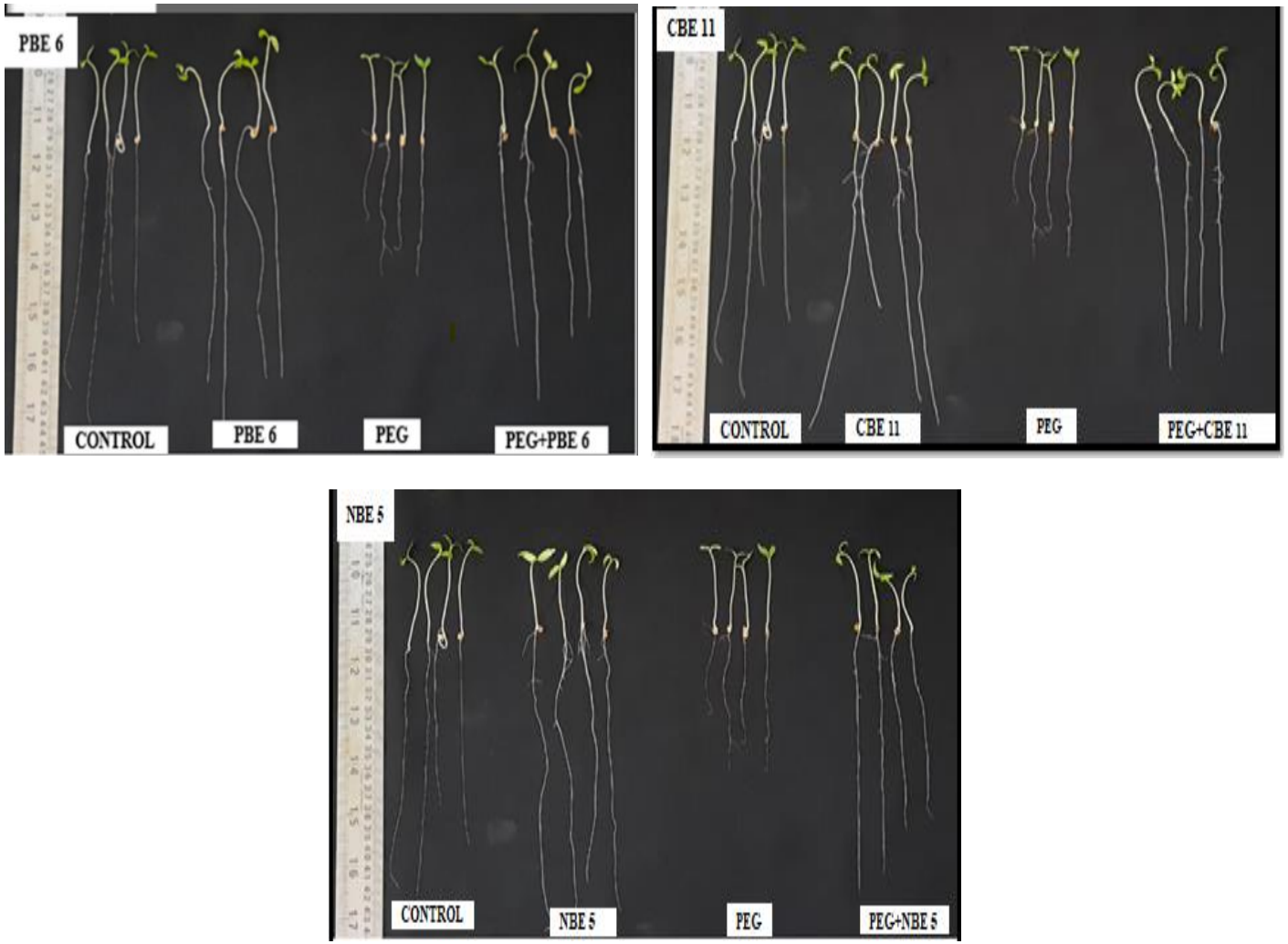


Plate 6: Effect of bacterial endophytes on growth of tomato (Arka Saurabh) at 7th day after germination under drought stress (15.3% % PEG 8000).

Rice leaf

Rice stem

Rice root

Tomato leaf

Tomato stem

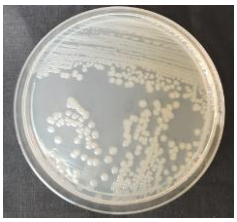
Tomato root

Plate 7: Bacterial growth emerging from tissue of rice and tomato seedlings.

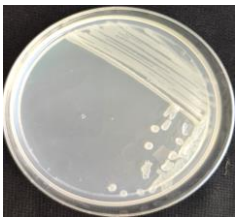
PBE 2 mother culture

PBE 2 Re-isolated culture

PBE 4 mother culture



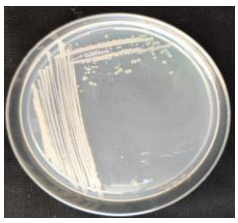
PBE 4 Re-isolated culture



PBE 6 mother culture

PBE 6 Re-isolated culture

PBE 8 mother culture



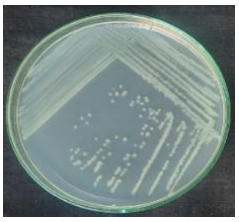
PBE 8 Re-isolated culture

CBE 11 mother culture

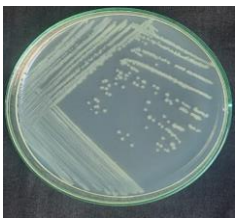
CBE 11 Re-isolated culture

NBE 5 mother culture

NBE 5 Re-isolated culture



NBE 7 mother culture



NBE 7 Re-isolated culture

NBE 20 mother culture

NBE 20 Re-isolated culture

NBE 23 mother culture

NBE 23 Re-isolated culture

Plate 8: Colonies of re-isolated bacteria in comparison with mother (original) culture.

6, CBE 11 and NBE 5 showed increased root and shoot growth at 15.35 % PEG (Table 12; Plate 6) by indicating their ability to induce drought tolerance. The results suggested that the inoculation of bacterial endophytes play a major role in imparting drought tolerance and the growth promotion. Endophyte inoculation was attributed to production of phytohormones and antioxidants in plants (Waqas *et al.,* 2012; Jagadheesh, 2014).

4.6 Confirmation of endophytes by re-isolation from inoculated seedlings

Bacteria were re-isolated from root, shoot and leaves of inoculated rice and tomato seedlings on nutrient agar medium (Plate 7) and confirmed by comparing their morphological characteristics with their mother culture (Plate 8) as reported by Dudeja *et al*. (2011)

4.7 Identification of selected bacterial endophytes

Identification of selected bacterial endophytes was done by morphology and microscopic observation at 100x magnification. Morphological characters revealed some isolates have varied colony colour between off white, creamy white, yellowish. Colony margin was of entire, elevation was observed to be of convex and flat in their nature (Table 13).

4.8 Molecular identification by 16S rRNA gene sequence

The Nine bacterial endophytes (Table 13) were identified by 16S rRNA gene sequences. The genes encoding for 16S rRNA in prokaryotes are most widely used in molecular taxanomy, because they are 1) sufficiently conserved, 2) functionally constant, 3) universally distributed and 4) have adequate length to provide a view of evolution encompassing all living microorganisms (Madigan *et al.,* 2009). Therefore, in the present study all the nine abiotic stress tolerant bacteria were identified based on the homology of the sequences available in NCBI GenBank.

The 16S rRNA partial gene sequence of the bacterial endophyte isolated from Pangong region, PBE 2 (706 bp) showed 99.41% homology of *Bacillus cereus*. (Fig.5a) sequences available in NCBI GenBank. The phylogenetic tree constructed using sequences

of 10 *Bacillus* species by neighbour joining tree method, showed that it is closely related to *Bacillus cereus* strain ATCC 14579 available at NCBI database (Fig.5b). Therefore, the isolate PBE 2 was identified as *Bacillus cereus*.

Table 13: Morphological characterization of selected salinity and drought tolerant bacterial endophytes.

Sl. No.	Isolates	Colour	Margin	Elevation	Cell shape/ Gram's reaction
1	PBE 2	Creamy white	Entire	Convex	Gram positive rods
2	PBE 4	Creamy white	Entire	Convex	Gram negative rods
3	PBE 6	Pale yellow	Entire	Convex	Gram positive rods
4	PBE 8	White	Entire	Flat	Gram negative rods
5	CBE 11	Yellowish	Entire	Flat	Gram negative rods
6	NBE 5	Pale yellow	Entire	Convex	Gram negative rods
7	NBE 7	Greenish yellow	Entire	Convex	Gram negative rods
8	NBE 20	Creamy white	Entire	Convex	Gram negative rods
9	NBE 23	Creamy white	Entire	Flat	Gram negative rods

Note: PBE = Pangong Bacterial Endophyte, CBE = Chang La Bacterial Endophyte, NBE = Namika La Bacterial Endophyte.

The PBE 4 isolate having 1461 bp showed 98.46 per cent homology with *Pseudomonas chlororaphis* available at the NCBI data base. The phylogenetic tree constructed with 10 species showed that it was closely related to *Pseudomonas chlororaphis strain* (Fig. 6a and 6b).

The PBE 6 bacterial isolate sequence with 1472 bp showed 98.78 per cent homology with *Lysinibacillus macroides* available at the NCBI data base. Thus, the bacterium was identified as *Lysinibacillus macroides* and the phylogenetic tree constructed

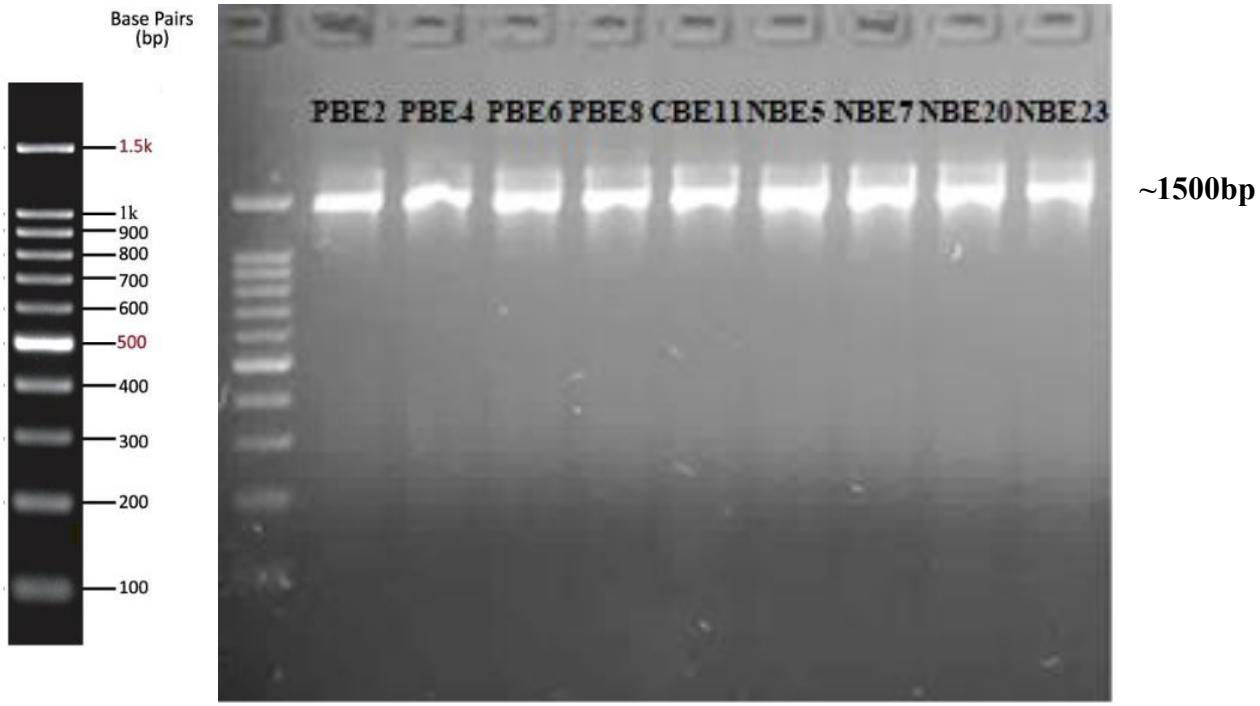
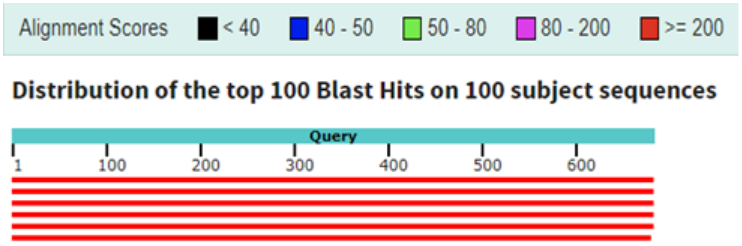


Plate 9: Amplified product of 16S rDNA of bacterial isolates viz., PBE 2, PBE 4, PBE 6, PBE 8, CBE 11, NBE 5, NBE 7, NBE 20 and NBE 23.

PBE 2 (*Bacillus cereus*)

GATGAGTGCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCA
CTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA
CATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAAGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTGATCTTAGTTGCCATCATTAAGTTGGCACTCTAAGGTGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTG
CTACAATGGACGGTACAAAGAGCTGCAAGACCGCAGGTGGAGCTAATCTCATAAAACCG
TTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATC
GCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAA
GGTGGGACAGATGATTGGGGTGAAGTCGTACAAA



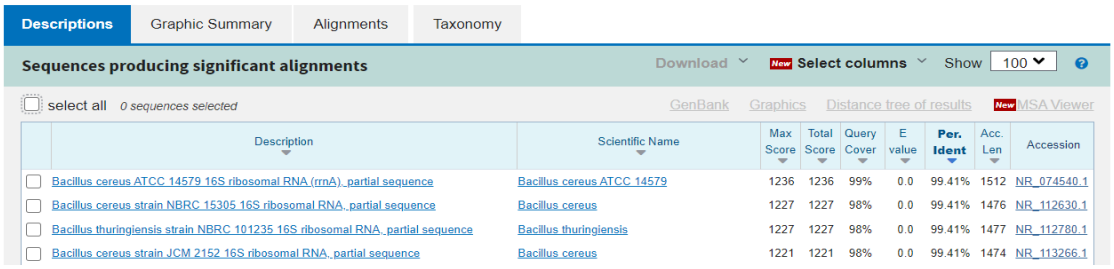


Fig. 5(a). 16S rRNA partial gene sequence of PBE 2 showing 99.41 % homology with *Bacillus cereus* ATCC 14579 (706 bp)

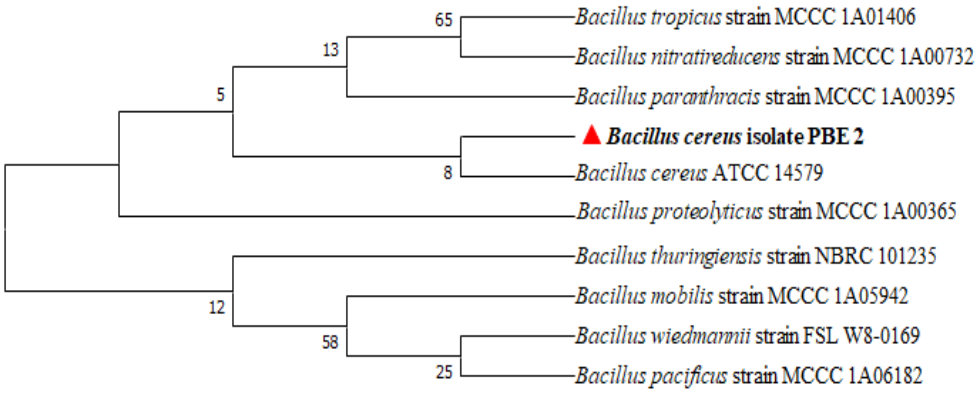


Fig. 5(b). Phylogenetic tree of *Bacillus cereus* isolate PBE 2

PBE 4 (*Pseudomonas chlororaphis*)

TTTAAAGTTTGAATATCTGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCG
GTAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGG
TAGTGGGGGATAACGTTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGG
ACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTC
ACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG
CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCT
AATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCG
GTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTT
AAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTAT
GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTG
GCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGT
GGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCGGGAACATTGAGACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCC
TTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGT
CGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCACAAAACCGATCGTAGTCCGGATC
GCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTG
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CTAGTCTAACCTTCGGGAGGACGGTTACCACGGTGTGAT

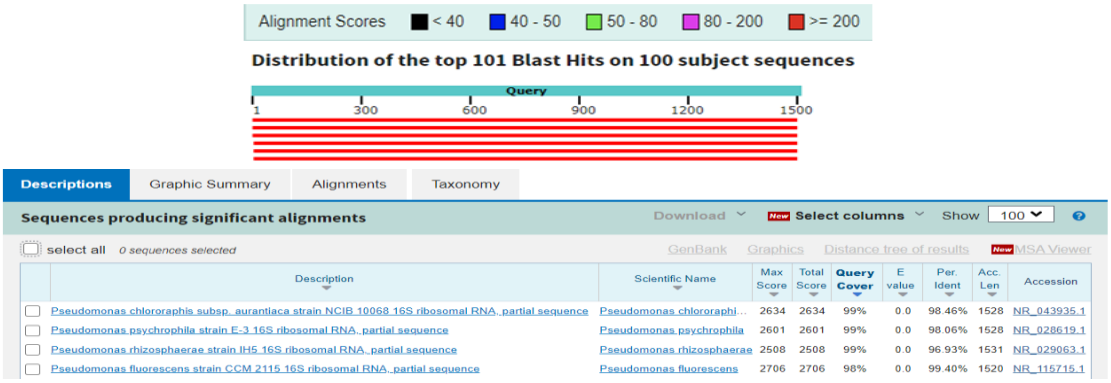

Fig. 6(a). 16S rRNA partial gene sequence of PBE 4 showing 98.46 % homology with *Pseudomonas chlororaphis strain* NCIB 10068 (1461 bp)

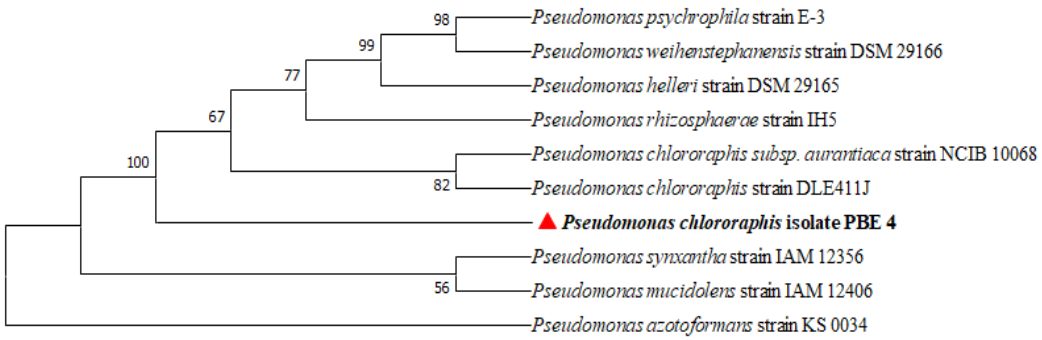

Fig. 6(b). Phylogenetic tree of *Pseudomonas chlororaphis* isolate PBE 4

PBE 6 (*Lysinibacillus macrolides*)

GACGAACGCGGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGAC
GTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTACCTTATAGTTTGGGATAACTCCGGGAAACC
GGGGCTAATACCGAATAATCTCTATTTCACTTCATGGTGAAATACTGAAAGACGGTCTCGGCTGTCGCTA
TAAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCC
GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATC
GTAAAACTCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTA
TTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGG
GTCATTGGAAACTGGGGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGC
GTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGAGGCGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA
GGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAG
ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAC
GCGAAGAACCTTACCAGGTCTTGACATCCCGTTGACCACTGTAGAGATATGGTTTTCCCTTCGGGGAC
AACGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GACGATACAAACGGTTGCCAACTCGCGAGAGGGAGCTAATCCGATAAAGTCGTTCTCAGTTCGGATTG
TAGGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA
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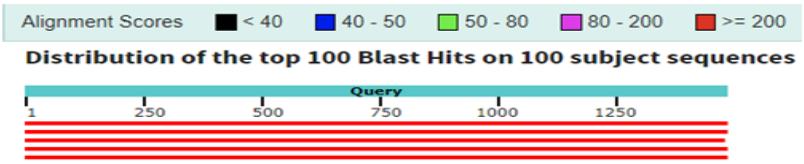

Fig. 7(a). 16S rRNA partial gene sequence of PBE 6 showing 98.78 % homology with *Lysinibacillus macroides* LMG 18474(1472 bp)

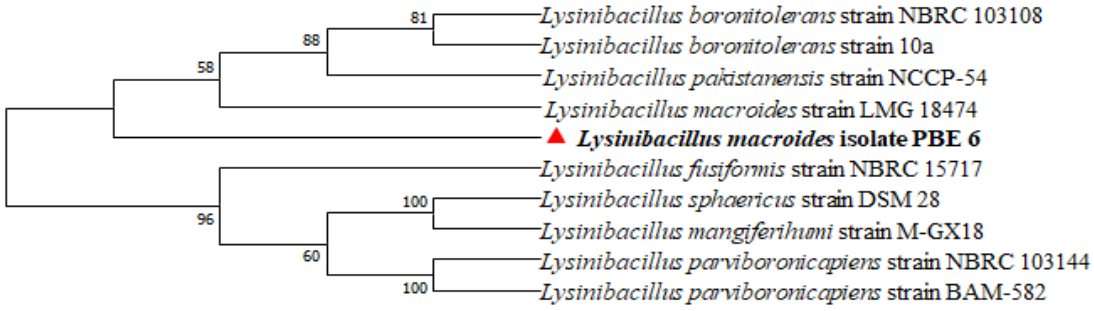

Fig. 7(b). Phylogenetic tree of *Lysinibacillus macroides* isolate PBE 6

PBE 8 (*Enterobacter hormaechei*)

ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTT
TGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACT
GGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCC
ATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCT
AGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGG
CCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTGA
CGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC
CCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAAT
TCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGG
ACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
CGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTA
AGTCGACCGCCTGGAGAGTACGGCCGCAGGATTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGA
GAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGC
GGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGAC
CTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
CATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTG
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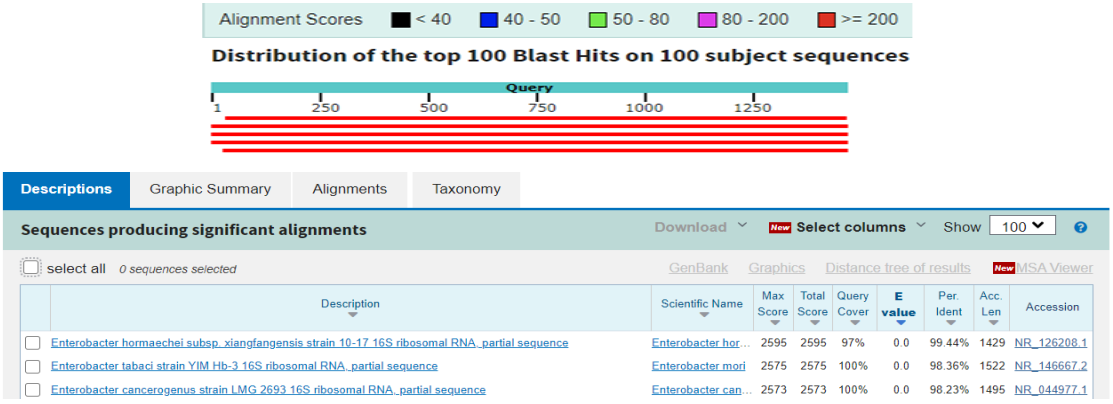

Fig. 8(a). 16S rRNA partial gene sequence of PBE 8 showing 99.44 % homology with *Enterobacter hormaechei strain* 10-17 (1466 bp)

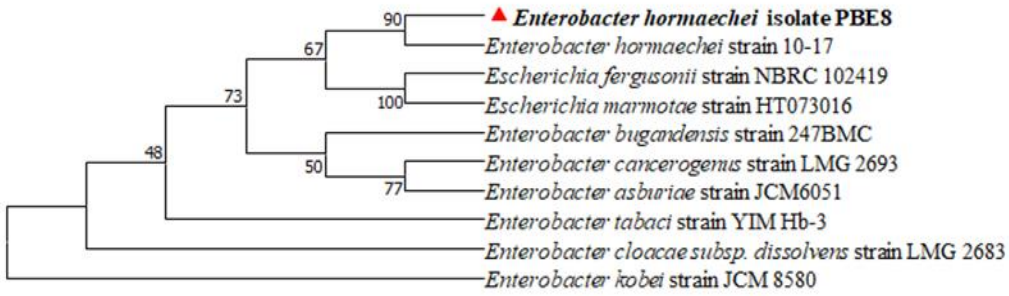

Fig. 8(b). Phylogenetic tree of *Enterobacter hormaechei* isolate PBE8

CBE 11 (*Stenotrophomonas maltophilia*)

GCTCAGAGTGAACGCTGGCGGTAGGCCTAACACATGCAAGTCGAACGGCAGCACAGGAGAGCTTGCTCTCT
GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTTTTTCGTGGGGGATAACGTAGGGAAAC
TTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGCGATTGAATGAGCC
GATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATG
ATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATG
GGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGA
AAGAAATCCAGCTGGCTAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGC
CAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGT
CGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAGTGTG
GTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGG
CAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCATTTGGCACGCAGTATCGAAGCTAACGCGTTAA
GTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTG
GAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAG
ATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAG
ACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACA
CACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTC
AGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTG
CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGCAGG
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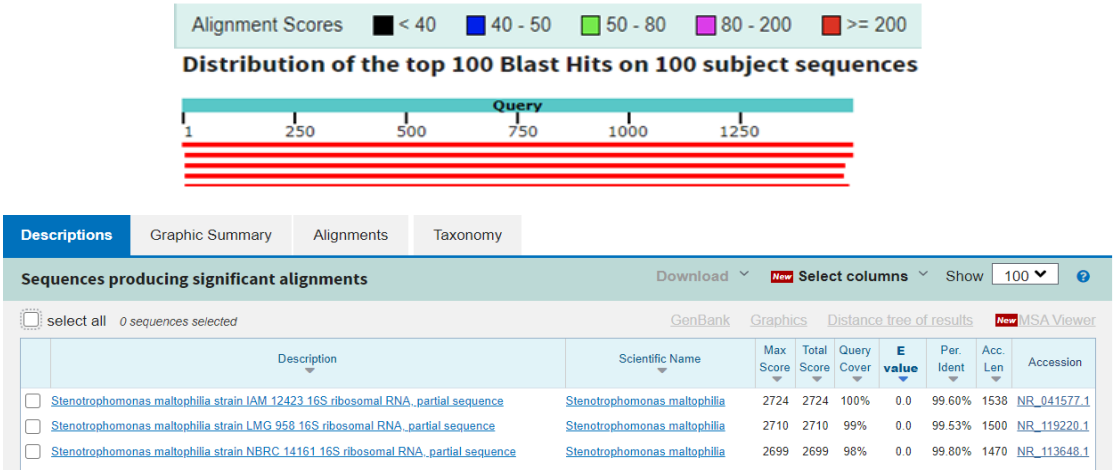

Fig. 9(a). 16S rRNA partial gene sequence of CBE11 showing 99.60 % homology with *Stenotrophomonas maltophilia* strain IAM 12423 (1493 bp)

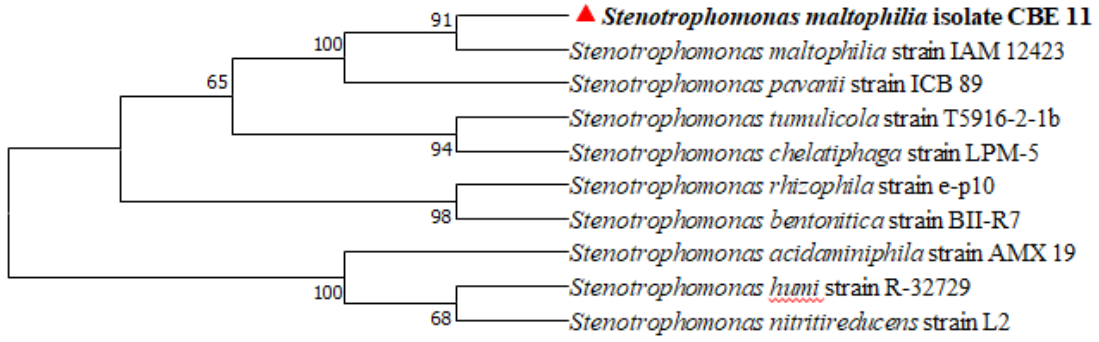

Fig. 9(b). Phylogenetic tree of *Stenotrophomonas maltophilia* isolate CBE 11

NBE 5 (*Acinetobacter lwoffii*)

TGAGTTTTGTTATCTGCTCAGATTGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGGGG
AAGAGTAGCTTGCTACTTGACCTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAG
TGGGGGACAACATCTCGAAAGGGATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACC
TTCGGGCCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACC
AAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAGCCATGCCG
CGTGTGTGAAGAAGGCCTTTTGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACCGAGATTAAT
ACTCTTGGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGT
AATACAGAGGGTGCAAGCGTTAATCGGATTTACTGGGCGTAAAGCGCGCGTAGGTGGCCAATTAA
GTCAAATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATACTGGTTGGCTAGAGTATGG
GAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGC
GAAGGCAGCCATCTGGCCTAATACTGACACTGAGGTGCGAAAGCATGGGGAGCAAACAGGATTAG
ATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCTTTGAGGCTTTAGTGGC
GCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTG
GTCTTGACATAGTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACATACAGGTGCT
GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTT
TCCTTATTTGCCAGCGGGTTAAGCCGGGAACTTTAAGGATACTGCCAGTGACAAACTGGAGGAAG
GCGGGGACGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGTCGG
TACAAAGGGTTGCTACCTCGCGAGAGGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTGGA
GTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGTAGGTA
GTCTAACCTTAGGGGGGACGCTTACCACGGTGT

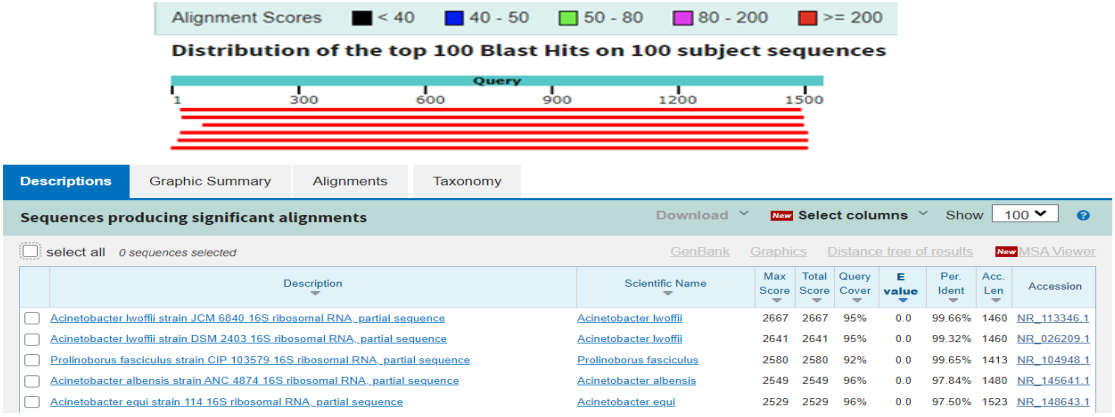

Fig. 10 (a). 16S rRNA partial gene sequence of NBE 5 showing 99.68 % homology with *Acinetobacter lwoffii* JCM 6840 (1489 bp)

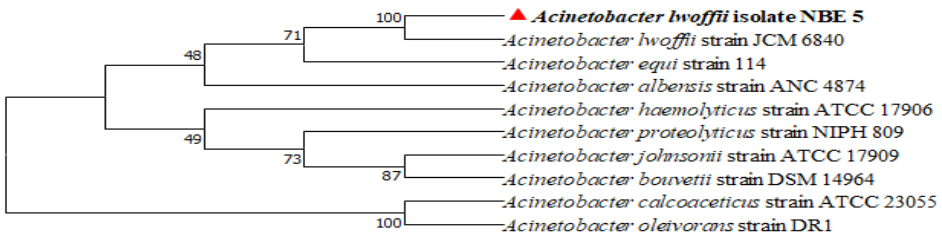

Fig. 10 (b). Phylogenetic tree of *Acinetobacter lwoffii* isolate NBE 5

NBE 7 (*Pseudomonas fluorescens*)

AATCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGAGAGAAGCTTG
CTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTC
GGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATC
AGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCCGTAACTGG
TCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGT
AAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAAT
AAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACT
GGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTG
CATTCAAAACTGACTGACTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGT
AGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGG
AGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTT
AAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCG
AAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCGGGAACATTG
AGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGC
AACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTC
GGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCACAAAACCGATCGTAGTCCGGATCGCAG
TCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAAC
CTTCGGGAGGACGGTTACCACGGTGTGATTCATGACTGGGGTGAAGTCGTACAAAGGT

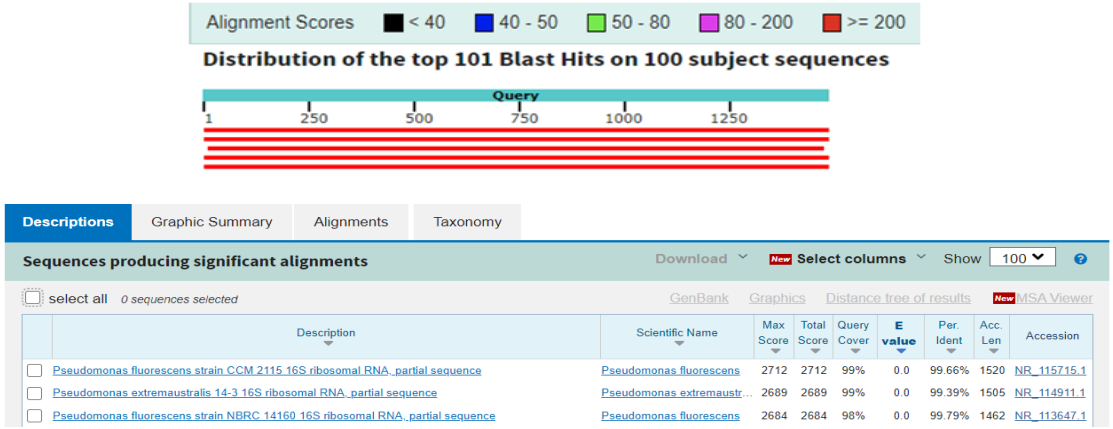

Fig. 11(a). 16S rRNA partial gene sequence of NBE 7 showing 99.66 % homology with *Pseudomonas fluorescens* strain CCM 2115 (1486 bp)

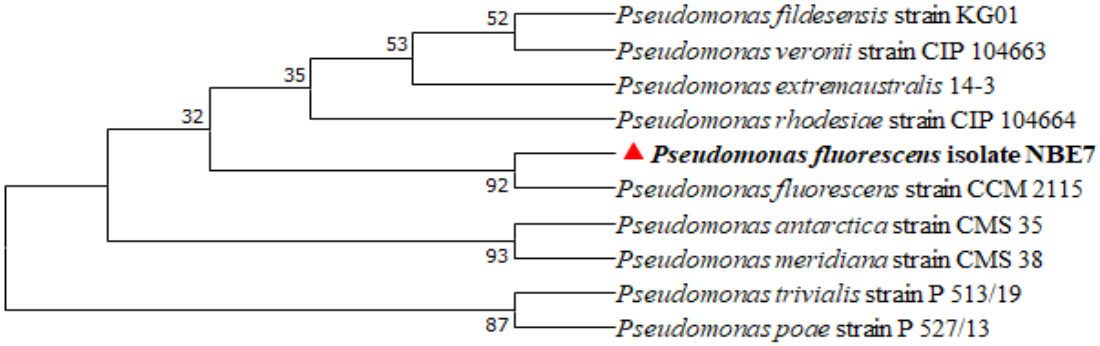

Fig. 11 (b). Phylogenetic tree of *pseudomonas fluorescens* isolate NBE 7

NBE 20 (*Enterobacter cloacae*)

GGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCT
GCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTAC
TGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCAT
CGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTG
GTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTG
TAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTCGTCGATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACT
GCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTG
TGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGAGAGTACGGCCGCAAGGT
TAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGC
GAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCT
GAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCG
CATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAG
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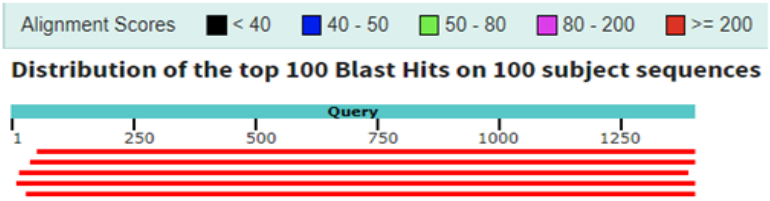

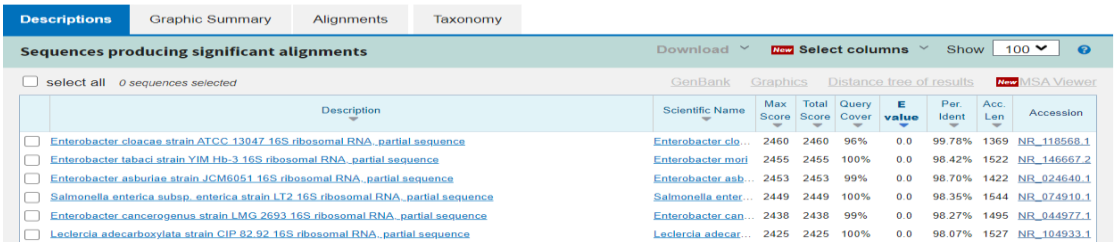

Fig. 12(a). 16S rRNA partial gene sequence of NBE 20 showing 99.78 % homology with *Enterobacter cloacae strain* ATCC 13047 (1395 bp)

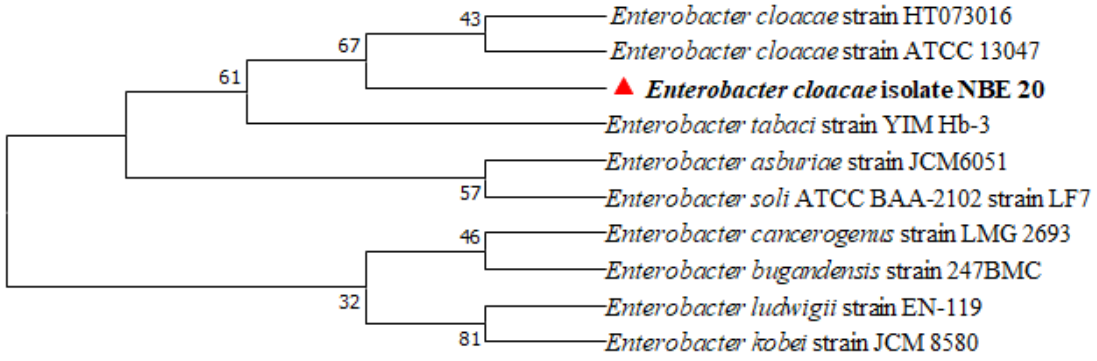

Fig. 12(b). Phylogenetic tree of *Enterobacter cloacae* isolate NBE 20

NBE 23 (*Enterobacter asburiae*)

AAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGG
GTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTG
AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGG
CGATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGCTAACTCCGTGCCA
GCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGG
CGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGG
CTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
ATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGA
GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAAC
TCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAA
GAACCTTACCTACTCTTGACATCCAGAGAACTTTCCAGAGATGGTTTGGTGCCTTCGGGAACTCT
GAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAA
ACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCT
ACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAG
TCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATG
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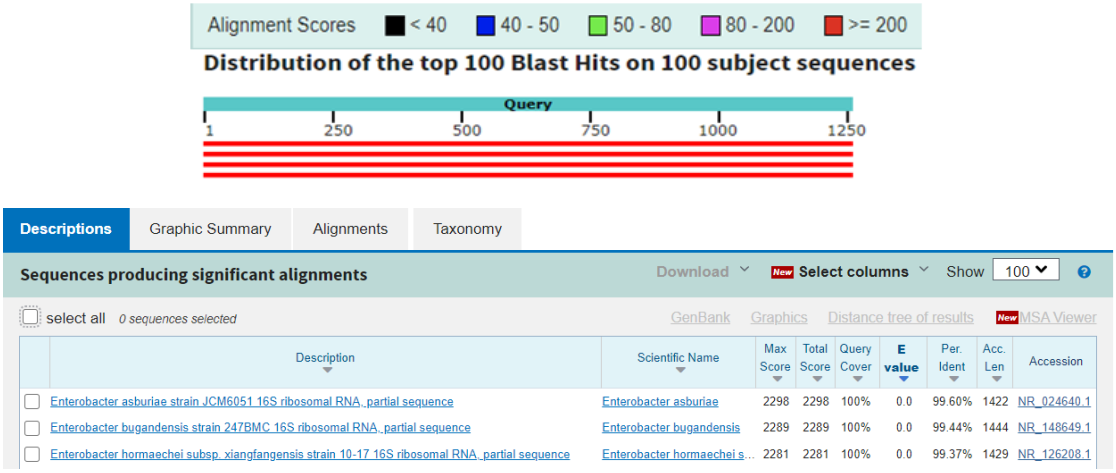


Fig. 13(a). 16S rRNA partial gene sequence of NBE 23 showing 99.60 % homology with *Enterobacter asburiae* JCM6051 (1258 bp)

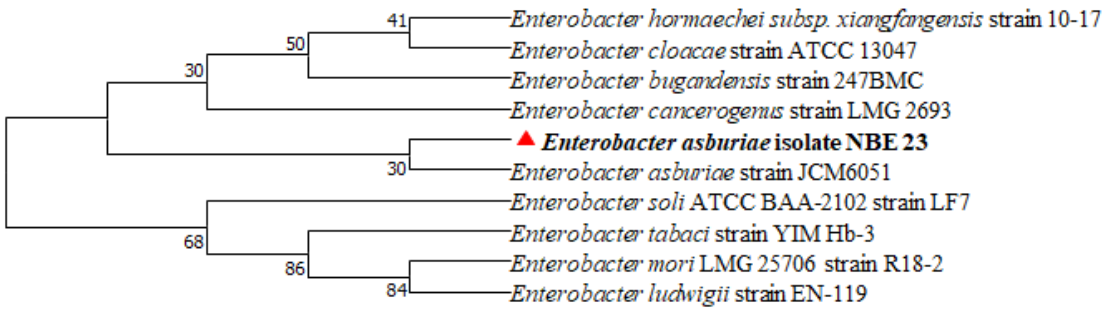


Fig. 13(b). Phylogenetic tree of *Enterobacter asburiae* isolate NBE 23

with 10 bacterial sequences available at NCBI showed as *Lysinibacillus macroides* LMG 18474 (Fig. 7a and 7b).

The PBE 8 isolate with 1466 bp showed 99.44 per cent homology with *Enterobacter hormaechei* and phylogenetic tree constructed with 16S rRNA sequences of 10 Bacteria available at NCBI data base showed as *Enterobacter hormaechei strain* 10-17 (Fig. 8a and 8b).

The partial sequences of the 16S rRNA of the bacterial isolate from Chang La region, CBE 11 consisting of 1493 bp showed 99.60 per cent homology with *Stenotrophomonas maltophilia* and the phylogenetic tree constructed with 10 organisms 16S rRNA sequences showed it as *Stenotrophomonas maltophilia* strain IAM 12423 available in the NCBI database (Fig. 9a and 9b).

The 16S rRNA partial gene sequence of the bacterial isolate from Namkila La region, NBE 5 (1489 bp) showed 99.68 per cent homology with earlier reported *Acinetobacter lwoffii*. The phylogenetic tree constructed using the sequences of 10 organisms showed that it was closely related to *Acinetobacter lwoffii* JCM 6840 available in the NCBI database (Fig.10a and 10b).

The NBE 7 bacterial isolate 16S rRNA sequences having 1486 bp showed 99.66 per cent homology with *Pseudomonas fluorescens* available at the NCBI data base. Thus, the bacterium was identified as *Pseudomonas fluorescens* and the phylogenetic tree constructed with 16S rRNA gene sequences of 10 bacteria showed that it was closely related to *Pseudomonas fluorescens* strain CCM 2115 available in the NCBI database (Fig. 11a and 11b).

The bacterial isolate NBE 20 having 1395 bp 16S rRNA gene sequences showed 99.78 % homology with *Enterobacter cloacae* available at the NCBI data base. The phylogenetic tree constructed with 10 organisms 16S rRNA gene sequences showed that the sequences are matching with *Enterobacter cloacae strain* ATCC 13047 available in the NCBI database (Fig. 12a and 12b).

The NBE 23 isolate with 1258 bp showed 99.60 per cent homology with *Enterobacter asburiae* and phylogenetic tree constructed with 10 organisms showed that it was closely related to with *Enterobacter asburiae* JCM 6051 available in the NCBI database (Fig.13a and 13b).

Thus, the nine selected endophytic bacteria were identified based on the homology of 16S rRNA gene sequences available at NCBI data base. The 16S rRNA gene is said to be golden standard for identification of bacterial species as these sequences are conserved and have sufficient length to understand the identity of bacteria.

4.9 Determination of growth promotion and stress tolerance imparting characters of selected bacteria

4.9.1 Phosphate solubilization

Phosphorus is the second most important nutrient of plant next to nitrogen. Many abiotic stress tolerant bacterial endophytes are reported to solubilize phosphate in the rhizosphere (Orhan, 2016; Kang *et al.*, 2021). In the present study, phosphate solubilization was noticed in seven bacteria. However, two bacteria, *E. hormaechei* and *S. maltophilia* did not show any phosphate solubilization.

4.9.2 Hydrogen cyanide production

Hydrogen cyanide indirectly promotes plant growth and play an important role in pathogen suppression. In the current study nine salinity and drought tolerant endophytes were able to produce HCN. The HCN production was observed in various endophytes in the earlier studies also (Chrouqi *et al*., 2017).

4.9.3 Siderophore production

Siderophores are minute, high affinity iron chelating compounds secreted by microorganism which help in chelating few elements essential for plants**.** In this study, only seven bacterial endophytes *viz., B. cereus, E. hormaechei, S. maltophilia, A. lwoffii, E. cloacae, E. asburiae P. fluorescenes* out of nine produced siderophores. Similarly,

halotolerant endophytes *viz.*, *Bacillus velezenis* and *Bacillus siamensis* exhibited siderophore production (Masum *et al.*, 2018).

4.9.4 Ammonia production

Ammonia produced by the bacteria will be utilized by plants through GOGAT (Glutamine oxoglutarate aminotransferase) pathway and incorporated as amino acids. Results obtained in the present study exhibited production of ammonia by all the nine bacterial endophytes. Chrouqi *et al.* (2017) have also reported ammonia production in eight bacteria isolated from the rhizosphere of wheat.

Table 14: Plant growth promoting traits of salinity and drought tolerant bacterial endophytes.

Sl. No.	Bacterial endophytes	Plant growth promoting traits			
		Phosphate solubilization	HCN	Siderophore	Ammonia production
1	*B. cereus*	+	+	+	+
2	*P. chlororaphis*	+	+	-	+
3	*L. macroides*	+	+	-	+
4	*E. hormaechei*	-	+	+	+
5	*S. maltophilia*	-	+	+	+
6	*A. lwoffii*	+	+	+	+
7	*P. fluorescenes*	+	+	+	+
8	*E. cloacae*	+	+	+	+
9	*E. asburiae*	+	+	+	+

Note: (+) = Presence, (-) =Absence

4.9.5 Proline production

Osmolytes concentration increases in the plants during abiotic stress and mainly involved in monitoring homeostasis of cellular contents in them (Manivannan *et al.*, 2007).

The proline content is increased in bacterial endophytes subjected to abiotic stress (Table 15). However, the bacteria which were not exposed to abiotic stress showed lesser proline content indicating that the proline is an important constituent imparting abiotic stress tolerance. The proline synthesis results in osmotic adjustment, free radical scavenging and stabilization of subcellular structures in cells to overcome the detrimental effects (Hare *et al.,* 1998). Ceylan *et al.* (2012) observed the maximum proline production in *Bacillus aryabhattai* S11 and *Oceanobacillus iheyensis* S1 when exposed to salt stress. Danish *et al.* (2020) reported increased proline accumulation in *Pseudomonas aeruginosa* grown under drought stress.

Table 15: Proline accumulation of salinity tolerant bacterial endophytes without and with stress induced conditions.

Bacterial endophytes	**Proline accumulation (µg mL^{-1})**	
Salinity tolerant	Without NaCl	With 2.0 M NaCl
E. hormaechei	22.30^{d}	28.40^{d}
P. fluorescenes	31.80^{a}	37.50^{b}
E. cloacae	25.60^{c}	33.40^{c}
E. asburiae	29.20^{b}	38.30^{a}
CD(P<0.05)	0.49	0.36
Drought tolerant	Without PEG-8000	With 20% PEG-8000
B. cereus	28.70^{d}	34.30^{d}
P. chlororaphis	33.90^{a}	39.80^{a}
L. macrolides	32.70^{b}	35.50^{c}
S. maltophilia	29.60^{c}	37.70^{b}
A. lwoffii	21.70^{e}	30.80^{e}
CD(P<0.05)	0.33	0.36

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

4.9.6 Determination of Indole acetic acid, Gibberellic acid, Abscisic acid and Salicylic acid production by endophytes

Phytohormones play a vital role on growth and development of plants. The quantified data of Indole acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA) and salicylic acid (SA) production using high performance liquid chromatography (HPLC) is presented in the table 16. The bacterial endophytes grown on the medium amended with precursor L-tryptophan showed the highest production of IAA under abiotic (Salt and drought) stress compared to bacteria grown under normal medium. This envisaged that the tryptophan amendment in medium can enhance the IAA production. Indole acetic acid is the most prevalent kind of auxin, that influences many aspects of plant growth and development. Many bacteria generate and release IAA as a secondary metabolite by exploiting L-tryptophan found in root exudates (Fu *et al.,* 2015).

Gibberellic acid 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 thereby 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 (Table 16). These results are in agreement with those of Goswami *et al.* (2014) who reported bacteria *Kocuria turfanensis* produced the maximum IAA in presence of tryptophan under salt stress. Danish *et al.* (2020) reported the maximum production of IAA without supplementation of L-tryptophan under drought stress by *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Leclercia adecarboxylata.* Ambawade and Pathade (2015) reported that *Bacillus siamensis* BE 76 isolated from the banana plant (*Musa spp*.) produce gibberellic acid of 0.108 µg mL^{-1}. Shahzad *et al.* (2017) reported that there was variation in production of ABA (0.32 ± 0.015 to 0.14 ±0.030 µg mL^{-1}) under normal and saline condition by the bacterial endophyte *Bacillus amyloliquefaciens* RWL-1. Forchetti *et al.* (2010) reported the maximum SA production by endophytic bacteria, *Achromobacter xylosoxidans* and *Bacillus pumilus* isolated from

Table 16: IAA, Gibberellic acid, Abscisic acid and Salicylic acid production by salinity and drought tolerant bacterial endophytes.

Bacterial endophytes	Indole-3-acetic acid (µg mL^{-1})				Gibberellic acid (µg mL^{-1})		Abscisic acid (µg mL^{-1})		Salicylic acid (µg mL^{-1})	
Salinity tolerant	T (-)	T (+)	T (-)	T (+)	Without stress	With stress (2.0M NaCl)	Without stress	With stress (2.0M NaCl)	Without stress	With stress (2.0M NaCl)
	Without stress		With stress (2.0M NaCl)							
E. hormaechei	6.80^{c}	27.60^{d}	15.10^{c}	95.20^{d}	33.20^{c}	60.80^{c}	1.90^{c}	3.40^{d}	30.90^{a}	37.70^{c}
P. fluorescenes	5.50^{d}	36.40^{c}	21.90^{a}	98.90^{c}	30.90^{d}	57.00^{d}	1.80^{c}	3.90^{c}	23.30^{d}	33.70^{d}
E. cloacae	7.10^{b}	41.20^{b}	14.60^{d}	115.90^{b}	43.30^{b}	63.50^{b}	2.50^{b}	5.30^{b}	24.30^{c}	39.90^{b}
E. asburiae	8.60^{a}	46.70^{a}	16.80^{b}	143.60^{a}	55.90^{a}	76.80^{a}	3.30^{a}	5.60^{a}	25.90^{b}	40.70^{a}
CD(P<0.05)	0.13	3.45	0.23	2.34	1.87	2.45	0.21	0.16	0.78	0.24
Drought tolerant	Without stress		With stress (20% PEG)		Without stress	With stress (20% PEG)	Without stress	With stress (20% PEG)	Without stress	With stress (20% PEG)
B. cereus	14.10^{a}	60.20^{a}	30.60^{a}	119.90^{a}	60.90^{a}	80.90^{a}	8.90^{a}	10.10^{a}	34.10^{a}	44.90^{a}
P. chlororaphis	12.70^{b}	52.80^{c}	25.60^{c}	99.80^{c}	55.50^{c}	77.80^{b}	6.50^{c}	8.80^{c}	32.60^{c}	41.80^{c}
L. macrolides	9.90^{d}	49.90^{e}	20.90^{e}	86.60^{e}	52.70^{e}	75.20^{d}	4.40^{e}	5.80^{e}	28.80^{e}	37.70^{e}
S. maltophilia	10.80^{c}	55.70^{b}	29.90^{b}	106.70^{b}	58.70^{b}	76.60^{c}	7.50^{b}	9.60^{b}	33.90^{b}	42.20^{b}
A. lwoffii	7.70^{e}	50.80^{d}	22.10^{d}	88.70^{d}	54.60^{d}	73.10^{e}	5.30^{d}	6.50^{d}	30.90^{d}	38.90^{d}
CD (P<0.05	0.69	0.54	0.87	0.97	0.65	0.76	0.45	0.25	0.14	0.19

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

roots of sunflower grown under drought stress. Gupta and Sinha (2020) reported increased production of salicylic acid under salt stress (27.3 µg L^{-1}) by rhizospheric bacteria *Rubrivivax gelatinosus.*

4.10 Evaluation of selected abiotic stress tolerant bacterial endophytes in rice and tomato under greenhouse conditions

The following bacterial endophytes which performed better under *in vitro* conditions against salinity and drought stress in rice and tomato were evaluated under greenhouse conditions.

Salinity stress		**Drought stress**	
Rice	**Tomato**	**Rice**	**Tomato**
Enterobacter hormaechei	*Enterobacter cloacae*	*Bacillus cereus*	*Lysinibacillus macroides*
Pseudomonas fluorescens	*Enterobacter asburiae*	*Pseudomonas chlororaphis*	*Stenotrophomonas maltophilia*
		Stenotrophomonas maltophilia	*Acinetobacter lwoffii*

4.11 Influence of bacterial endophytes on growth under salt stress in rice and tomato

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 un-inoculated plants. Number of tillers at 60, 90 and 120 DAT significantly enhanced due to *E. hormaechei* inoculation followed by *P. fluorescens* (Fig. 14; Plate 10). 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 *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 17). The photosynthetic

pigments (Chl a, b, total chl and carotenoid) content significantly increased in *E. hormaechei* inoculated plants (Table 18). 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 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 15). 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. This study showed that the *E. hormaechei* was more efficient for rice than *P. fluorescens.*

To study the salt stress tolerance in Tomato, *E. cloacae* and *E. asburiae* were selected. The plants inoculated with *E. cloacae* and *E. asburiae* showed significantly increased plant height. Average number of leaves per plant was significantly increased in *E. asburiae* inoculated plants which was followed by *E. cloacae* under both normal as well as salt stress as compared to un-inoculated plants. The number of branches also increased in *E. asburiae* inoculated plants compared to un-inoculated plants (Fig 16; Plate 11). Increased growth parameters in endophyte inoculated plants under salt stress could be due to production of phytohormones (Khan *et al.*, 2012).

E. cloacae and *E. asburiae* inoculation significantly increased the number of fruits and fruit yield compared to un-inoculated plants (Table 19). Carotenoid and RWC were significantly enhanced in *E. asburiae* treated plants compared to uninoculated plants (Table 20). The proline content did not differ between treatments under normal conditions but under salt stress, *E. asburiae* inoculated plants showed significantly increased proline

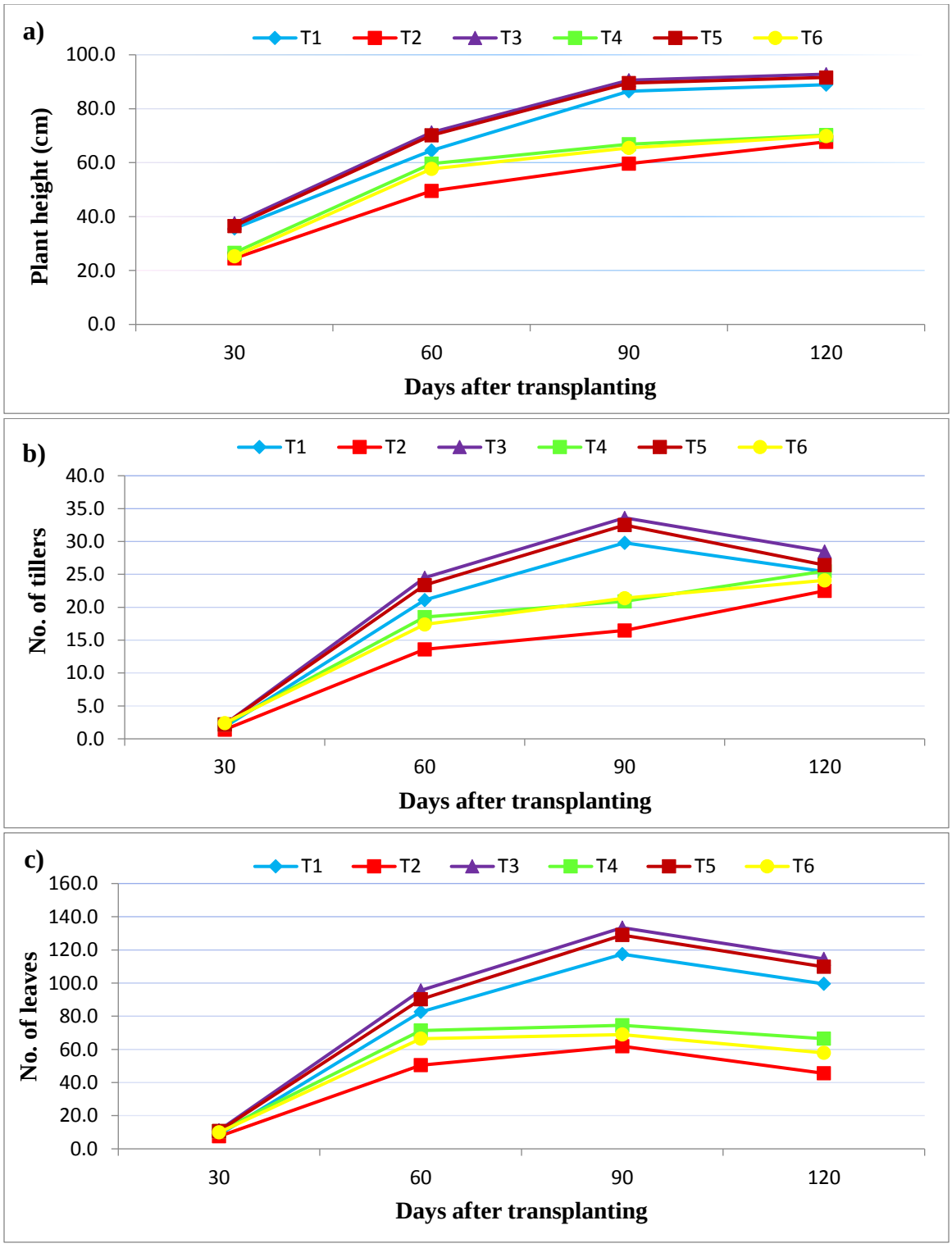


Fig. 14: Effect of bacterial endophytes on growth parameters (a) Plant height (b) No. of tillers (c) No. of leaves of rice under salinity stress (4 dS/m).

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*

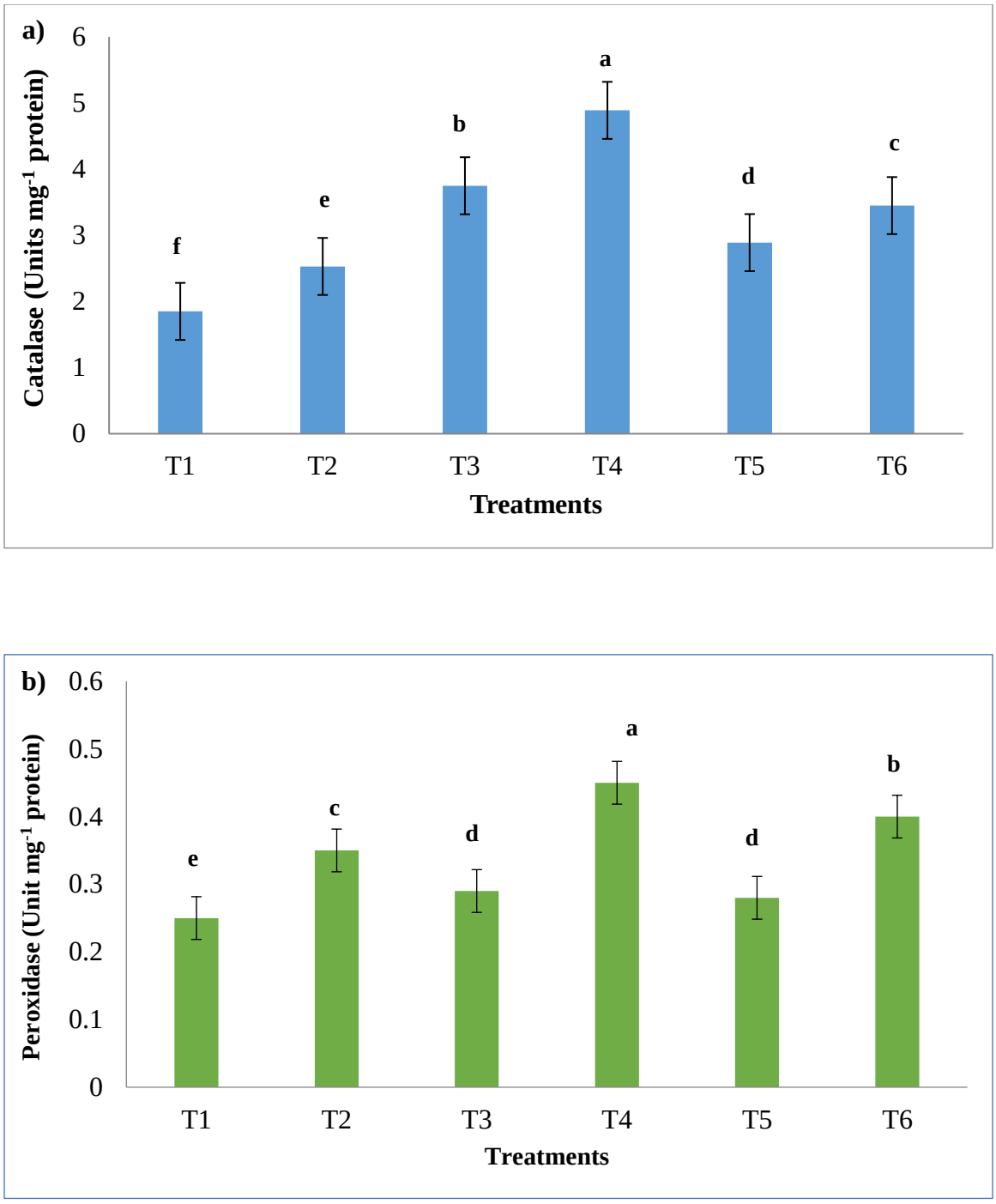
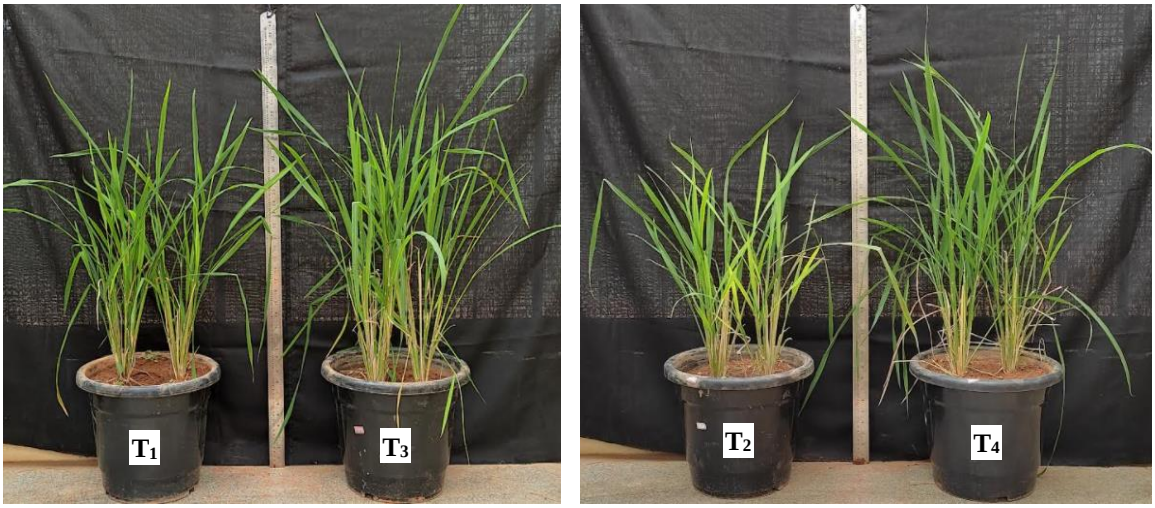
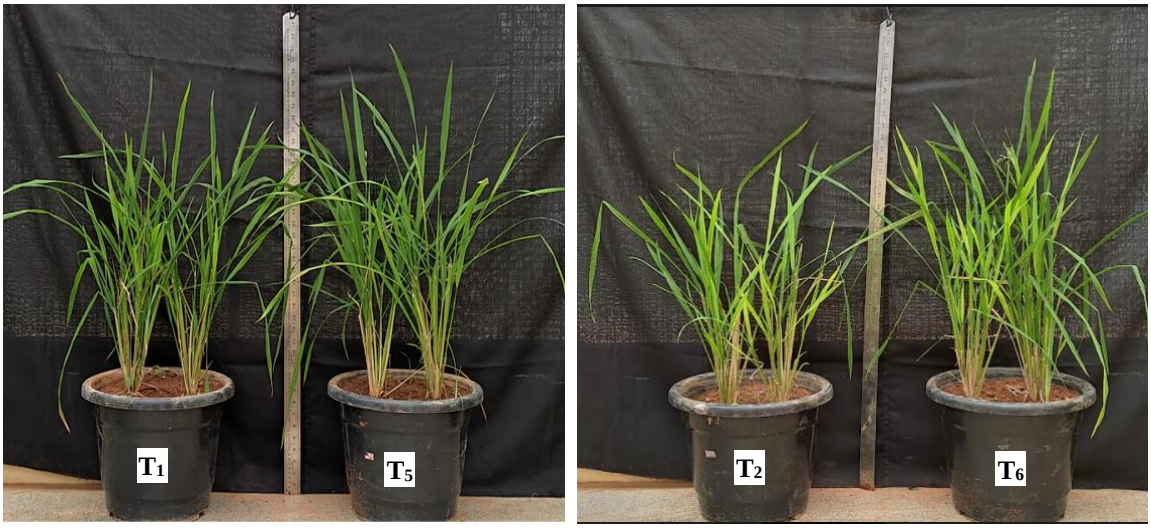


Fig. 15: Effect of bacterial endophytes on (a) Catalase and (b) Peroxidase activity in rice under salt stress (4 dS/m).

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*



Control | *E. hormaechei* | Salt stress (4 dS/m) | Salt stress (4 dS/m)+ *E. hormaechei*



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

Plate 10: 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.

Table 17: 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)

Table 18: Effect of bacterial endophytes on physiological parameters of rice under salinity stress (4 dS/m).

Treatments	Chl a (mg/g FW)	Chl b (mg/g FW)	Total chl (mg/g FW)	Carotenoid content (mg/g FW)	RWC (%)	Proline content (µmol/g FW)	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)

Table 19: Effect of bacterial endophytes on yield parameters of tomato under salt stress (4 dS/m).

Treatments	No. of fruits/plant	Fruits yield/plant(g)
T_1= Control	21.94^{c}	735.35^{c}
T_2= Salt stress (4 dS/m)	12.31^{f}	369.45^{f}
T_3= *E. cloacae*	24.60^{b}	861.37^{b}
T_4= Salt stress (4 dS/m) + *E. cloacae*	17.60^{e}	616.35^{e}
T_5= *E. asburiae*	25.36^{a}	896.40^{a}
T_6= Salt stress (4 dS/m) + *E. asburiae*	19.60^{d}	686.33^{d}
CD (P<0.05)	0.61	17.53

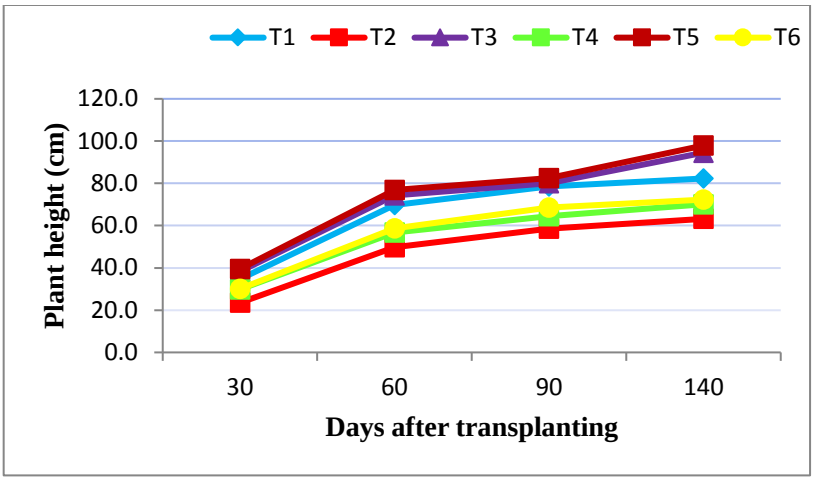
Note: Means with the same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT)

Table 20: Effect of bacterial endophytes on physiological parameters of tomato under salinity stress (4 dS/m).

Treatments	Chl a (mg/g FW)	Chl b (mg/g FW)	Total chl (mg/g FW)	Carotenoid content (mg/g FW)	RWC (%)	Proline content (µmol/g FW)	Electrolyte leakage (%)
T_1= Control	0.74^{a}	0.32^{ab}	0.92^{a}	0.31^{b}	87.90^{d}	6.36^{d}	35.45^{b}
T_2= Salt stress (4dS/m)	0.55^{c}	0.25^{c}	0.72^{c}	0.27^{c}	71.50^{e}	10.25^{c}	55.60^{a}
T_3= *E. cloacae*	0.74^{a}	0.34^{b}	0.92^{a}	0.34^{a}	89.60^{b}	6.35^{d}	32.21^{d}
T_4= Salt stress (4 dS/m) + *E. cloacae*	0.63^{b}	0.35^{b}	0.74^{c}	0.30^{b}	87.60^{d}	11.84^{b}	35.50^{b}
T_5= *E. asburiae*	0.75^{a}	0.40^{a}	0.95^{a}	0.35^{a}	90.80^{a}	6.35^{d}	30.15^{e}
T_6= Salt stress (4 dS/m) + *E. asburiae*	0.65^{b}	0.35^{b}	0.84^{b}	0.31^{b}	88.80^{c}	11.93^{a}	34.80^{c}
CD (P<0.05)	0.04	0.04	0.03	0.02	0.41	0.05	0.30

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

a)



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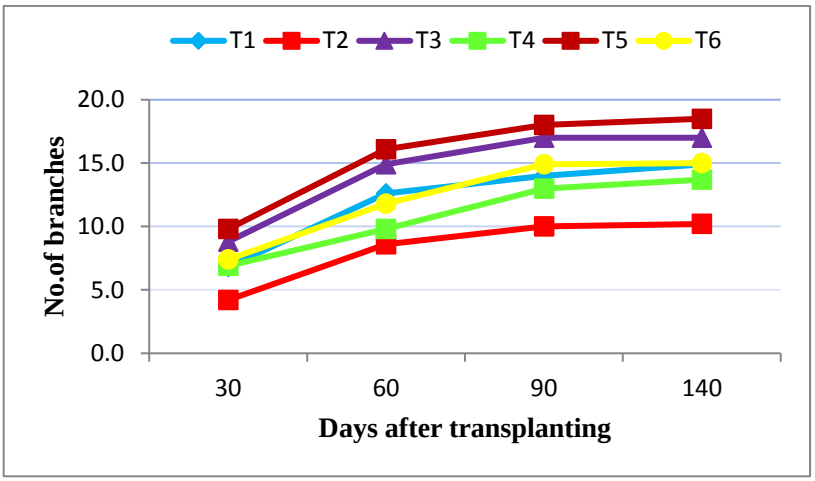

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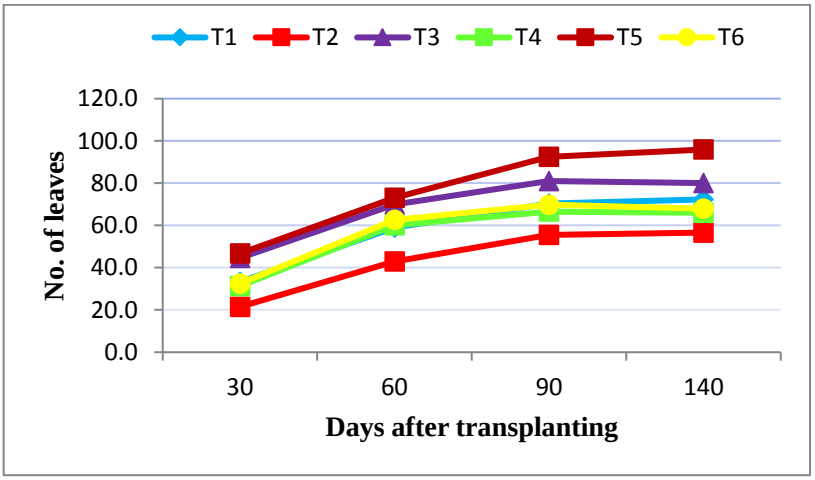
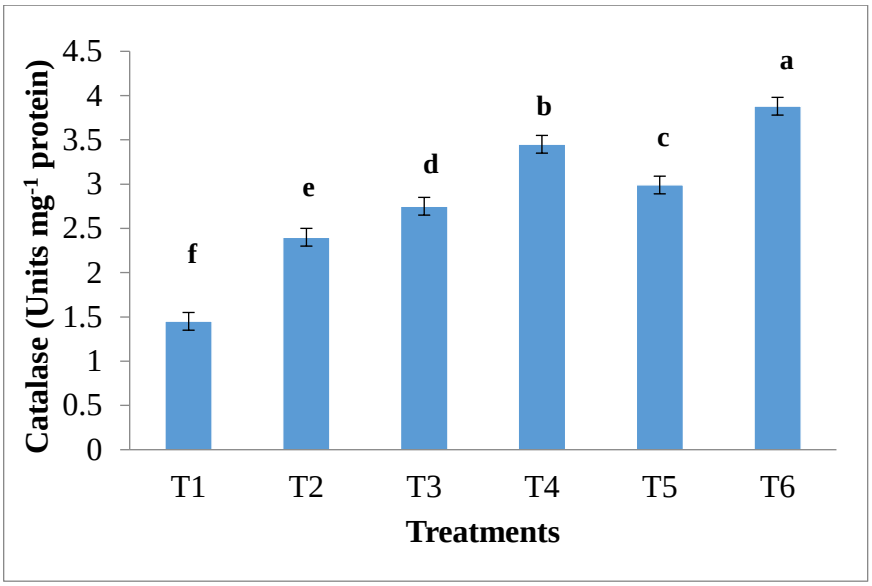


Fig 16: Effect of bacterial endophytes on (a) Plant height (b) No. of branches (c) No. of leaves of tomato under salt stress (4 dS/m).

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

a)



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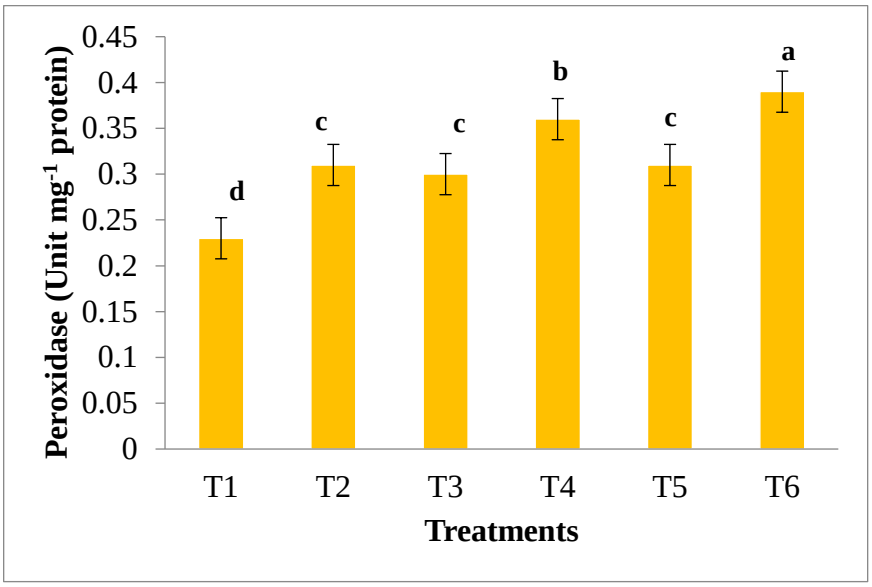
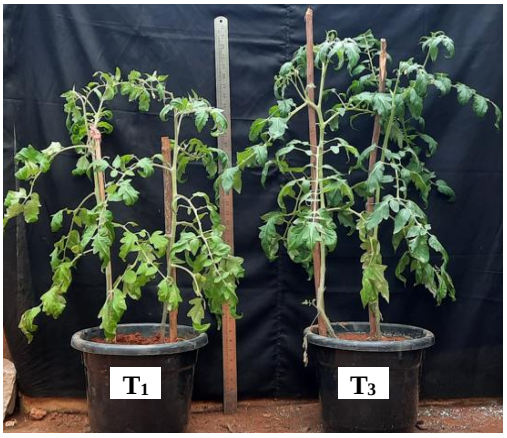
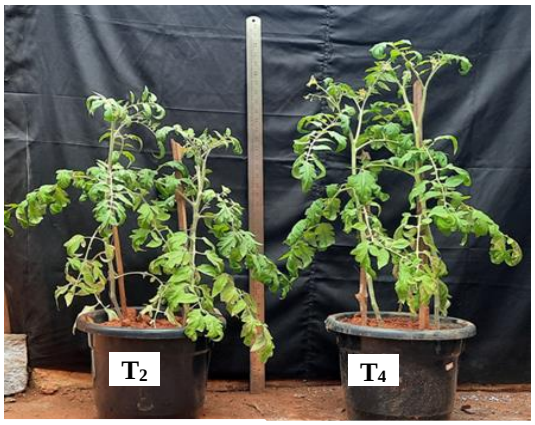


Fig. 17: Effect of bacterial endophytes on biochemical parameters (a) Catalase and (b)Peroxidase of tomato under salt stress (4 dS/m).

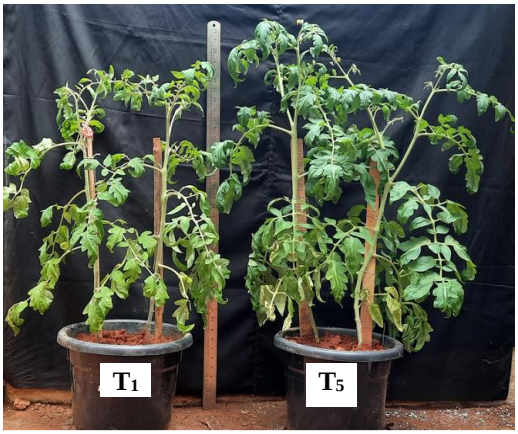
Note: T1=Control, T2= Salt stress (4dS/m), T3=*E. cloacae*, T4=Salt stress (4 dS/m)+ *E. cloacae*, T5=*E.asburiae*, T6=Salt stress (4 dS/m)+ *E. asburiae*



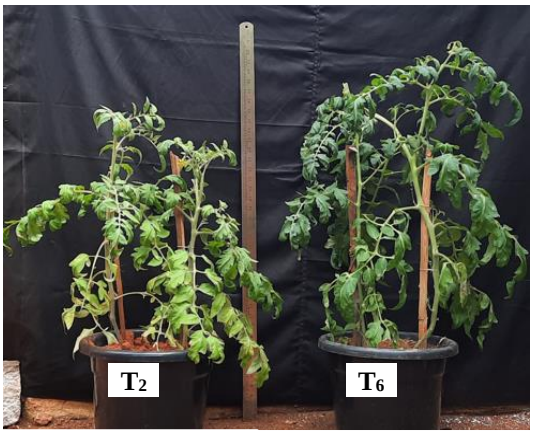
Control *E. cloacae*



Salt stress (4 dS/m) Salt stress (4 dS/m) + *E. cloacae*



Control *E. asburiae*



Salt stress (4 dS/m) Salt stress (4 dS/m)+ *E. asburiae*

Plate 11: Effect of bacterial endophytes *E. cloacae* and *E. asburiae* on growth of tomato under normal and salt stress (4dS/m) condition at 60 DAT.

content. The *E. asburiae* treated plants also showed less electrolyte leakage compared to un-inoculated plants. This was attributed to increased photosynthetic pigments and accumulation of osmolytes by endophyte mediation (Yandigeri *et al.,* 2012). The catalase and peroxidases play a vital role in scavenging ROS (Abogadallah, 2011). Treatment of bacterial endophyte to salt sensitive tomato seedlings showed significant increase in activity of these enzymes (Fig 17). The symbiotic association of bacterial endophytes with host plants is attributed to upregulation of the activity of catalase and peroxidases to scavenge ROS under salt stress (Choudhury *et al.,* 2017). Amjad *et al.* (2017) reported that *Bacillus pumilus* and *Exiguobacterium sp.* inoculated to tomato plants under salinity stress increased plant growth, biomass, and photosynthetic rate. In this study, the endophyte *E. asburiae* was found better compared to *E. cloacae* for tomato.

4.12 Influence of bacterial endophytes against drought stress in rice and tomato

Three bacteria *viz., B. cereus, P. chlororaphis, S. maltophilia* selected for evaluation in rice were used in this experiment. Plants treated with bacterial endophyte *S. maltophilia* showed significant increase in plant height which was followed by *P. chlororaphis* and *B. cereus* as compared to untreated plants at different growth intervals (30, 60, 90 and 120 DAT). Significantly higher number of leaves were recorded in *S. maltophilia* treated plants. Number of tillers also increased after 60 DAT in *S. maltophilia* and *P. chlororaphis* inoculated plants (Fig 18; Plate 12). The increased growth parameters in endophyte inoculated plants under moisture stress might be production of auxins, gibberellins and decreased endogenous abscisic acid (De Battista *et al.,* 1990).

The endophytes treated plants showed significant increase in yield parameters compared to untreated plants (Table 21). The *S. maltophilia* inoculated plants increased the number of panicles, number of seeds and seed yield under drought stress compared to un-inoculated plants which was followed *by P. chlororaphis.* The proline accumulation under water stress condition was also found higher in endophyte treated plants. The proline acted as storage compound for protein synthesis and it might have been used as a source of carbon and nitrogen for development of seeds (Schellenbaum *et al.,* 1998). Further, all the plants treated with bacterial endophytes showed significant increase in physiological parameters such as Chl a, b, total chl, carotenoid compared to untreated plants (Table 22). Among

Table 21: Effect of bacterial endophytes on yield parameters of rice under drought stress (50 % FC).

Treatments	No. of panicles	No. of seeds/ plant	Seed yield (g)/ plant
T_1= 100 % FC	10.13^{d}	575.50^{d}	13.50^{d}
T_2= 50 % FC	5.36^{h}	271.10^{h}	4.60^{h}
T_3= 100 % FC+ *B. cereus*	12.45^{c}	725.60^{c}	15.90^{c}
T_4= 50 % FC+ *B. cereus*	8.50^{g}	398.40^{g}	7.80^{g}
T_5= 100 % FC+ *P. chlororaphis*	12.90^{b}	738.50^{b}	16.30^{b}
T_6= 50 % FC+ *P. chlororaphis*	8.70^{f}	410.50^{f}	8.10^{f}
T_7= 100 % FC+ *S. maltophilia*	13.00^{a}	799.60^{a}	17.40^{a}
T_8= 50 % FC+ *S. maltophilia*	8.82^{e}	425.20^{e}	8.60^{e}
CD (P<0.05)	0.09	11.70	0.33

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

Table 22: Effect of bacterial endophytes on physiological parameters of rice under drought stress (50 % FC).

Treatments	Chl a (mg/g FW)	Chl b (mg/g FW)	Total chl (mg/g FW)	Carotenoid content (mg/g FW)	RWC (%)	Proline content (µmol/g FW)
T_1= 100 % FC	0.76^{b}	0.33^{a}	0.94^{a}	0.34^{a}	87.50^{d}	7.34^{e}
T_2=50 % FC	0.63^{c}	0.28^{b}	0.73^{c}	0.26^{b}	71.50^{h}	12.25^{c}
T_3 =100 % FC+ *B. cereus*	0.75^{b}	0.36^{a}	0.92^{a}	0.33^{a}	88.40^{c}	8.35^{d}
T_4 =50 % FC+ *B. cereus*	0.63^{c}	0.33^{a}	0.75^{c}	0.31^{a}	82.20^{g}	13.23^{b}
T_5=100 % FC+ *P. chlororaphis*	0.76^{b}	0.36^{a}	0.93^{a}	0.34^{a}	89.60^{b}	8.34^{d}
T_6=50 % FC+ *P. chlororaphis*	0.65^{c}	0.33^{a}	0.76^{c}	0.31^{a}	83.70^{f}	13.94^{a}
T_7= 100 % FC+ *S. maltophilia*	0.83^{a}	0.36^{a}	0.94^{a}	0.31^{a}	89.80^{a}	8.35^{d}
T_8=50 % FC+ *S. maltophilia*	0.63^{c}	0.34^{a}	0.83^{b}	0.34^{a}	83.90^{e}	13.95^{a}
CD (P<0.05)	0.05	0.04	0.04	0.03	0.12	0.38

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

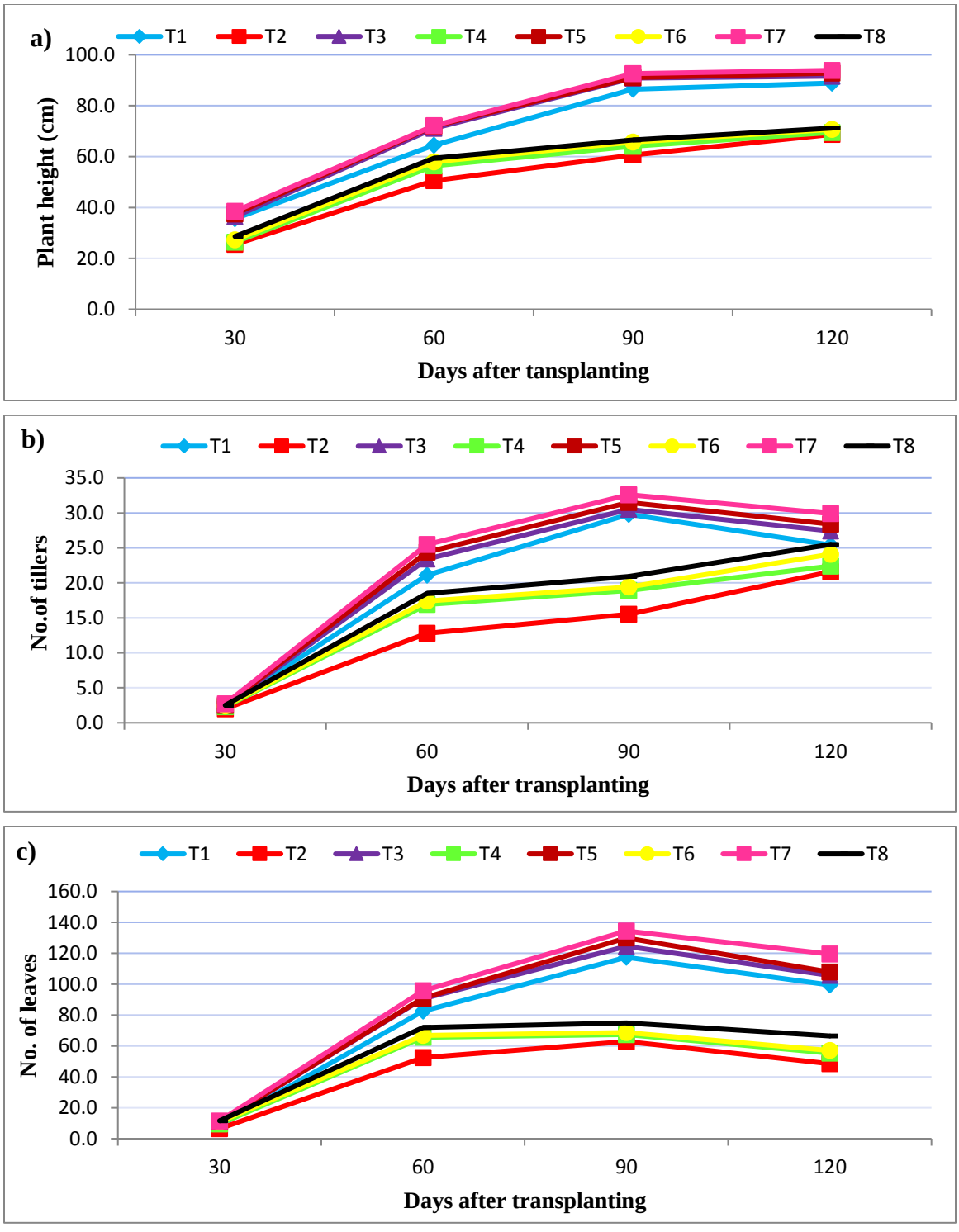
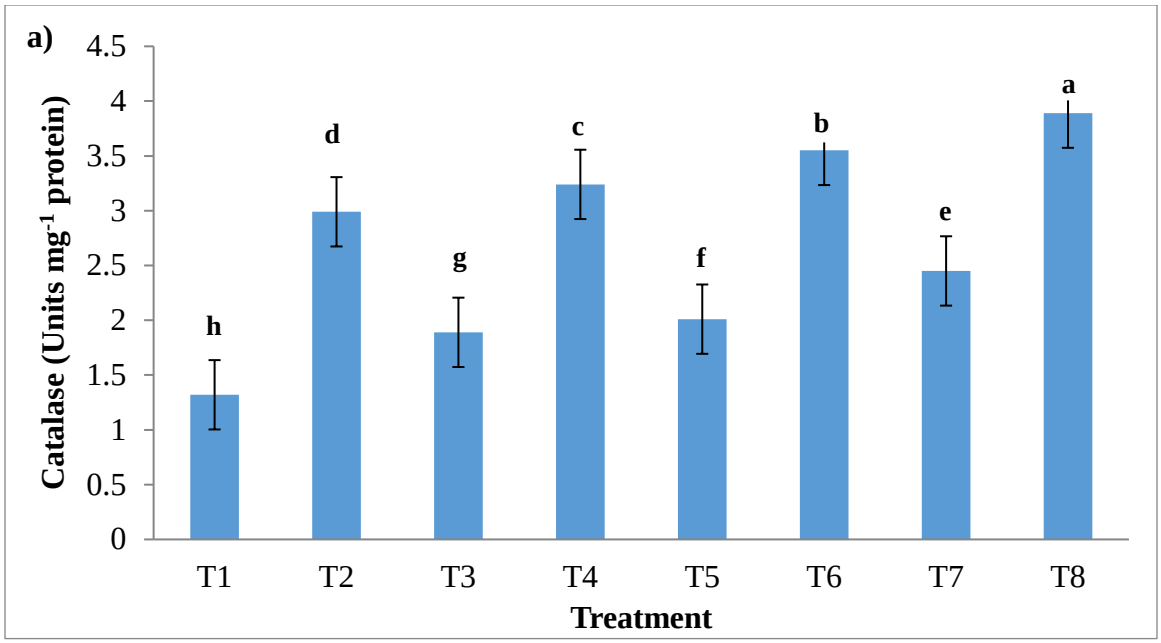


Fig. 18: Effect of bacterial endophytes on growth parameters (a) Plant height (b) No. of tillers (c) No. of leaves of rice under drought stress (50 % FC).

Note: T1= 100 % FC, T2=50 % FC, T3 =100 % FC+ *B. cereus*, T4 =50 % FC+ *B. cereus*, T5=100 % FC+ *P. chlororaphis*, T6=50 % FC+ *P. chlororaphis*, T7= 100 % FC+ *S. maltophilia*, T8=50 % FC+ *S. maltophilia*



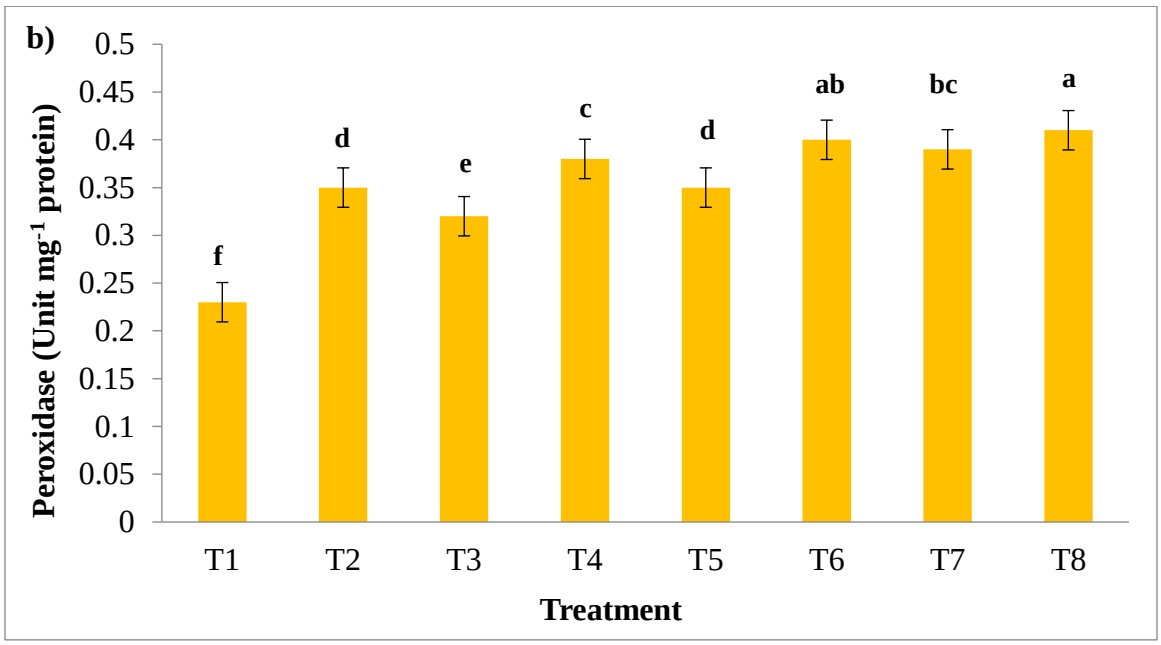
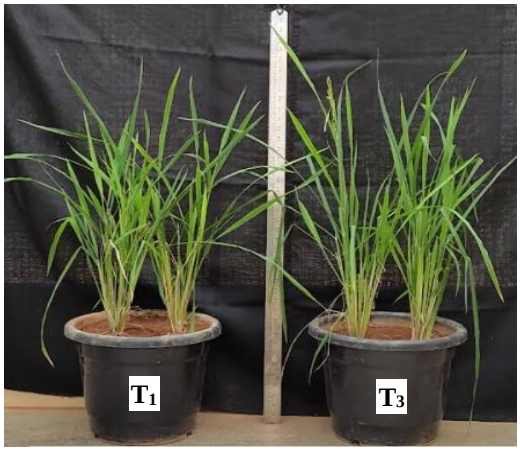
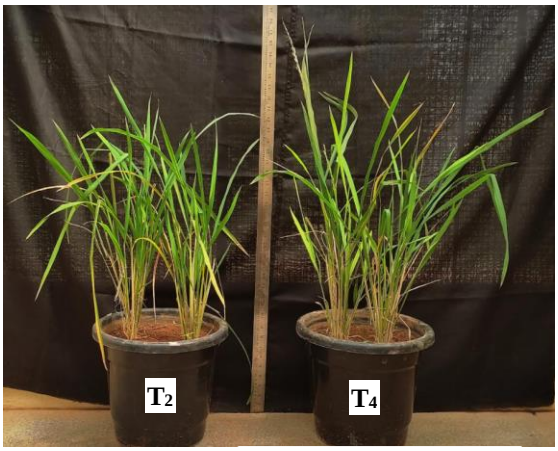


Fig 19: Effect of bacterial endophytes on biochemical parameters (a) Catalase and (b) Peroxidase of rice under drought stress (50 % FC)

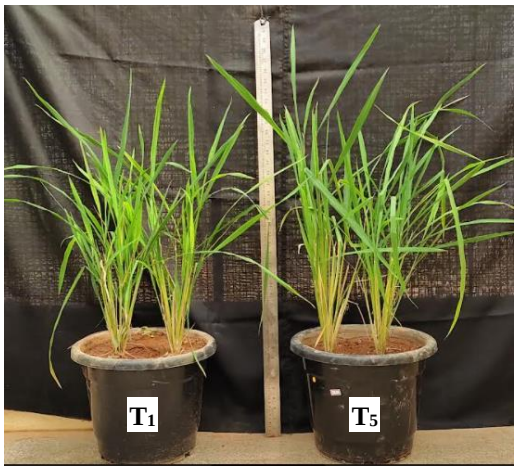
Note: T1= 100 % FC, T2=50 % FC, T3 =100 % FC+ *B. cereus*, T4 =50 % FC+ *B. cereus*, T5=100 % FC+ *P. chlororaphis*, T6=50 % FC+ *P. chlororaphis*, T7= 100 % FC+ *S. maltophilia*, T8=50 % FC+ *S. maltophilia*



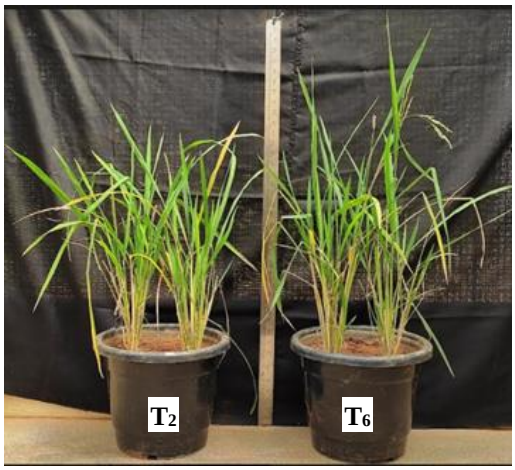
100 % FC *B. cereus*



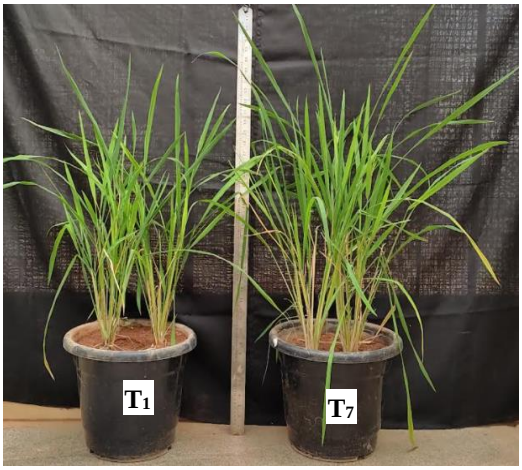
50 %FC 50 %FC + *B. cereus*



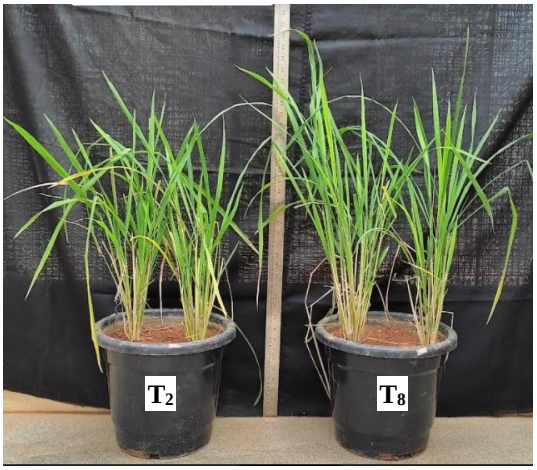
100 % FC *P. chlororaphis*



50 %FC 50 %FC + *P. chlororaphis*



100 % FC *S. maltophilia*



50 %FC 50 %FC + *S. maltophilia*

Plate 12. Effect of bacterial endophyte *B. cereus, P. chlororaphis* and *S. maltophilia* on growth of rice under control (100 % FC) and drought stress (50 % FC) condition at 60 DAT.

three bacteria selected for pot culture study, the *S. maltophilia* showed better results in withstanding drought stress.

Drought induced oxidative stress leading to production of reactive oxygen species (ROS) which affected physiological and biochemical process. Oxidative stress can be overcome by production of antioxidant producing enzymes (Choudhury *et al.,* 2017). In the present study, the *S. maltophilia* inoculated plants showed increased production of catalase, peroxidase, and proline (Fig 19). This suggested *S. maltophilia* as an efficient endophyte to mitigate drought stress in rice.

For tomato, three bacteria *viz., L. macroides, S. maltophilia* and *A. lwoffii* were for green house experiment. The plants treated with bacterial endophyte *A. lwoffii* showed significant increase in plant height, number of branches and number of leaves compared to un-inoculated plants at 100 % FC and 50 % FC which was followed by *S. maltophilia* and *L. macrolides* (Fig 20; Plate 13). The increase in growth parameters could be due to production of phytohormones and other organic acids under stress by downregulating endogenous ABA levels and increasing glutathione and sugar content (Khan *et al.,* 2020)

The two bacteria *A. lwoffii* and *S. malt*ophilia significantly increased the number of fruits per plant and fruit yield compared to un-inoculated plants under 100 % FC as well as 50 % FC (Table 24) indicating the endophytes influenced on increased fruits in tomato. The endophyte *A. lwoffii* also increased the photosynthetic pigments (Table 24) compared to un-inoculated plants. RWC is an important indicator of water status in plants as it reflects the balance between water supply to the leaf tissue and transpiration rate (Ferus *et al.,* 2019). Endophytes inoculated plants showed higher Relative Water Content compared to un-inoculated plants. At 50 % FC the *A. lwoffii* inoculated plants showed higher relative water and proline content.

The drought stress results in generation of reactive oxygen species (ROS) thereby affecting plant's physiological and biochemical processes (Abogadallah, 2011). In the present study, the *A. lwoffii* inoculated plants showed increased production of catalase and peroxidase (Fig 21) which regulated oxidative stress (Morsy *et al.,* 2020). Anna

Table 23: Effect of bacterial endophytes on yield parameters of tomato under drought stress (50 % FC).

Treatments	No. of fruit/plant	Fruit yield/plant(g)
T_1= 100 % FC	22.94^{c}	802.90^{d}
T_2= 50 % FC	13.31^{g}	399.30^{h}
T_3= 100 % FC +*L. macroides*	25.00^{b}	875.00^{c}
T_4= 50 % FC+ *L. macroides*	17.90^{f}	644.40^{g}
T_5= 100 % FC+ *S. maltophilia*	27.80^{a}	973.00^{a}
T_6= 50 % FC+ *S. maltophilia*	18.60^{e}	706.80^{e}
T_7= 100 % FC +*A. lwoffii*	27.90^{a}	948.00^{b}
T_8= 50 % FC+ *A. lwoffii*	19.90^{d}	703.33^{f}
CD (P<0.05)	0.35	2.25

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

Table 24: Effect of bacterial endophytes on physiological parameters of tomato under drought stress (50 % FC).

Treatments	Chl a (mg/g FW)	Chl b (mg/g FW)	Total chl (mg/g FW)	Carotenoid content (mg/g FW)	RWC (%)	Proline content (µmol/g FW)
T_1= 100 % FC	0.74^{a}	0.32^{c}	0.92^{a}	0.32^{a}	87.50^{d}	6.34^{e}
T_2= 50 % FC	0.56^{c}	0.26^{d}	0.73^{c}	0.27^{b}	70.00^{h}	11.22^{d}
T_3= 100 % FC +*L. macroides*	0.74^{a}	0.36^{ab}	0.93^{a}	0.35^{a}	88.60^{c}	6.35^{e}
T_4= 50 % FC+ *L. macroides*	0.64^{b}	0.32^{c}	0.75^{c}	0.33^{a}	73.50^{g}	11.94^{c}
T_5= 100 % FC+ *S. maltophilia*	0.75^{a}	0.37^{a}	0.94^{a}	0.34^{a}	88.77^{b}	6.34^{e}
T_6= 50 % FC+ *S. maltophilia*	0.62^{b}	0.33^{bc}	0.75^{c}	0.32^{a}	74.60^{f}	12.42^{b}
T_7= 100 % FC +*A.* lwoffii	0.76^{a}	0.35^{abc}	0.94^{a}	0.35^{a}	89.80^{a}	6.35^{e}
T_8= 50 % FC+ *A. lwoffii*	0.63^{b}	0.36^{ab}	0.82^{b}	0.36^{a}	75.90^{e}	12.94^{a}
CD (P<0.05)	0.05	0.04	0.05	0.04	0.08	0.45

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

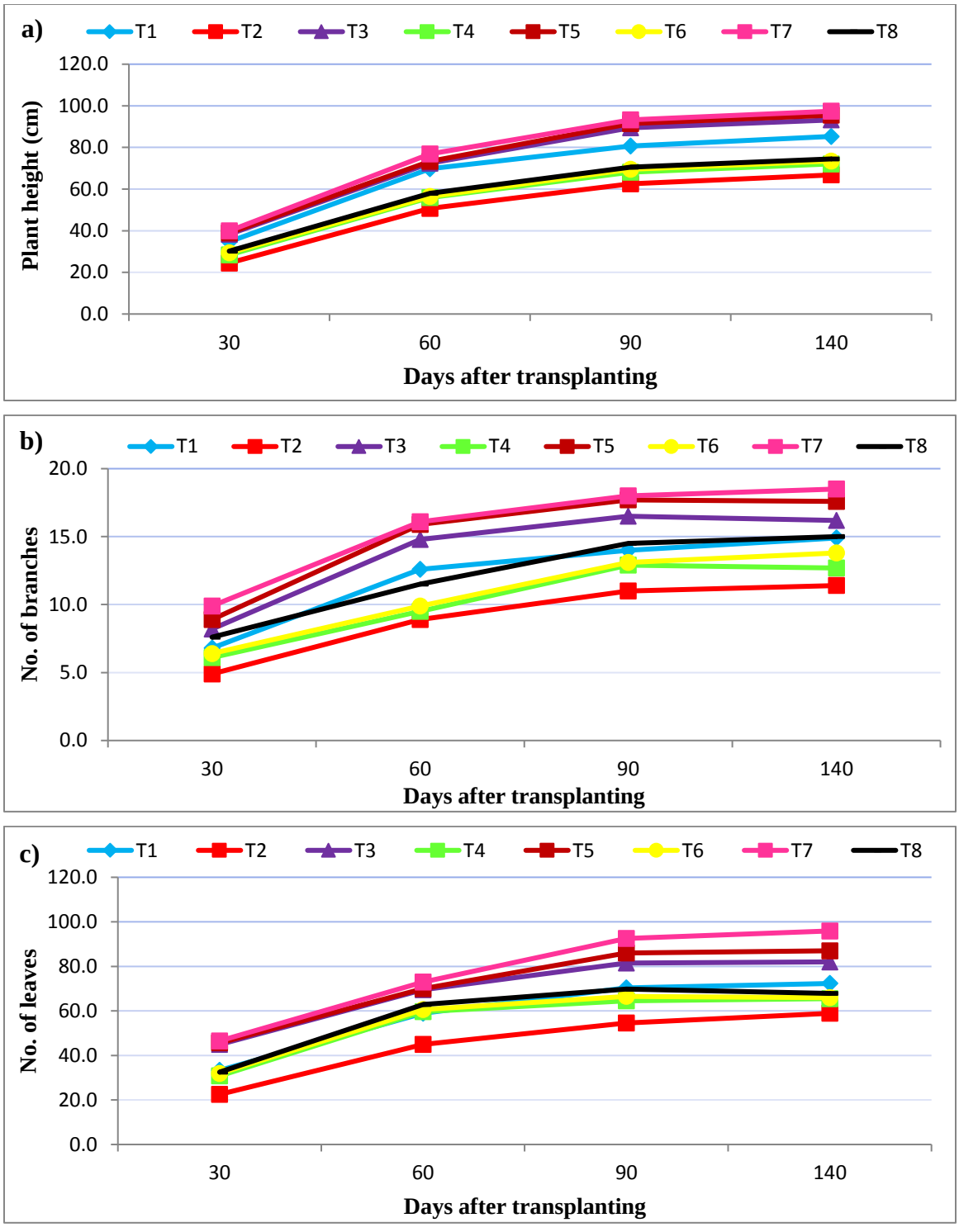


Fig. 20: Effect of bacterial endophytes on (a) Plant height (b) No. of branches (c) No. of leaves of tomato under drought stress (50 % FC).

Note: T1= 100 % FC, T2= 50 % FC, T3= 100 % FC +*L. macroides*, T4= 50 % FC+ *L. macrolides*, T5= 100 % FC+ *S. maltophilia*, T6= 50 % FC+ *S. maltophilia*, T7= 100 % FC+*A.lwoffii*, T8=50 %FC+*A. lwoffii*

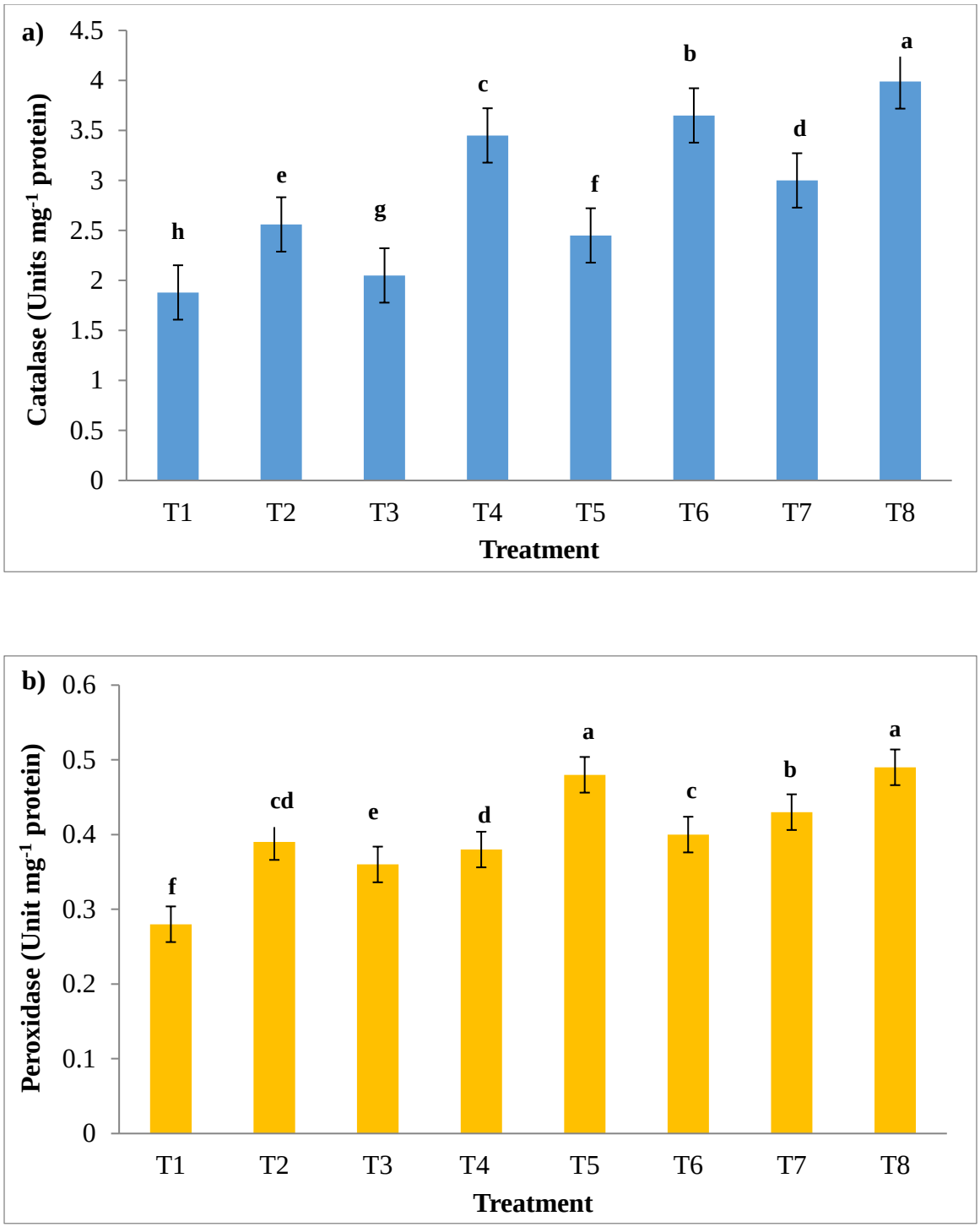
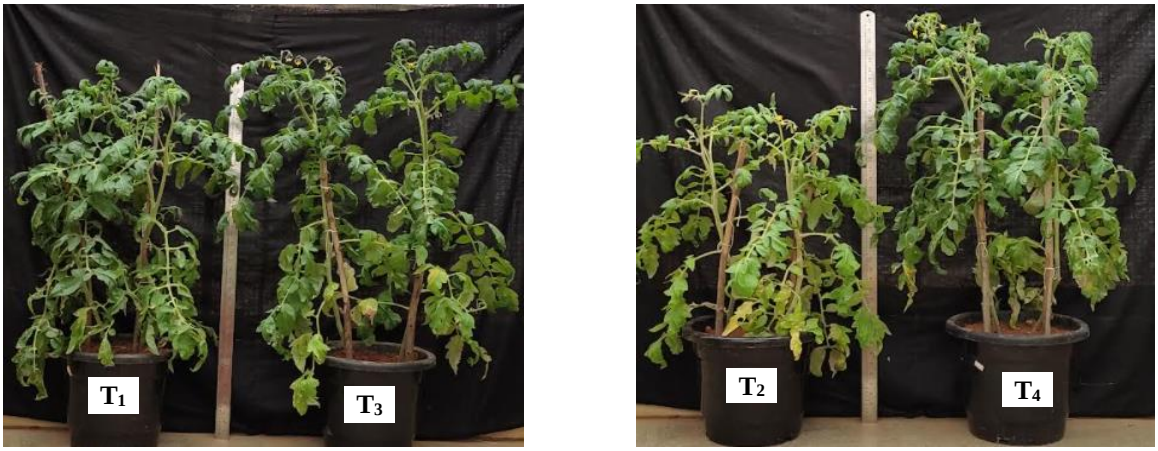
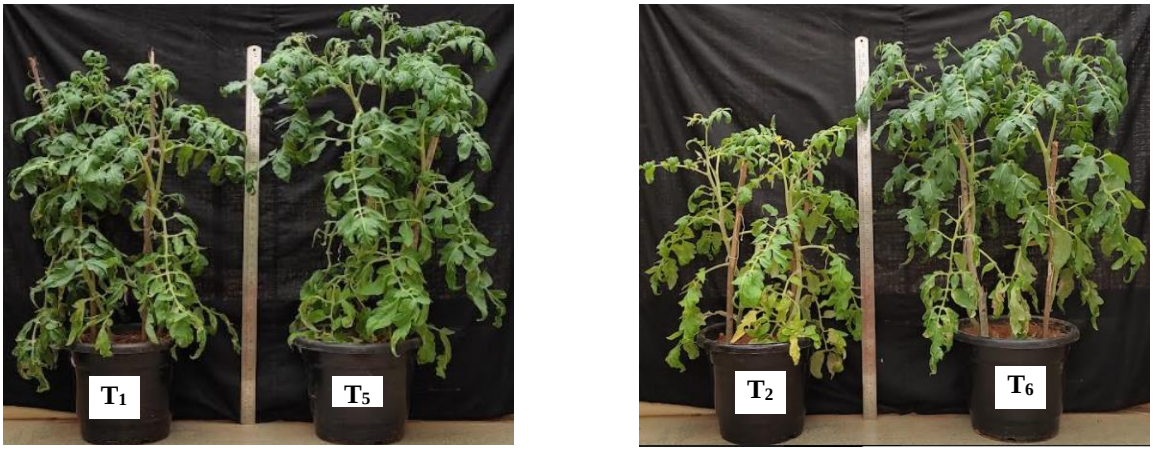


Fig. 21: Effect of bacterial endophytes on biochemical parameters (a) Catalase and (b) Peroxidase of tomato under drought stress (50 % FC)

Note: T1= 100 % FC, T2= 50 % FC, T3= 100 % FC +*L. macroides*, T4= 50 % FC+ *L. macrolides*, T5= 100 % FC+ *S. maltophilia*, T6= 50 % FC+ *S. maltophilia*, T7= 100 % FC+*A.lwoffii*, T8=50 %FC+*A. lwoffii*



100 % FC *L. macroides* 50 % FC 50 % FC + *L. macroides*



100 % FC *S. maltophilia* 50 % FC 50 % FC + *S. maltophilia*

100 % FC *A. lwoffii* 50 % FC 50 % FC + *A. lwoffii*

Plate 13: Effect of bacterial endophytes *L. macrolides, S. maltophilia* and *A. lwoffii* on growth of tomato under control (100 % FC) and drought stress (50 % FC) condition at 60 DAT.

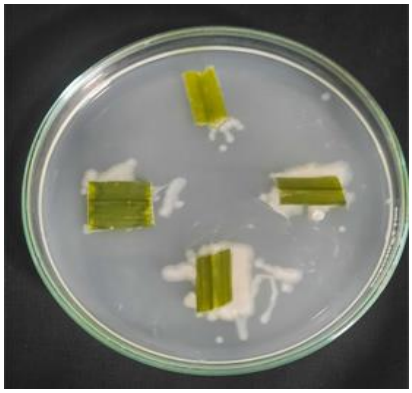
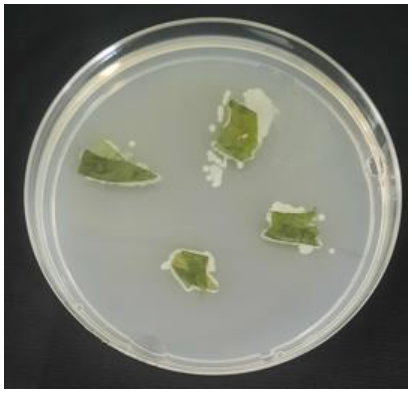
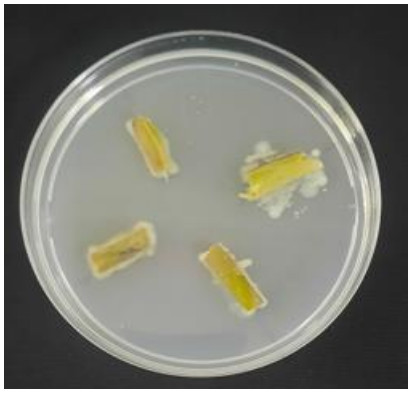
Leaves		
Stem		
Root		

Plate 14: Re-isolated bacterial colonies from leaves stem and root of rice (left) and tomato (right) plants at 45 DAT.

et al. (2020) observed significant increase in shoot and root biomass, better photosynthetic efficiency and higher water-use efficiency in stress induced tomato plants. Thus, the drought stress experiment on tomato proved *A. lwoffii* as efficient endophyte bacterium.

4.13 Re-isolation of bacterial endophytes

The presence of inoculated bacteria in plant tissue was confirmed by re-isolation from the root and stem of inoculated rice and tomato plants at 45 DAT. The isolates were confirmed by comparing their colony characters with the mother culture and further confirmation was made by molecular identification of re-isolated bacteria (Appendix IV).

V SUMMARY

Symbiotically conferred stress tolerance in plants by the association of microbes (endophytes) inside tissue of plants is often referred to as habitat adapted symbiosis. The endophytes (fungi and bacteria) isolated from the plants growing under harsh environmental conditions can provide fitness to withstand abiotic stresses. In the present study, bacterial endophytes were isolated from the Himalayan cold desert plants (Pangong, Changla and Namkila La) and explored for their ability to impart drought and salt stress tolerance in abiotic stress sensitive rice (IR-64) and tomato (Arka saurabh) crops.

Fifty-eight bacterial endophytes were screened against salinity and drought stress tolerance at different concentration of NaCl (0.5, 1.0, 1.5, 2.0 and 2.5M) and PEG-8000 (5%, 10%, 15%, 20%, 25%). Of which, nine isolates *viz.*, PBE 4, PBE 6, PBE 8, PBE 15, CBE 12, NBE 7, NBE 20, NBE 21 and NBE 23 showed growth at 2.0 M NaCl and eight isolates *viz.*, PBE 2, PBE 4, PBE 6, PBE 8, PBE 14, CBE 11, CBE 13 and NBE 5 showed growth at 20% PEG concentrations. Therefore, these endophytes were selected for further experimentation.

Salt and drought tolerance of rice and tomato seedlings was standardized by using pre-germinated seeds. The pre-germinated seeds were subjected to different concentrations of NaCl (25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM and 200 mM) and PEG (5%, 10%, 15%, 20%, 25%). The shoot, root and seedling length of rice and tomato were recorded on 14^{th} day for rice and 7^{th} day for tomato. The length of the seedling decreased with increased PEG and NaCl concentrations. Therefore, LC_{50} values for NaCl and PEG were determined by probit analysis. The LC_{50} value for NaCl for rice was 150 mM and for tomato was 117 mM. The LC_{50} value of PEG was 14.3% for rice and 15.3% for tomato. These LC_{50} values were used further for screening bacterial endophytes against salinity and drought tolerance.

The Nine salt tolerant bacterial endophytes were also screened for salt tolerance using paper towel method with appropriate concentrations of NaCl. The pre-germinated seeds of rice inoculated with bacterial endophytes were placed on paper towel dipped in

NaCl and incubated for growth. Of which only two isolates *viz.,* PBE 8 and NBE 7 induced salt tolerance in rice seedlings. Similarly, pre-germinated tomato seeds treated with nine endophytes were placed on paper towel dipped in appropriate concentration of NaCl. Of which two endophytes *viz.,* NBE 20 and NBE 23 showed salt tolerance in tomato seedlings by increasing the seedling length.

For drought tolerance, the eight selected endophytes were further screened by inoculating them to pre-germinated rice and tomato seedlings and placing on paper towel dipped in PEG of appropriate concentration. Out of which three endophytes, PBE 2, PBE 4 and CBE 11 showed drought tolerance in rice and three endophytes PBE 6, CBE 11, NBE 5 showed drought tolerance in tomato. Presence of inoculated endophytes in rice and tomato seedlings was confirmed by re-isolating and comparing their colony characters with respective mother cultures.

These nine isolates were identified by 16S rRNA gene sequence as *Bacillus cereus* (PBE 2), *Pseudomonas chlororaphis* (PBE 4), *Lysinibacillus macroides* (PBE 6), *Enterobacter hormaechei* (PBE 8), *Stenotrophomonas maltophilia* (CBE 11), *Acinetobacter lwoffii* (NBE 5), *Pseudomonas fluorescenes* (NBE 7), *Enterobacter cloacae* (NBE 20) and *Enterobacter asburiae* (NBE 23). Further these endophytes were evaluated for growth promoting traits like phosphate solubilization, HCN, siderophore and ammonia production. Out of nine, two bacteria, *E. hormaechei* and *S. maltophilia* did not show any phosphate solubilization. Other seven bacterial endophytes *viz., B. cereus, E. hormaechei, S. maltophilia, A. lwoffii, E. cloacae, E. asburiae P. fluorescenes* produced siderophores and all the isolates produced ammonia, proline and phytohormones under salt (2.0 M NaCl) and drought (20% PEG) stresses.

These nine bacteria were evaluated against drought and salt stress under greenhouse conditions. In general endophytes inoculated rice and tomato plants showed increased growth, yield, physiological and biochemical traits compared to un-inoculated plants. Out of nine bacteria evaluated for salt tolerance in pot culture, *E. hormaechei* and *E. asburiae* were found superior in imparting salt stress (4 dS/m) in rice and tomato respectively. The endophytes *S. maltophilia* and *A. lwoffii* were found better in imparting drought tolerance

(50 % FC) in rice and tomato respectively. Presence of these endophytes in tissues of inoculated rice and tomato were confirmed by re-isolation and molecular identification. This study suggested that four endophytes could be used to mitigate abiotic stresses (salinity and drought) in rice and tomato.

Future line of work

1. Field evaluation of *E. hormaechei* and *E. asburiae* for salinity stress tolerance and *S. maltophilia* and *A. lwoffii* for drought tolerance in rice and tomato is required to understand the practical utility.
2. The molecular mechanism involved in drought and salinity tolerance and growth promotion by bacteria is required to be explored by multi-omics approaches like genomics, transcriptomics, proteomics and metabolomics

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APPENDIX I

Culture medium

Nutrient agar	
Peptone	5.0 g
Beef extract	3.0 g
NaCl	5.0 g
Distilled water	1000.0 mL
Agar	15.0 g
pH	7.0

APPENDIX II

STAINING AND TEST REAGENTS

1. Gram stain

• Crystal violet

Solution A

Crystal violet (90 % dye content)	2.0 g
Ethyl alcohol (95 %)	20.0 mL

Solution B

Ammonium oxalate	0.8 g
Distilled water	80.0 mL

Dissolve crystal violet and ammonium oxalate in ethyl alcohol and distilled water, respectively. Mix solution A and B.

• Gram's iodine

Iodine	1.0 g
Potassium iodine	2.0 g
Distilled water	300.0 mL

• Ethyl alcohol (95 %)

Ethyl alcohol (100 %)	95.0 mL
Distilled water	5.0 mL

• Safranin

Safranin (2.5 % solution in 95 % ethyl alcohol)	10.0 mL
Distilled water	100.0 mL

2. Nessler's reagent

Potassium iodide	50.0 g
Distilled water (ammonia free)	25.0 mL
Mercuric chloride (saturated)	35.0 g
Potassium hydroxide (50 % aqueous)	400.0 mL

3. Picric acid solution (HCN production)

Picric acid	0.5g
Distilled water	100ml

APPENDIX III

BACTERIAL GENOMIC DNA ISOLATION

1. Extraction buffer

Stock	Required volume	For 100 mL
1. 1 M Tris HCl (pH 8)	0.1 M (100 mM)	10 mL
2. 0.5 M EDTA	0.1 M (100 mM)	20 mL
3. 5 M NaCl	0.25 M (25 mM)	5 mL
4. 1 % SDS - 1g		
5. 1 % PVP - 1g		

Make up the volume to 100 mL with distilled water

2. Potassium acetate solution (100 mL)

Potassium acetate (5 M) - 60 mL

Glacial acetic acid - 11.5 mL

Make up the volume using distilled water

3. TE buffer

One mL of 1 M Tris HCL plus 200 µL of 0.5 M EDTA and make up the volume to 100 mL with distilled water.

4. Ribonuclease A: Used at concentration of 10 µg mL^{-1}

5. 70 % chilled ethanol: Add 30 mL of distilled water to 70 mL of absolute ethanol and chilled in freezer.

AGAROSE GEL ELECTROPHORESIS

1. TAE buffer (50 X)

Tris base - 242 g

Glacial acetic acid - 57.1 mL

EDTA 0.5 M (pH 8.0) - 100 mL

2. TBE Buffer (5 X)

Tris base - 54 g

Boric acid - 27.5 g

EDTA 0.5 M (pH 8.0) - 20 mL

Distilled water - 1000 mL

3. Loading dye (5 X)

Bromophenol blue - 0.25 %

Xylene cyanol - 0.25 %

Glycerol - 30 %

4. Reagents:

- **Vanadomolybdic acid**

Solution A was prepared by dissolving 25 g of ammonium molybdate in 400 mL of distilled water. Solution B was prepared by dissolving 1.25 g of ammonium metavenadate in 300 mL boiling water. Solution B was cooled and then 250 mL concentrated HNO3 added. Finally, solution A and B were mixed and the mixture was diluted to 1 L.

- **Mixed indicator**

One gram of Bromocresol green + 1 g of Methyl red. Mixed together and made up the volume to 100 mL with ethanol

APPENDIX IV

1. Re-isolated sequence of isolate *Bacillus cereus* PBE 2

CGTGGATGAGTGCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAACAC
TCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCAAGCG
GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA
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CTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTC
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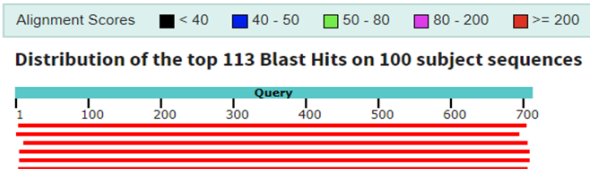
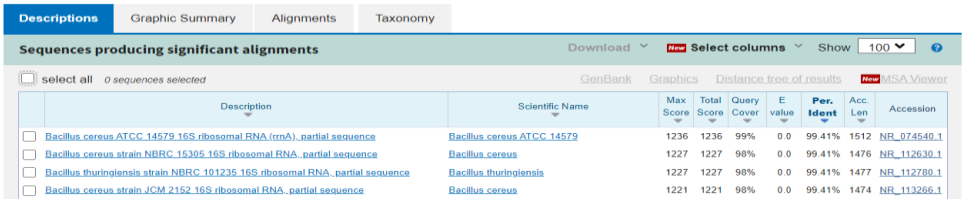
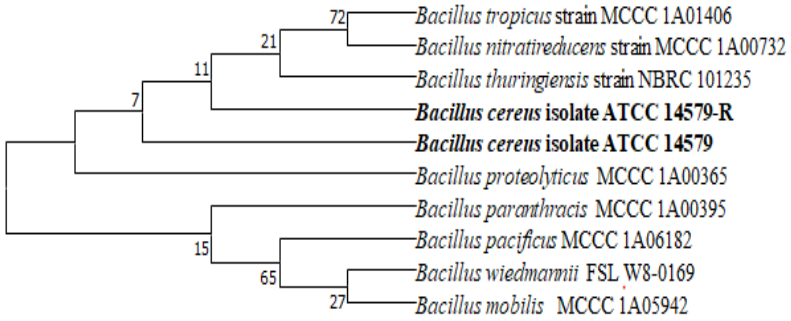




Re-isolated 16S rRNA gene partial sequence (706 bp) of endophytic bacteria *Bacillus cereus* isolate ATCC 14579-R



Reconfirmation of isolate Bacillus cereus ATCC 14579-R

2. Re-isolated sequence of *Pseudomonas chloraphis* PBE4

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AGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTG
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TGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGA
GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCT
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TGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAG
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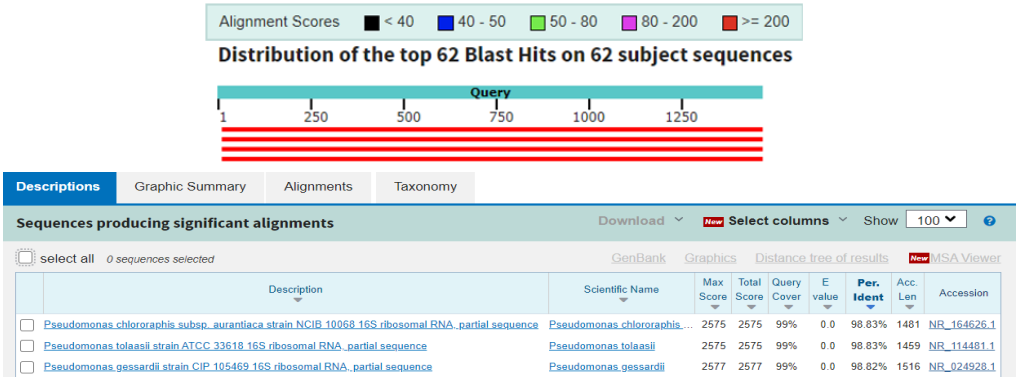
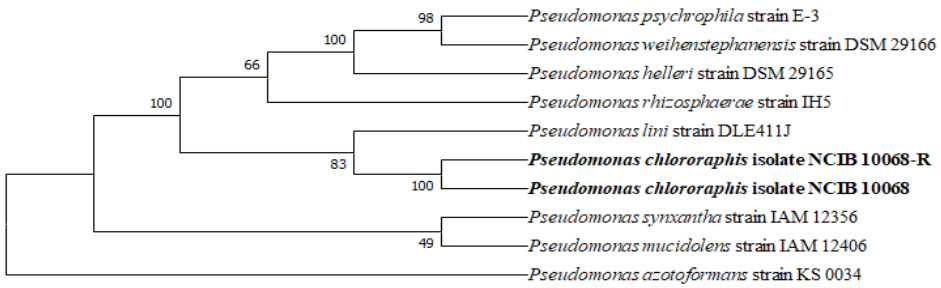


Re-isolated 16S rRNA gene partial sequence (1457 bp) of endophytic bacteria *Pseudomonas chloraphis* isolate NCIB 10068-R



Reconfirmation of isolate *Pseudomonas chloraphis* NCIB 10068-R

3. Re-isolated sequence of *Lysinibacillus macroides* PBE 6

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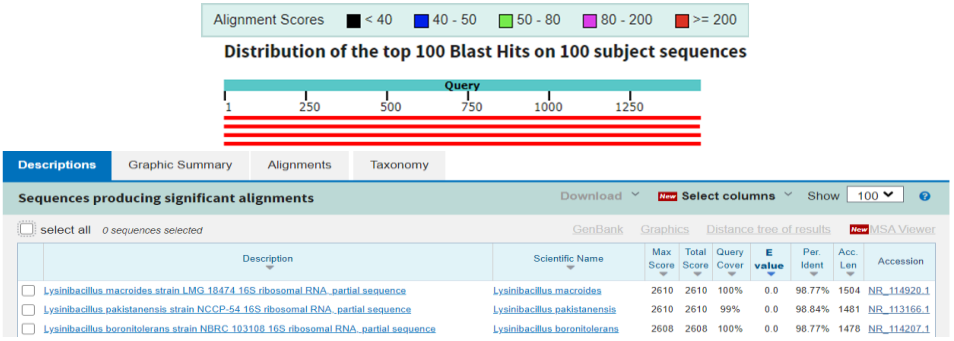
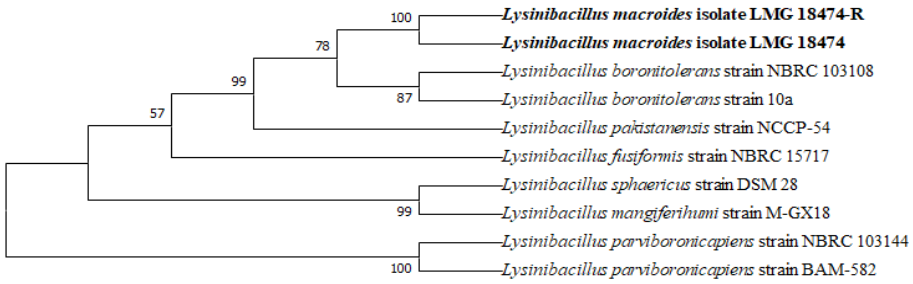


Re-isolated 16S rRNA gene partial sequence (1466 bp) of endophytic bacteria *Lysinibacillus macroides* LMG 18474-R



Reconfirmation of isolate *Lysinibacillus macroides* LMG 18474-R

4. Re-isolated sequence of *Enterobacter hormaechi* PBE 8

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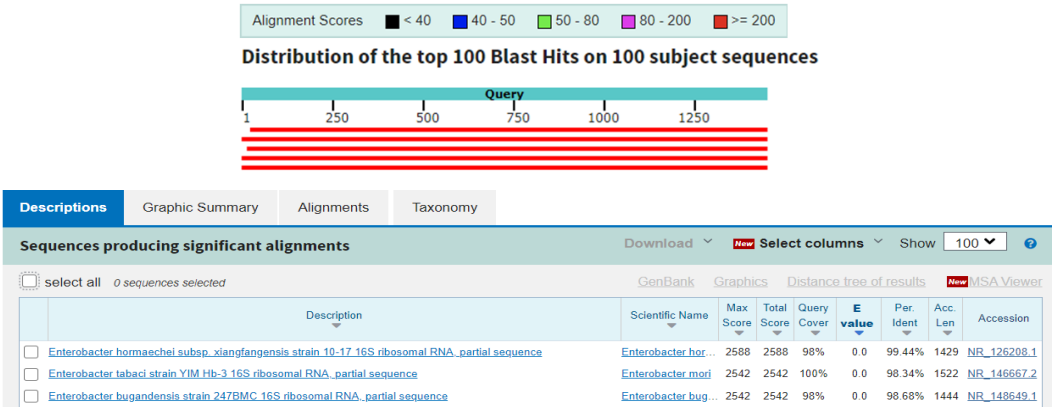
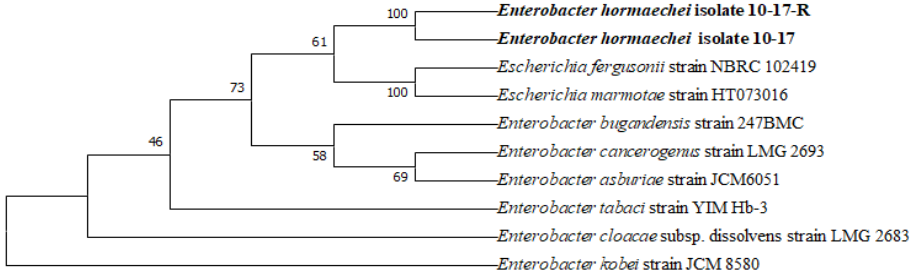


Re-isolated 16S rRNA gene partial sequence (1488 bp) of endophytic bacteria *Enterobacter hormaechi* 10-17-R



Reconfirmation of isolate *Enterobacter hormaechi* 10-17-R

5. Re-isolated sequence of *Stenotrophomonas maltophillia* CBE 11

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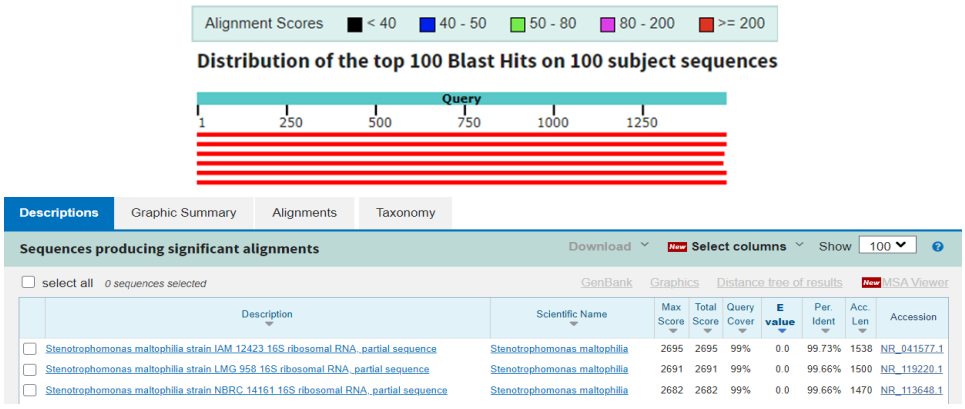
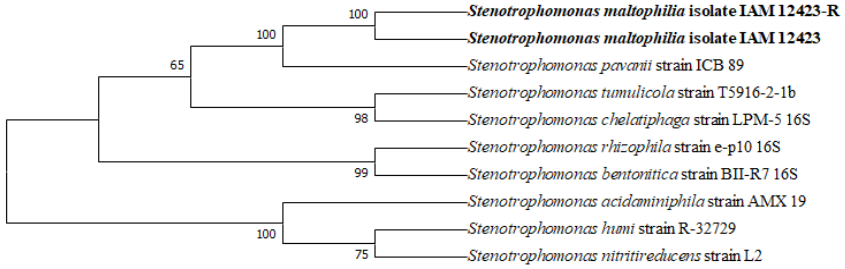


Re-isolated 16S rRNA gene partial sequence (1471 bp) of endophytic bacteria *Stenotrophomonas maltophillia* IAM 12423-R



Reconfirmation of isolate *Stenotrophomonas maltophillia* IAM 12423-R

6. Re-isolated sequence of *Acineobacter lwoffii* NBE 5

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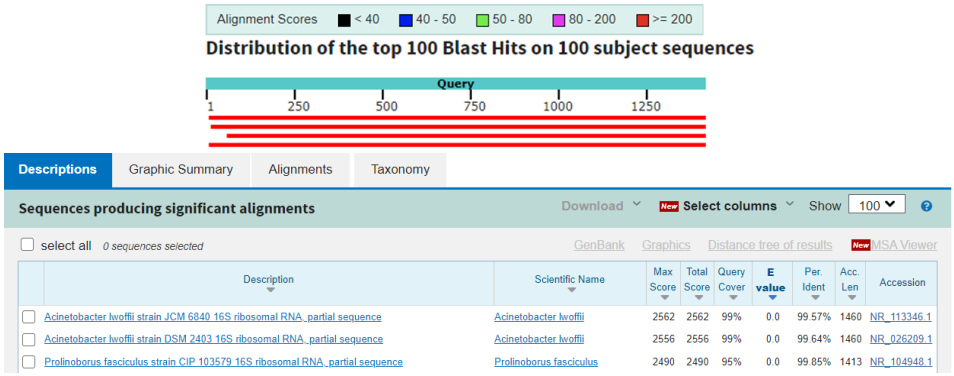
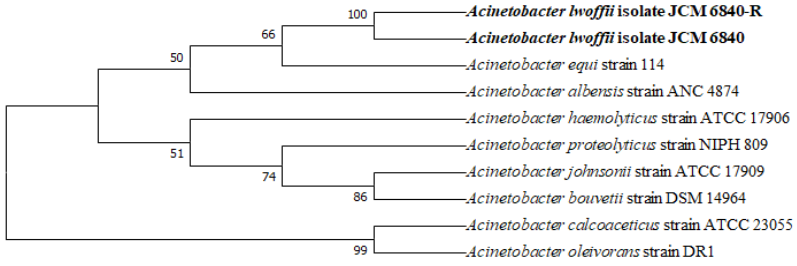


Re-isolated 16S rRNA gene partial sequence (1411 bp) of endophytic bacteria *Acineobacter lwoffii* JCM 6840-R



Reconfirmation of isolate *Acineobacter lwoffii* JCM 6840-R

7. Re-isolated sequence of *Pseudomonas fluorescens* NBE 7

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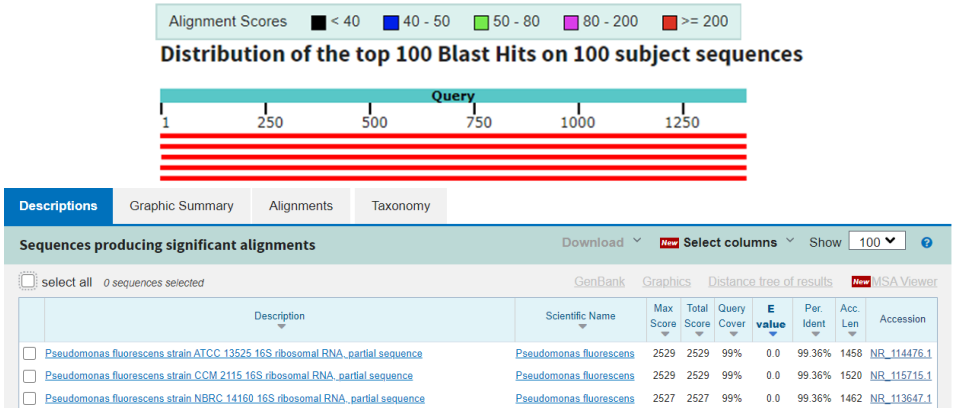
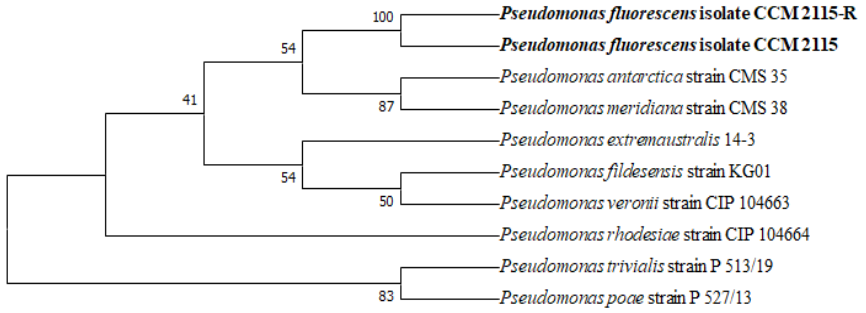


Re-isolated 16S rRNA gene partial sequence (1394 bp) of endophytic bacteria *Pseudomonas fluorescens* ATCC 13525-R



Reconfirmation of isolate *Pseudomonas fluorescens* ATCC 13525-R

8. Re-isolated sequence of *Enterobacter cloacae* NBE 20

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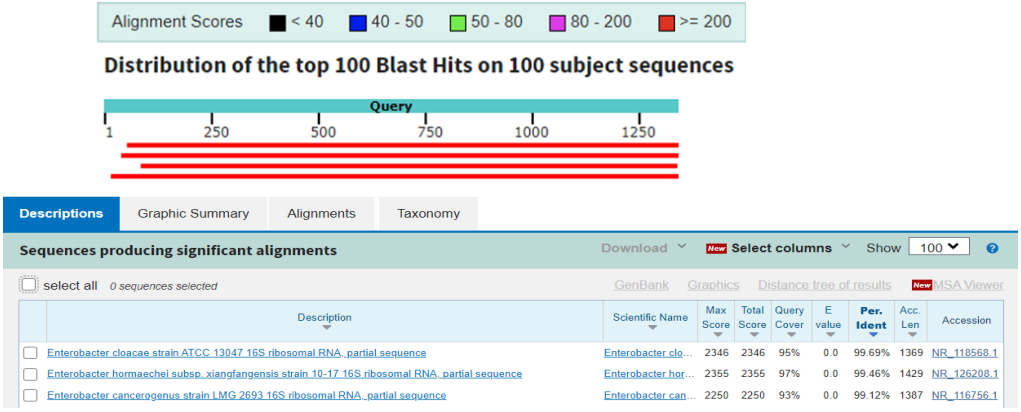
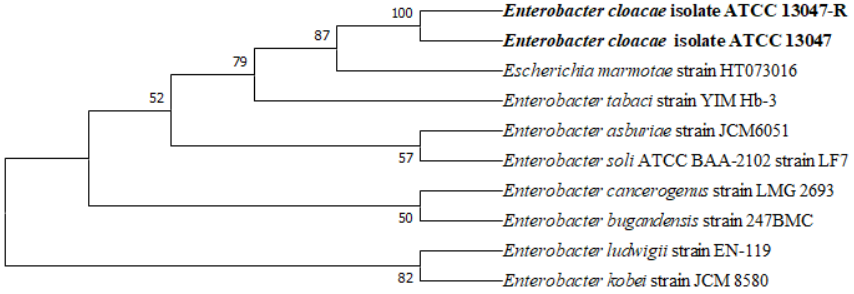


Re-isolated 16S rRNA gene partial sequence (1336 bp) of endophytic bacteria *Enterobacter cloacae* ATTC 13047-R



Reconfirmation of isolate *Enterobacter cloacae* ATTC 13047-R

9. Re-isolated sequence of *Enterobacter asburiae* NBE 23

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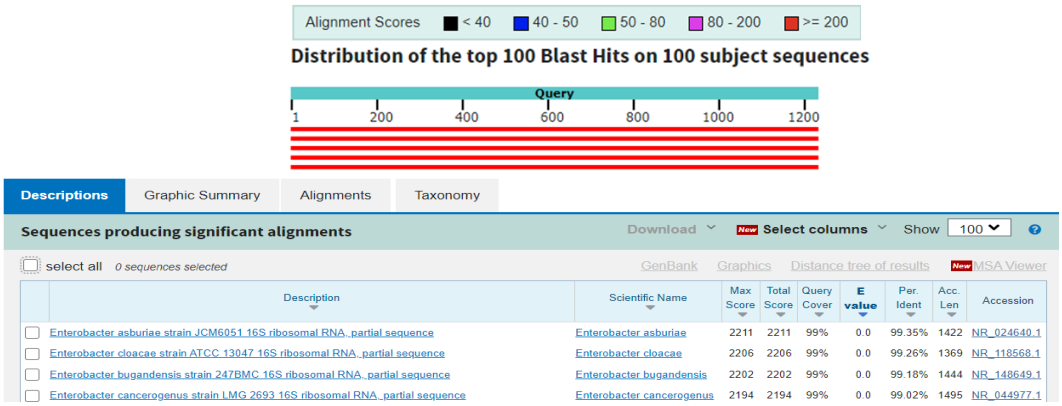
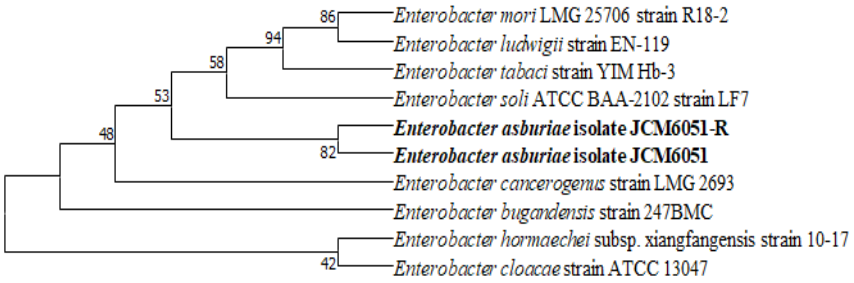


Re-isolated 16S rRNA gene partial sequence (1220 bp) of endophytic bacteria *Enterobacter asburiae* JCM6051-R



Reconfirmation of isolate *Enterobacter asburiae* JCM6051-R

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Characterization of Bacterial Endophyte Imparting Drought Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

Drought is a major abiotic stress which affects plant growth and productivity. It is proven in recent years, that microorganisms (fungi and bacteria) which can symbiotically inhabit the plant system and impart tolerance against abiotic stresses. In the present study, 58 bacterial endophytes isolated from Himalayan cold deserts were screened *in-vitro* using Polyethylene glycol (PEG MW-8000) against drought tolerance at different concentrations (5%, 10%, 15%, 20%, 25%). Out of 58 isolates, 8 isolates showed drought tolerance up to 20 per cent PEG. The drought tolerance of sensitive crops like, Rice (var. IR-64) seedlings were standardized by paper towel method using pre-germinated seeds. Further, pre-germinated seeds of rice seedings inoculated with the eight-drought tolerant endophytic bacteria were grown at 14.3 per cent PEG in paper towel method, of which one endophyte (CBE 11) significantly showed increased seedling length compared to uninoculated seeds. The endophyte was identified as *Stenotrophomonas maltophilia* by 16S rRNA gene sequence. It was found that this bacterial endophyte can impart drought tolerance in rice.

Keywords : Bacterial endophytes, Polyethylene glycol (PEG MW-8000), Rice (var. IR 64), *Stenotrophomonas maltophilia*

THE term 'Endophyte' is derived from the Greek words 'endon' (within) and 'phyte' (plant)). Endophytes include 'fungi or bacteria, which for all or part of their life cycle invade the tissues of living plants, but do not exhibit symptoms of disease (Kandel *et al*., 2017). Endophytic bacteria have been isolated from roots, leaves, stems, a few from flowers, fruits and seeds (Imran *et al.,* 2019). Endophytic bacteria may accompany certain metabolic properties, such as promoting plant growth, controlling soil-borne pathogens, or helping host plant to defeat stress (Ullah *et al.,* 2019). Further more, the interaction between plants and bacteria aid plants to settle in ecosystem restoration processes. These interactions may enhance the ability of plants to utilize nutrients from the soil by increasing root development, nitrate uptake or solubilizing phosphorus.

The abiotic stress such as drought cause imbalance in natural status of the plant affecting growth and productivity. It is estimated that 50 per cent of world rice production is affected by drought. Drought is a meteorological term that denotes a period without rain which results in water deficit stress. It is an environmental event with water availability below the optimum requirement for the full expression of yield potential (Blum, 2011). It also alters different physiological processes in rice leading to significant crop losses.

Drought tolerance is the ability of a plant to with stand moisture stress conditions. It is an important trait found in crop plants. Plants under drought stress are highly regulated by osmoregulation, antioxidative systems and secondary metabolite contents as reported elsewhere (Takahashi *et al.,* 2020). Drought stress induces an increase in ROS production resulting in various degree of oxidative damage in different genotypes of crops including rice. Therefore, it is important to understand the mechanisms that trigger physiological responses to drought stress and dehydration conditions. In the light of the above, the present study is initiated to screen and characterize bacterial endophytes important to impart drought tolerance in drought sensitive rice variety IR 64.

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Material and Methods

Collection of Bacterial Endophytes

Fifty-eight bacterial endophytes isolated from the plants growing in harsh environment of north Himalayan cold deserts of Pangong, Changla and Namika La regions and maintained by the School of Ecology and Conservation (SEC) Laboratory, Department of Crop Physiology, University of Agricultural Sciences (UAS-B), Gandhi Krishi Vignana Kendra, Bengalore - 560 065. These bacteria were rejuvenated on Nutrient agar medium. The physiographical of the cold desert regions are given in Table 1.

Table 1

Geographical information on Himalayan cold desert regions (where plants are collected for endophytes isolation)

Location	Latitude (° N)	Longitude (° E)	Altitude (M)
Pangong	33°43' 2.74"	78 °53' 29.08"	4250
Changla	34°30"	77° 55°	5216
Namika La	34°22'	76° 35	3700

Screening of Bacterial Endophytes for Drought Tolerance

The selected bacterial strains were tested for their drought tolerance in liquid cultures. Five ml of Nutrient broth (NB) supplemented with PEG (MW-8000) at 5, 10, 15, 20, 25 percentage concentrations were inoculated with each bacterial strain. The standard NB was used as control. The cell growth was determined measuring optical density OD_{600} by using a spectrophotometer (Bandeppa *et al.*, 2017). All tests were conducted in triplicates.

Standardization of PEG Concentration for Rice

Tolerance level of PEG for rice seedlings was determined by paper towel method. Different concentrations (5, 10, 15, 20 and 25%) of PEG solution was prepared in sterile distilled water (Muddarsu and Manivannan, 2017). These concentrations were corresponding to the osmotic potential -0.04 MPa, -0.14 MPa, -0.30 MPa, -0.51 MPa, -0.77 MPa, respectively. Two germination papers were taken for each concentration and 500 ml each of PEG solutions having different concentrations (5, 10, 15, 20 and 25%) were added to germination paper and excess solution was removed. Control was maintained by soaking the germination paper in distilled water. Then 10 each pre-germinated seeds of rice were placed on germination paper and incubated at 30 °C in the growth chamber. The shoot and root length of seedlings were recorded at 14 days after incubation. Lethal concentration (LC 50) value of PEG - 8000 was calculated for the shoot, root and seedling length using statistical software IBM SPSS statistics 20 (https://www.ibm.com/in-en/analytics/spss-statistics-software)

Inoculation of Bacterial Endophytes to Rice Seedlings

The seeds of rice were surface sterilized and incubated for germination at ambient temperature. Then the sprouted seeds were treated with bacterial suspension having ~$8x10^7$ CFU/ml population for three hours (Walitang *et al.*, 2017). The corresponding control was treated with sterile distilled water. After then the endophytes treated seeds were subjected to drought stress by placing on germination paper amended with 14.3 per cent PEG (LC 50 value) and incubated at room temperature for 14 days. There were two replications for each treatment and each replication comprised of 10 seedlings. Root and shoot length were recorded on 14 days intervals.

Confirmation of Inoculated Endophyte in Seedlings by Re-isolation

The plants were cut into one cm bits (root, stem and leaf) surface sterilized and placed on nutrient agar and incubated at 30 °C for 24 h. The bacterial colony emerged out of cut ends were sub-cultured and confirmed by comparing with the mother culture (Walitang *et al.*, 2017).

Molecular Identification of Bacterial Endophyte using 16S rRNA Gene Sequence

Extraction of Genomic DNA and PCR Amplification : Total genomic DNA of the bacterial endophyte

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was extracted by alkaline lysis method (Sambrook and Fristsol Maniatis, 1989) and the DNA concentration was determined by Nano drop. The primers already reported for 16S rRNA sequence from the NCBI (http://www.ncbi.nlm.nih.gov) were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly for the PCR reactions (26 bp forward primer 5' GTTAGATCTTGGCTCAGGA CGAACGC 3' and 24 bp reverse primer 5' GATCCAGCCGCACCTTCCGATACG 3'). PCR was performed in 20 µl reaction mixture containing 2.0 µl of 1X PCR Taq buffer with $MgCl_2$ (1.5 mM), 2.0 µl of 10 mM dNTP's mix (200 µM), 0.5 µl of primers (both forward and reverse), 0.3 µl of Taq DNA Polymerase (1U Genei Bengaluru), 1.0 µl of Template DNA, 13.7 µl of Sterile distilled water. Amplification was carried out with an initial denaturation at 96 °C for four minutes followed by 35 amplification cycles consisting of 94 °C for one minute, 60 °C for 30 seconds and 72 °C for one minute and a final extension at 72 °C for 10 minutes. Then the amplified product of DNA was electrophoresed using one per cent agarose gel and documented using gel documentation system. The DNA was eluted by using gel elution kit (The Gene JET™ Gel Extraction Kit, Thermo Scientific) and the amplified product was got sequenced by Chromgene Biotech Pvt. Ltd., Bengaluru, Karnataka. The sequences obtained were analysed for homology using NCBI GenBank.

Statistical Analysis

The data was statistically analysed using WASP: 2.0 (Web Agri Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php) and means were separated by Duncan Multiple Range Test (DMRT).

Results and Discussion

Screening of Bacterial Endophytes for Drought Tolerance

Fifty-eight bacteria isolated from different regions of Himalayan cold desert were screened for drought tolerance using different concentrations of PEG. Of which eight bacterial isolates (PBE 2, PBE 4, PBE 6, PBE 8, PBE 14, CBE 11, CBE 13 and NBE 5) showed tolerance at 20 per cent PEG (Fig. 1). This includes five isolates (PBE 2. PBE 4, PBE 6, PBE 8 and PBE 14) from Pangong region (Table 2), two isolates (CBE 11 and CBE 13) from Changla (Table 3) and one isolate (NBE 5) from Namkila La (Table 4). Other 50 isolates grown up to 15 per cent and did not show any growth at 20 per cent PEG indicating that they are susceptible to increased concentration. This may be due to increased membrane instability and decreased activity of superoxide dismutase (Sun *et al.*, 2010) which resulted in decreased growth. Aswathy *et al.*, (2020) reported that the bacterial endophytes isolated from leaves of *Ananas comosus* with stand water potential up to -1.5 MPa.

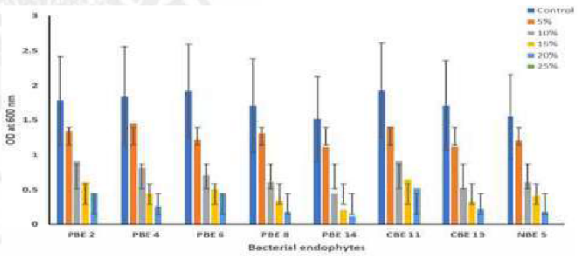

Fig 1. Growth of drought tolerant bacterial endophytes at different concentrations of PEG. Lines over bars indicates standard error mean ± SE(n=3)

Effect of Bacterial Endophytes on Drought Sensitive Rice (IR-64)

Standardization of Polyethylene Glycol (PEG MW 8000) Concentration for Rice : Rice seedlings were screened for drought stress using PEG (MW 8000) at 0, -0.04, -0.14, -0.30, -0.51 and -0.77 MPa concentrations. The length of root and shoot was decreased with increased PEG concentrations (Table 5). The untreated rice seedlings showed highest seedling length whereas lowest seedling length was observed with increased concentrations. LC 50 value of PEG concentration was found to be 14.3 per cent. The results are in agreement with Muddarsu and Manivannan (2017) who reported that eight Chilli cultivars screened for drought stress using PEG showed decreased root and shoot length with increased concentrations.

TABLE 2

Effect of different concentrations of PEG MW- 8000 on growth of endophytic bacteria isolated from Pangong region plants

Bacterial Isolates	Optical density of bacterial growth					
	Control (0%)	5%	10%	15%	20%	25%
PBE 1	0.70	0.38	0.23	0.10	0.0	0.0
PBE 2	1.78	1.34	0.91	0.61	0.45	0.0
PBE 3	0.65	0.58	0.32	0.18	0.0	0.0
PBE 4	1.84	1.45	0.82	0.45	0.26	0.0
PBE 5	0.86	0.68	0.55	0.24	0.01	0.0
PBE 6	1.92	1.22	0.71	0.51	0.45	0.0
PBE 7	1.08	0.34	0.25	0.16	0.00	0.0
PBE 8	1.71	1.31	0.61	0.34	0.18	0.0
PBE 9	0.65	0.48	0.24	0.12	0.0	0.0
PBE 10	0.66	0.54	0.32	0.21	0.0	0.0
PBE 11	0.89	0.52	0.46	0.18	0.0	0.0
PBE 12	0.98	0.55	0.42	0.12	0.0	0.0
PBE 13	0.58	0.44	0.38	0.18	0.0	0.0
PBE 14	1.52	1.11	0.45	0.21	0.12	0.0
PBE 15	0.65	0.48	0.32	0.17	0.0	0.0
PBE 16	0.64	0.45	0.23	0.12	0.0	0.0
PBE 17	0.68	0.44	0.22	0.10	0.0	0.0
PBE 18	0.74	0.33	0.16	0.10	0.0	0.0
PBE 19	0.89	0.58	0.24	0.12	0.0	0.0

Note: PBE = Pangong Bacterial Endophytes

TABLE 3.

Effect of different concentrations of PEG MW-8000 on growth ofendophytic bacteria isolated from Changla region plants

Bacterial Isolates	Optical density of bacterial growth					
	Control (0%)	5%	10%	15%	20%	25%
CBE 1	1.14	0.39	0.38	0.02	0.0	0.0
CBE 2	0.71	0.55	0.35	0.08	0.0	0.0
CBE 3	1.18	0.53	0.37	0.07	0.0	0.0
CBE 4	0.95	0.41	0.42	0.01	0.0	0.0
CBE 5	0.68	0.66	0.69	0.01	0.0	0.0
CBE 6	0.79	0.58	0.66	0.01	0.0	0.0
CBE7	0.58	0.35	0.15	0.01	0.0	0.0
CBE 8	0.78	0.51	0.44	0.10	0.0	0.0
CBE 9	1.10	0.53	0.31	0.10	0.0	0.0
CBE 10	1.01	0.80	0.40	0.18	0.0	0.0
CBE 11	1.93	1.41	0.91	0.65	0.52	0.0
CBE 12	0.50	0.17	0.16	0.11	0.0	0.0
CBE 13	1.71	1.12	0.52	0.33	0.22	0.0
CBE 14	0.35	0.29	0.18	0.12	0.0	0.0

Note: CBE = Changla Bacterial Endophytes

TABLE 4

Effect of different concentrations of PEG MW-8000 on growth of endophytic bacteria isolated from Namika La region plants

Isolates	Optical density of bacterial growth					
	Control (0%)	5%	10%	15%	20%	25%
NBE 1	0.55	0.25	0.12	0.02	0.0	0.0
NBE 2	0.45	0.24	0.11	0.02	0.0	0.0
NBE 3	0.55	0.45	0.12	0.05	0.0	0.0
NBE 4	1.05	0.98	0.50	0.04	0.0	0.0
NBE 5	1.55	1.21	0.61	0.41	0.18	0.0
NBE 6	1.02	0.65	0.45	0.24	0.0	0.0
NBE 7	0.56	0.32	0.21	0.14	0.0	0.0
NBE 8	0.23	0.10	0.08	0.05	0.0	0.0
NBE 9	0.45	0.32	0.22	0.12	0.0	0.0
NBE 10	0.89	0.40	0.32	0.10	0.0	0.0
NBE 11	0.44	0.35	0.25	0.01	0.0	0.0
NBE 12	0.65	0.52	0.44	0.04	0.0	0.0
NBE 13	0.78	0.69	0.58	0.35	0.0	0.0
NBE 14	0.77	0.58	0.44	0.21	0.0	0.0
NBE 15	0.74	0.35	0.25	0.10	0.0	0.0
NBE 16	0.56	0.24	0.12	0.04	0.0	0.0
NBE 17	0.47	0.21	0.11	0.06	0.0	0.0
NBE 18	0.85	0.78	0.54	0.07	0.0	0.0
NBE 19	0.96	0.44	0.24	0.24	0.0	0.0
NBE 20	0.74	0.33	0.22	0.12	0.0	0.0
NBE 21	0.32	0.24	0.18	0.05	0.0	0.0
NBE 22	0.25	0.12	0.08	0.02	0.0	0.0
NBE 23	0.96	0.69	0.34	0.11	0.0	0.0
NBE 24	0.66	0.34	0.07	0.03	0.0	0.0
NBE 25	0.55	0.24	0.14	0.06	0.00	0.00

Note: NBE = Namika La Bacterial Endophytes

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TABLE 5

Effect of different concentrations of PEG (MW-8000) on seedling length of Rice (IR-64)

Treatments	Root length (cm)	Shoot length (cm)	Seedling length (cm)
Control	10.0 [a]	14.5 [a]	24.5 [a]
5% (-0.04Mpa)	9.0 [b]	12.5 [b]	21.5 [b]
10% (-0.14Mpa)	7.0 [c]	10.5 [c]	17.5 [c]
15% (-0.30Mpa)	6.0 [d]	9.8 [d]	15.8 [d]
20% (-0.51Mpa)	4.5 [e]	9.1 [e]	13.6 [e]
25% (-0.77Mpa)	3.0 [f]	7 [f]	10 [f]
CD (P<0.05)	0.119	0.154	0.119

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

In-vitro Inoculation Bacterial Endophytes to Drought Sensitive Rice

Out of eight bacterial endophytes, three endophytes (CBE 11, PBE 2 and PBE 4) inoculated seedlings recorded maximum seedling length (32.5, 27.5 and 27.1 cm, respectively). The CBE11 showed significantly highest length which was selected for characterization (Table 6). PEG induced 14.3 per cent drought stress seedlings showed less seedling growth compared to control (without PEG) seedlings. Timmusk *et al.* (2014) reported higher root length and density improve water and nutrient uptake which could allow plants to tolerate drought stress. Similarly, three *Pseudomonas* sp. used for mitigating drought stress showed significant growth performance of finger millet compared to unino culated plants (Chandra *et al.,* 2018). The bacterium was re-isolated from root, shoot and leaves from inoculated rice seedlings and confirmed by morphological and microscopic observations while comparing with mother culture.

Molecular Characterization of Selected Endophyte using 16S rRNA Primer

Bacteria can be identified using morphological as well as molecular tools. The genes encoding for 16S rRNA in prokaryotes have been used extensively for sequence based evolutionary analysis because they are (1) Universally distributed, (2) Functionally constant, (3) sufficiently conserved and (4) Have adequate length to provide a view of evolution

TABLE 6

Effect of inoculation of bacterial endophytes on root and shoot length of Rice (IR-64) after 14 days of incubation

Treatments	Without drought stress			With drought stress at 14.3% PEG MW-8000 concentration		
	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)
Control	13.5 [d]	12.0 [d]	25.5 [d]	10.5 [d]	9.2 [c]	19.7 [d]
PBE 2	15.0 [b]	12.1 [c]	27.1 [c]	13.0 [b]	9.0 [d]	22.0 [c]
PBE 4	14.5 [c]	13.0 [b]	27.5 [b]	12.5 [c]	9.9 [a]	22.4 [b]
PBE 6	13.1 [e]	10.0 [e]	23.1 [f]	10.5 [d]	9.2 [b]	19.7 [e]
PBE8	15.0 [b]	9.5 [f]	24.5 [e]	10.0 [f]	6.0 [e]	16.0 [g]
PBE 14	13.5 [d]	9.5 [f]	23.0 [g]	10.0 [f]	5.6 [g]	15.6 [i]
CBE 11	18.5 [a]	14.0 [a]	32.5 [a]	15.0 [a]	9.0 [d]	24.0 [a]
CBE 13	10.5 [f]	9.2 [g]	19.7 [h]	10.0 [f]	5.7 [f]	15.7 [h]
NBE 5	9.0 [g]	10 [e]	19.0 [i]	10.5 [e]	6.0 [e]	16.5 [f]
CD(P<0.05)	0.162	0.144	0.129	0.124	0.119	0.136

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

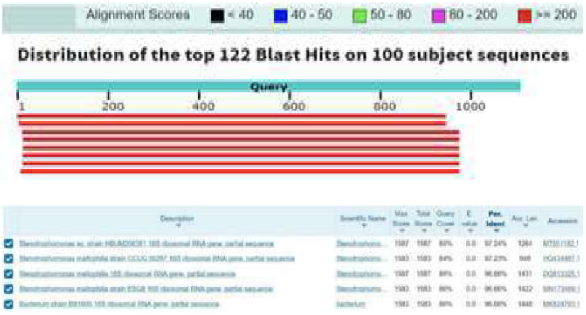

Fig. 2 (a) : 16S rRNA gene partial sequence of CBE 11 isolate showing 97% homologywith *Stenotrophomonas malthophilia* CCUG 50297 (518 bp)

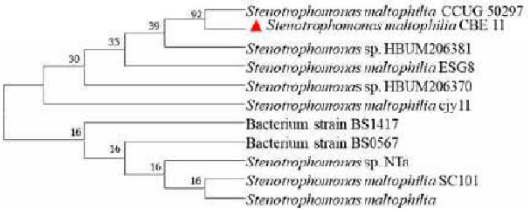

Fig. 2 (b) : Phylogenetic tree of *Stenotrophomonas maltophilia*

encompassing all living microorganisms (Madigan *et al.,* 2009). Molecular methods such as 16S rRNA/ 18S rRNA gene sequence is extensively used for identification of microorganisms (Nandan *et al.*, 2021). In the present study, drought tolerant endophyte (CBE 11) was identified by 16S rRNA gene sequence. The amplified product having 1,200 bp was compared with the sequences available at NCBI database and found 97.4 per cent homology with *Stenotrophomonas malthophilia*. The phylogenetic tree constructed with the sequences of 10 *Stenotrophomonas* spp. revealed that the isolate CBE11 is closely related to *S. malthophilia* (HQ434487) Therefore, the bacterium was confirmed as *S. malthophilia* (Fig. 2a and Fig. 2b). Santhosha gowda and Earanna (2017) identified the *Gluconoaceto bacter diazotrophicus* isolated from Maize using 16S rRNA gene sequence.

To conclude, bacterial endophytes isolated from different Himalayan regions were screened and characterized with distinct concentration of PEG, *Stenotrophomonas maltophilia* showed a significant result in Rice (IR-64). Therefore, bio-priming with this endophyte in rice can impart tolerances to drought stress.

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