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Functional characterization of exopolysaccharide and IAA producing bacterial strains of *Bacillus Cereus* for alleviating salt stress in spinach

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Abstract

Increasing soil salinity has been a serious problem over the globe and it is a potential barrier to food security. In India, a vast land around 6.74 million ha has been affected by salinity and it has been predicted that around 50% of the arable land will be sodic by the year 2050. Envisioning the national urgency and global microbiological intervention in salinity management, the present study has been conducted to assess the efficacy of exopolysaccharide (EPS) and IAA producing plant growth promoting rhizobacteria (PGPR) in alleviating salt stress in spinach. Two bacterial strains SP6 and SP8 originally isolated from potato rhizosphere were found to produce IAA and also exhibited salt induced EPS production. Biochemical and 16SrRNA gene analysis revealed that both the strains belong to *Bacillus cereus*. The strains when used as rhizoinoculant in Spinach, they have shown positive response towards minimising salt stress in spinach. The strain SP8, however, was more effective compared to the strain SP6. Furthermore, in addition to growth promotion in spinach under saline stress condition, they also protected the plant from salt stress injury by synthesising proline and preventing membrane damage as marked by decrease in malondialdehyde (MDA) content. Therefore, the study concluded that EPS and IAA producing PGPR strain *Bacillus cereus* SP8 may be exploited for restoring crop productivity in salinity affected agricultural fields.

Keywords: salinity, PGPR, IAA, exopolysaccharide, bacillus cereus, proline, MDA

Introduction

There are various environmental factors that limit agricultural productivity globally. Salinity is a major dynamic problem that causes degradation of the physical-chemical properties of soil resulting considerable decrease in crop productivity. Despite various reports, approximately 10% of the total arable lands estimating around 954.83 million ha are considered as authentic data that are affected by salinity, globally (Szabolcs, 1989; Shahid *et al.*, 2018) [44, 37]. In India, the estimated salt affected area is around 6.74 million ha and the estimate is escalating by 10% every year which may render around 50% of the arable land sodic by 2050 (Kumar and Sharma, 2020) [24]. The Govt. of India has set a goal to restore a total of 26 million degraded lands to ensure national food security. Natural withering and anthropogenic activities like global warming, rise in sea levels, chemical fertilizers are major constrains to achieve the national target. Amidst this serious problem of soil salinity and consequent threat to food security, natural beneficial Plant Growth Promoting Rhizobacteria (PGPR) have shown good promise to cater this problem in sustainable way. PGPR are group of bacteria that provide array of beneficiary mechanisms to improve plant growth, enhance plant yield and even can restore degraded soil by their intrinsic genetical mechanism (Backer *et al.*, 2018; Basu *et al.*, 2021) [4, 6]. PGPR has now been accepted globally for mitigating agricultural salinity and could be the future agricultural engineering for soil reclamation as extensively reviewed by many scientists in recent times (Shilev, 2020) [38]. ACC deaminase producing PGPR in particular have proved its efficacy in restoring soil salinity by many researchers (Egamberdieva *et al.*, 2019; Gupta and Pandey, 2019) [15, 17]. Exopolysaccharide (EPS) are complex biopolymers produced by many rhizobacteria has recently drawn attention of the microbiologist for its versatility in alleviating saline, drought stress in soil (Banerjee *et al.*, 2019; Ilyas *et al.*, 2020; Zainab *et al.*, 2020) [5, 20, 50]. In this present context of escalating soil degradation and urgency of soil restoration, the present study has been untaken to elucidate the role of EPS producing PGPR bacteria in catering salt stress in spinach and to scrutinise the possibility of EPS producing PGPR strains for restoring crop production in salinity affected agricultural soil.

Materials and Methods

Isolation of rhizosphere bacteria from potato plants

Root adhering soil form potato plants was collected from the potato field of Haripal Block, District-Hooghly, West Bengal, India. Ten grams of the soil was used for dilution plating in nutrient agar medium and distinct colonies were picked up for further analysis.

Detection of salt tolerance

Detection of salt tolerance of the strains were carried out following standard procedures. Equal amount of young broth culture of the bacterial strains was inoculated into conical flasks containing 50 ml of Salt minimal media (Dipotassium phosphate- 7gm/lit, Monopotassium phosphate- 2gm/lit., sodium citrate- 0.5gm/lit, Magnesium Sulphate- 10gm/lit and Ammonium Sulphate- 1gm/lit) supplemented with varying concentration of NaCl salt (0.25, 0.50, 0.75, 1.00, 1.25, 1.5 M) separately. Flasks were kept in a shaker incubator at 37 ºC. After 72 h of incubation, growth of the bacterial strain was observed for each set.

Screening and quantification of indole acetic acid (IAA) production by bacterial strains

IAA was quantified by slightly modified method by Bric *et al.* (1991) [10]. Briefly, bacterial cultures were grown in nutrient broth amended with 0.5mg/ml of tryptophan in shaking incubator for 72 hours at 37ºC. Supernatant of the cultures were collected by centrifugation at 10,000 rpm for 10 min. After that 2ml of supernatant was taken from each experimental set and mixed with 2 to 3 drops of orthophosphoric acid and 4 ml of Salkowski reagent (50 ml 35% perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Appearance of pink colour confirmed IAA production. IAA production was measured done by reading the OD at 530 nm with the help of spectrophotometer using IAA standard curve (10-100 µg/ml).

Screening of EPS production and quantification under salt stress

The bacterial isolates were streaked to yeast extract mannitol agar and grown for 30ºC for 48 hours for screening EPS production following the method of Sayyed *et al.* (2015) [36] with little modification. Appearance of mucoid colony confirmed positive test. For assessing the effect of salt stress in EPS synthesis by bacterial isolates, quantitative estimation of EPS was done. One loop full of overnight grown bacterial cultures were inoculated to flasks containing 50 ml of yeast extract-mannitol broth (YEMB) supplemented with varying concentration of NaCl (0, 0.25, 0.50 and 0.75 M) at 37ºC with constant shaking at 120 rpm. Viscosity of the culture flasks were monitored daily. After 3 days, the bacterial cultures were centrifuged (10000 rpm, 20 min) and the supernatants were collected for EPS estimation. Equal volume of 30% (v/v) isopropanol was slowly added to the supernatant with constant stirring and EPS was separated by spooling. Spooled samples were oven dried at 50ºC until a constant weight and was obtained for estimation of EPS. Two maximum EPS producing bacterial isolates grown under saline stress, SP6 and SP8, were finally selected for further studies

Spinach seed germination assay

Spinach seeds were purchased from local market of Sheoraphully, WB, India. and washed in 20% ethanol for 30 seconds for surface sterilization followed by shaking with 1% sodium hypochlorite, 0.1% Tween-20 solution for 10 minutes. The seeds were then washed thoroughly in autoclaved distilled water. Spinach seeds were soaked in young bacterial suspension of SP6 and SP8 (5×10^8 cfu/mL) separately for 3 hours. Germination assay was conducted using 150 seeds in three groups of 50 seeds each following the protocol of Lodo *et al.* (2013) [26] with necessary modification in ambient temperature in the month of November. The experimental groups contained SP6 and SP8 treated seeds and the control group contained only water treated seeds. After seven days, the number of germinated seeds were counted on basis of emergence of radicle (1 mm of length) out of the seed coat.

Plant Growth Assay

Twenty germinated seeds from each group of treatment *i.e.*, SP6 and SP8 treatment and control group were then planted in sterilized plastic pots (15cm height and 18cm diameter) filled with sterilized soil. To test the salt ameliorating potentiality of the selected bacterial isolates, bacteria treated germinated seeds and control seeds were irrigated either with 200mM of NaCl solution or water after two days of planting in the pots following the pot experiment method of Sinha and Mukherjee (2008). Plants were harvested after three weeks. The growth parameters such as length, fresh weight, dry weight, for root and shoot were noted. For dry weight measurement, plant parts were oven dried at 60°C till constant weight is achieved. The chlorophyll content of the mature leaves was determined. The experiments were carried out in triplicate.

Total Soluble Protein

Leaf Soluble protein was quantified according to established method of Bradford (1976) [9]. Two hundred milligrams of fresh leaves from treated and control groups were collected and homogenized in 5 ml chilled 0.1 M Na-phosphate buffer (pH -7). Then the homogenate was centrifuged at 5000 rpm for 10min at 4 °C and the supernatant was collected in a test tube for protein estimation. 0.1 ml of the supernatant was mixed with 5 mL of Coomassie brilliant blue solution (0.1 g Coomassie brilliant blue [G-250] in 50 mL of 90% ethanol and 85% phosphoric acid) and soluble protein was quantified by measuring the optical density at 595 nm. using a standard curve prepared taking bovine serum albumin (BSA) as standard.

Total Soluble Sugar

The quantitative estimation of total soluble sugar (TSS) content in the spinach leaves were done following the protocol of Watanabe *et al.* (2000) [46]. In short, two hundred milligrams of fresh leaves from treated and control groups were collected and homogenized in 80% ice-chilled ethanol. Then the homogenate was centrifuged at 5000 rpm for 10min at 4 °C and supernatant was collected in a measuring cylinder. The volume of the supernatant was made up to 10 ml with 80% ethanol. 1 ml supernatant was mixed with 3 ml anthrone reagent and the mixture were incubated for 10min at 100 °C in hot water bath. The reaction was stopped by placing in ice bath and soluble sugar was estimated by measuring the optical density at 620 nm using a standard curve prepared taking D-glucose as standard.

Proline Content

Proline content in spinach leaves were estimated by the method of Bates *et al.* (1973) [7]. Briefly, five hundred milligrams of fresh leaves from treated and control groups were collected and homogenized in 3.00 mL of 5% (w/v) sulfosalicylic acid. Then the homogenate was centrifuged at 10,000 rpm for 10min at 4 °C and supernatant was collected in a test tube. Following addition of ninhydrin and glacial acetic acid (1:1, v/v) to 500 µL of supernatant, the reaction mixture was boiled for 30 min. at 100 °C and then the reaction was stopped by putting it in ice for 5 mins. Solvent extraction of the reaction mixture was done using equal volume of toluene and the chromophore containing toluene was aspirated from the upper aqueous phase and proline was quantified by measuring the optical density at 520 nm using a standard curve prepared taking proline as standard.

MDA Content

MDA content in spinach leaves were estimated by the method of Heath and Packer (1968) [18]. Five hundred milligrams of fresh leaves from treated and control groups were collected and homogenized in in 5.0 ml of 0.1% (w/v) trichloroacetic acid (TCA). Then the homogenate was centrifuged at 10,000 rpm for 10min 4 °C and supernatant was collected in a test tube. Two millilitres of supernatant were mixed with 2.0 ml of 0.5% TBA (prepared in 20% TCA) and was boiled for 30 min at 95 °C in a water bath and then the reaction mixture was cooled quickly on ice bath. To discard the unwanted suspended impurities, the mixture was centrifuged at 10,000 rpm for 10min and absorbance of the supernatant was measured at 532 nm. To calibrate the observation, a blank mixture was prepared taking 0.025% TBA in 10% TCA and MDA was quantified as µmol/g FW using an extinction coefficient of $155Mm^{-1}$ cm^{-1}.

Morphological and biochemical characterization of bacterial isolate SP6 and SP8.

The morphological and biochemical characterization of the bacterial isolates SP6 and SP8 were carried out following standard microbiological procedure. Taxonomic identification of the bacterial strains was done following the standard method.

Molecular characterization of the bacterial isolate SP6 and SP8 by 16S rDNA gene sequencing and phylogenetic tree analysis

Total genomic DNA from the overnight culture of SP6 and SP8 were extracted. 16S rDNA of each strain was amplified using universal primers (Weisburg *et al*., 1991) [47], fD1 (5′-GAGTTTGATCCTGGCTCA–3′) AND Rp2 (5′-ACGGCTACCTTGTTACGACTT – 3′). The sequencing of the PCR product was done by SciGenom, India. Phylogenetic analysis of the sequence was done by aligning reference sequences downloaded from NCBI in CLUSTALW (Larkin *et al.*, 2007) [25]. MEGAX software (Kumar *et al*., 2018) was used to construct Phylogenetic tree by Maximum Likelihood method.

Statistical Analysis

Data were analyzed in RStudio of R version 4.0.2 (2020-06-22) using agricolae statistical package. The data were analyzed using one way ANOVA at p equal to 0.01and 0.001 significance level. Difference between treatment means were calculated using Tukey HSD test and compared at 5% probability level (p=0.5).

Result and Discussion

Potato rhizosphere bacterial colonies that appeared on NA plate were screened visually for colony morphology and EPS production. EPS positive isolates were further screened for salt tolerance and ten isolates which could tolerate up to 1mM NaCl were selected for studying salt induced EPS production efficiency. Ten EPS producing rhizosphere bacteria have shown to produce variable amount EPS under different NaCl concentrations (Figure 1). Similar reports have been represented by many researchers that salinity Stress can induce EPS production in bacteria (Kucuk 2020; Chambi *et al.*, 2021) [21, 13]. Finally, two bacterial isolates SP6 and SP8 which showed maximum EPS production under saline stress were selected for further study. The bacterial isolates SP8 and SP6 produced maximum of 3.42 and 3.17 mg/ml EPS respectively at 0.50 M NaCl but this production of EPS, however, decreased as NaCl strength was either decreased or increased beyond 0.50M NaCl concentration and the observation is consistent with earlier workers (Qurashi and Sabri 2012; Mukherjee *et al*., 2019) [31]. The bacterial isolates were also tested positive for plant hormone IAA production and estimated to produce IAA around 15.20 µg/ml by the strain SP6 and 18.37 µg/ml by the strain SP8 (Figure 2). Microbes synthesize and release

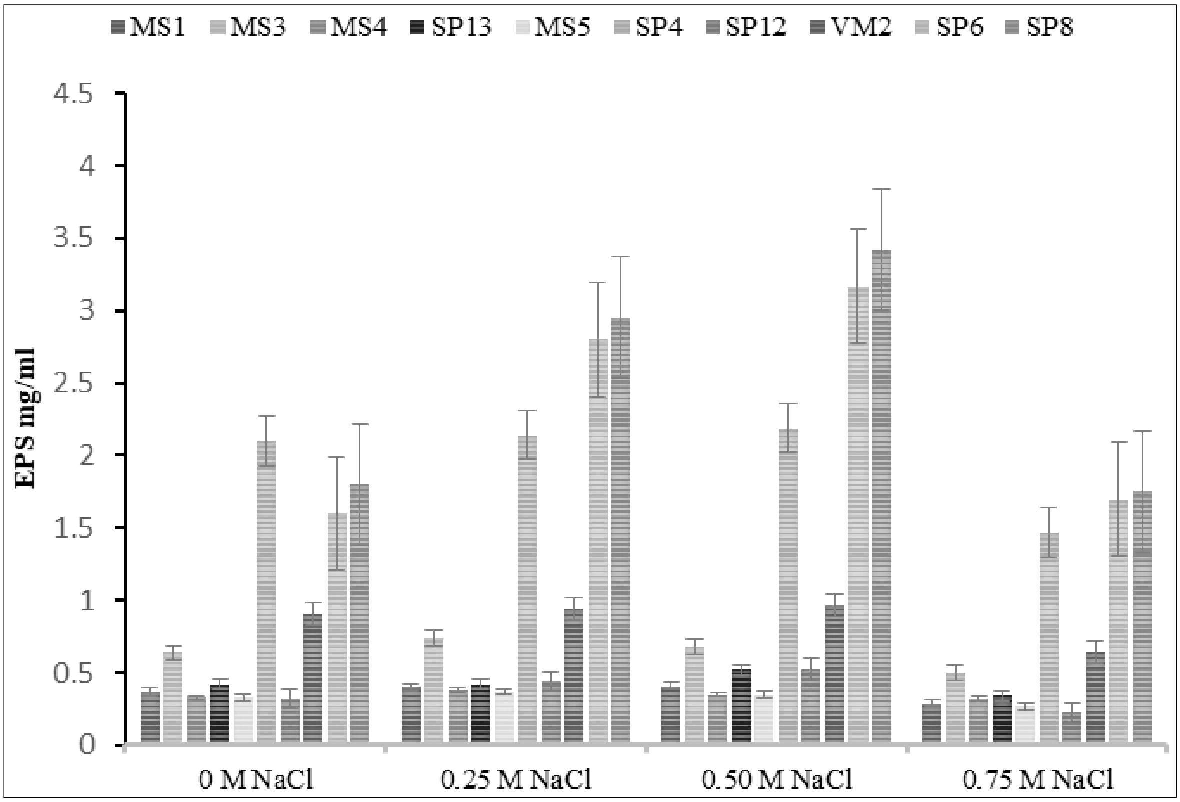

Fig 1: Effect of NaCl on EPS production by bacterial isolates

diverse compounds such as complex biopolymers, lectins, siderophores, polyhydroxyalkanoates (PHA) to cater environmental stress which act as protective jacket for their survival in the hostile environment (Yin *et al*., 2019) [49]. Among them exopolysaccharide (EPS) is a complex biopolymer synthesized by many bacteria under stress have drawn considerable attention of scientists in recent time owing to its multifarious applicability in different sectors. Despite diverse physiological role of EPS in management of environmental stress in bacteria (Costa *et al.*, 2018) [11], the physiological role of EPS in bacteria under saline stress remained obscure. Biofilm formation and exopolysaccharide synthesis is an intrinsic mechanism of salt tolerant bacteria to survive in saline environment. The synthesis of exopolysaccharide by microorganisms not only help their own survival but it offers various advantages to the plants when they are present in the rhizosphre. It has been reported that exopolysaccharide produced by PGPR can form soil aggregates and improve water retention capacity in saline soil (Costa *et al.*, 2018) [11]. EPS can bind to cations like Na^+ directly and hinder their toxic effect on plants and thereby alleviates salt stress (Mukherjee *et al*., 2019; Kumar *et al*., 2020) [24] through successful plant-microbe interaction (Upadhyay *et al*., 2011 & Arora *et al*., 2020) [45, 3].

Salt tolerant PGPR strains, SP6 and SP8 were tested for their efficacy to improve seed germination in spinach and it was found that both the strains improved spinach seed germination percentage. The strain SP8 was found to be slightly more efficient with 71% germination efficiency over the SP6 which had shown 66% germination of spinach seeds (Figure 3). Since the strains produced IAA so it might have improved seed germination percentage in spinach

Since both the strains could tolerate saline stress and could produce EPS so when tested both the strains found to alleviate salt stress in spinach in pot experiment (Table 1). The bacterial isolates were found to improve shoot length, root length, fresh weight, dry weight and chlorophyll content in spinach compared to uninoculated control. The bacterial strain SP8 however, was found to be more efficient in eliciting growth response increasing shoot length, root length, fresh weight, dry weight and chlorophyll content by 51.59%, 6.9%, 36%, 64% and 22.48% respectively compared to SP6 which increased all the growth parameters tested by 34.55%, 6.9%, 16%, 52% and 11% respectively over uninoculated control. The results are corroborating the observation reported by other researcher on spinach growth by PGPR (Cakmakci *et al.*, 2009; Shinde *et al.*, 2018) [39]. The two bacterial isolates also exhibited protection against salt stress by reducing the deleterious effects in spinach when grown under 200mM NaCl amended soil. The SP8 was the most efficient bacterial isolate to cater salt stress and it improved the plant health by increasing 57.91% shoot length, 45.78% root length, 22% fresh weight, 28.57% dry weight and 19.57% chlorophyll content compared to NaCl stressed uninoculated plants. The strain SP6, however, was less effective but has also shown the ability to protect plants grown under saline stress as evidenced by 26% increase in shoot length, 16% increase in root length and 12.07% increase in fresh weight, 14.28% increase in dry weight and 5.8% increase in chlorophyll content respectively over the NaCl treated plants. The results are concomitant with the observation of other researchers who have reported the role of PGPR in salt stress mitigation in different plants (Akram *et al.*, 2019; Shultana *et al.*, 2020) [2, 40]. The study of growth promotion of spinach by PGPR under saline stress however, is very limited but the present study corroborates with the observation of Muhammad *et al*. (2020) [28] who have recently reported salt stress alleviation by PGPR in spinach. The bacterial isolates could produce IAA and also have shown increased EPS production in saline condition which might have assisted the plants to restore their growth in saline environment (Kucuk 2020; Kumar *et al*., 2020) [21, 24]. Recently, it has been reported by Mukherjee *et al*. (2019) that not only EPS producing PGPR but even pure EPS could ameliorate salt stress in plants. Therefore, increased EPS synthesis by SP8 and SP6 under saline stress might have benefited spinach plant to cater salinity stress in this study.

Salinity stress is detrimental to the plants as it impairs normal physiological function and growth by altering the osmotic balance and hindering photosynthesis. Salt stress was found to inhibit plant growth in this study possibly by exerting sodium ion toxicity on cell enlargement, tissue differentiation, nutrient mobilization, cell membrane integrity and photosynthesis (Ahanger *et al*., 2017) [1]. Soil amendment of 200 mM NaCl was also found to reduce total chlorophyll content in the present study consistent with other reports (Sarker and Oba 2020) [35] probably by inhibiting ALA (5-aminolaevulinic acid) synthesis (Santos, 2004) [33] and increased chlorophyllase activity (Odjegba and Chukwunwike 2012) [30]. The PGPR bacteria SP8 and SP6 in this study were found to restore leaf chlorophyll content under saline stress like other PGPRs reported by many scientists (Zhang *et al*., 2018; Samaddar *et al*., 2019; Misra and Chauhan, 2020) [52, 32, 27]. The probable molecular mechanism of this favourable reversal of chlorophyll status by SP8 and SP6 might have been resulted from restoration of expression of PBGD (porphobilinogen deaminase) gene that encodes tetrapyrrole molecule of chlorophyll as reported in tomato by PGPR bacteria *Bacillus megaterium* strain A12 (BMA12) (Akram *et al.*, 2019) [2].

Protein content in the plant leaves were found to decrease in the saline condition (Figure 4) as reported by many researchers (Sarkar *et al*., 2020) [34]. However, leaf protein content was considerably increased by the bacterial isolates SP8 and SP6 in non-saline soil and they also restored the protein content in spinach leaves when grown under saline stress in the present study in agreement with the earlier work by Ilyas *et al.* (2020) [20]. Since IAA was reported to improve protein status in leaves of plants under salinity stress possibly through activating the nitrogen metabolizing enzymes (Gong *et al*., 2019), so it may be presumed that IAA producing SP8 and SP6 might have restored protein content in leaves through supply of IAA to spinach plants.

Salinity stress was found to reduce soluble sugar content in the leaves of spinach but SP8 and SP6 bacterized plants were successfully reverted the sugar status in the leaves to a maximum level (Figure 5) similarly as reported in previous studies by Misra and Chauhan (2020) [27]. In contrast to the observation of Misra and Chauhan (2020) [27], salinity was also found to increase soluble sugar in wheat (Ilyas *et al*., 2020) [20] as a mechanism to cater salt stress. However, PGPR treated wheat plants were found to accumulate more soluble sugars congruently with studies of Misra and Chauhan (2020) [27]. The explanation of sugar accumulation in this study by bacterized spinach plants may possibly by stimulation of carbohydrate metabolism and transportation of sugars and through accumulation of bacterial compatible sugars through root absorption (Ilangumaran and Smith

2017) [19]. Proline is an osmolyte and its accumulation is one of the adaptive features of plants to maintain osmotic balance and to overcome the membrane damaging effects of reactive oxygen species (ROS) generated by salt injury (Slama *et al*. 2015) [42] whereas augmentation of Malondialdehyde (MDA) indicate membrane damage by ROS induced lipid peroxidation (Zeeshan *et al* 2020) [51]. Therefore, both the proline content and MDA serve as biochemical signature in plants indicating the health status of plants grown in saline environment. Recently, application of salt tolerant PGPRs for mitigating salinity stress in plants through proline accumulation and MDA received considerable appreciation through the experimental validation of in different crops (Bharti *et al.*, 2016; Yasmin *et al*., 2020) [8, 48]. In congruence with the previous reports, the same trend of proline accumulation and reduction of MDA content were observed in spinach inoculated with salt tolerant bacterial isolates SP8 and SP6 when grown in NaCl amended soil. SP8 was more competent to diminish the toxic effects of salt treated spinach as it produced more proline (Figure 6) and less MDA than SP6 (Figure 7). Although most of the earlier reports highlighted IAA and ACC deaminase producing PGPRs for salt tolerant activities but the present study however, reports that EPS and IAA producing bacterial strains may protect plants from salt injury by altering the proline and MDA content which is supported by the recent study on rice by Sun *et al*. (2020) [43]. Detailed morphological and biochemical study of the bacterial isolates, SP6 and SP8 indicated that the strains possibly belong to the genera *Bacillus*. Analysis of phylogeny exploiting 16SrRNA gene confirmed that both the strains showed 100% homology with *Bacillus cereus* (Figure 8).

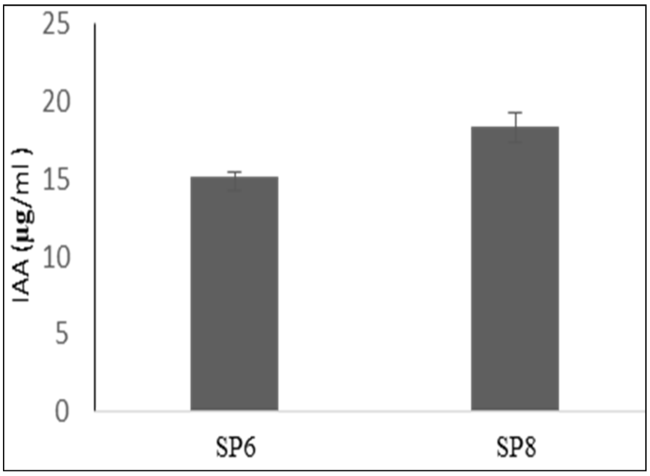

Fig 2: IAA production by bacterial isolates, SP6 and SP8

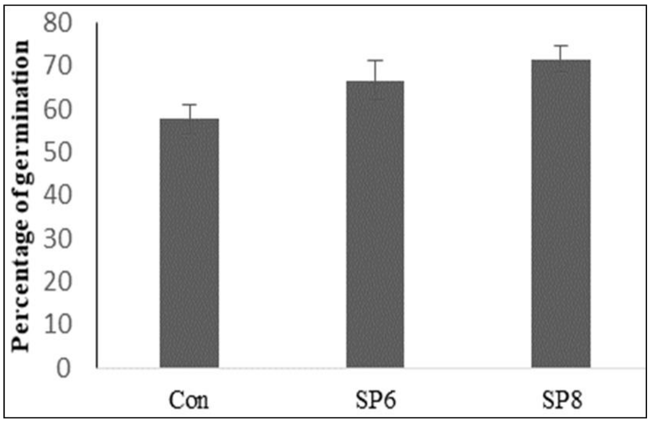

Fig 3: Effect of SP6 and SP8 on seed germination

Table 1: Effect of bacterial strains SP6 and SP8 on spinach growth under saline stress

	Shoot Length (cm)	**Root Length (cm)**	**Fresh Wt. (mg/plant)**	**Dry Wt. (mg/plant)**	**Chlorophyll content (mg/gm fresh tissue)**
CON	8.16^{c} (±0.12)	4.83^{a} (±0.18)	121.12^{c} (±1.01)	0.198^{b} (±0.01)	2.58^{c} (±0.03)
CON+SP6	10.98^{b} (±0.09)	5.13^{a} (±0.15)	140.71^{b} (±1.20)	0.248^{a} (±0.008)	2.88^{b} (±0.05)
CON+SP8	12.37^{a} (±0.19)	5.20^{a} (±0.14)	164.97^{a} (±1.06)	0.287^{a} (±0.01)	3.16^{a} (±0.04)
CON+200mM NaCl	5.18^{e} (±0.13)	1.66^{c} (±0.01)	98.93^{e} (±1.01)	0.14^{c} (±0.01)	1.89^{e} (±.0.05)
CON+SP6+200mM NaCl	6.55^{d} (±0.13)	1.93^{bc} (±0.05)	110.88^{d} (±1.16)	0.16^{bc} (±0.01)	2.00^{e} (±0.04)
CON+SP8+200mM NaCl	8.18^{c} (±0.11)	2.42^{b} (±0.10)	121.38^{c} (±0.84)	0.18^{b} (±0.01)	2.26^{d} (±0.07)
F-value	10.97	85.95	8.004	9.664	25.17
p	0.0016**	0.00***	0.006**	0.0029**	0.00***

Data are the mean of ten observations with standard error in parenthesis. Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 (Treatments with the same letter are not significantly different)

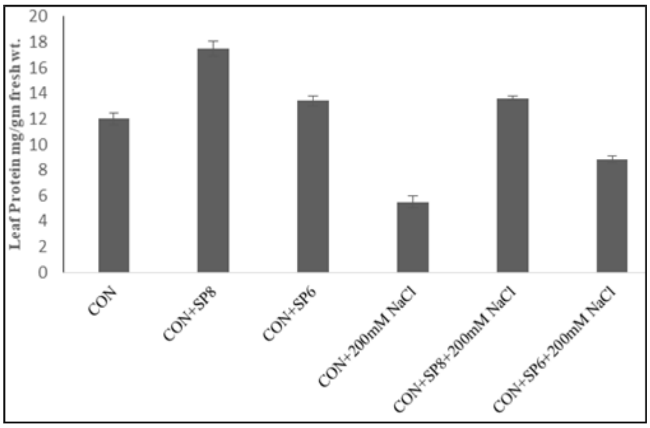

Fig 4: Effect of bacterial strains SP6 and SP8 on leaf protein content of spinach under saline stress

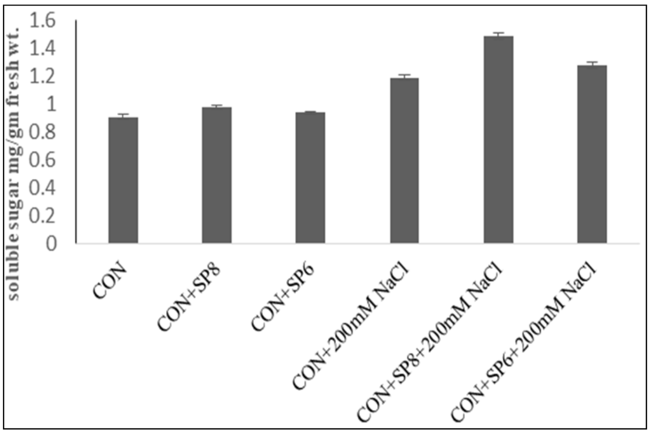

Fig 5: Effect of bacterial strains SP6 and SP8 on leaf soluble sugar content of spinach under saline stress

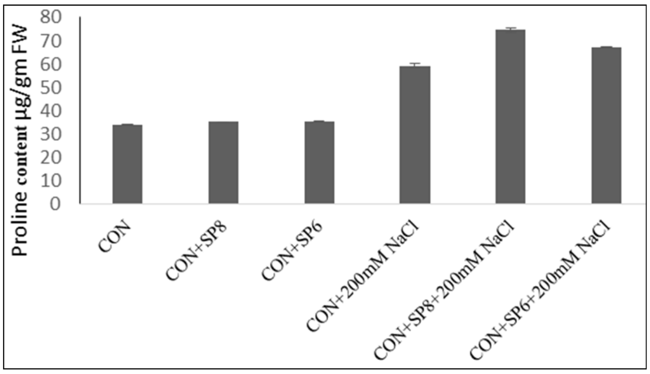

Fig 6: Effect of bacterial strains SP6 and SP8 on leaf proline content of spinach under saline stress

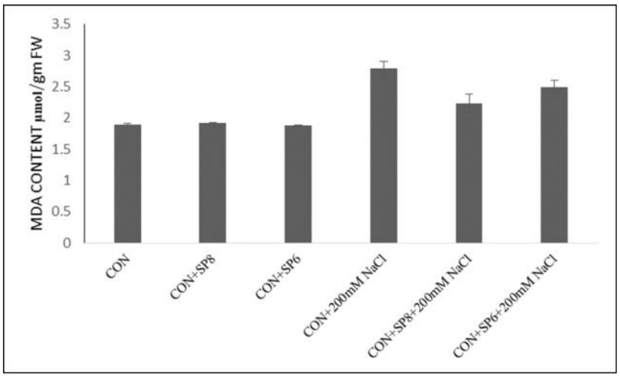

Fig 7: Effect of bacterial strains SP6 and SP8 on MDA content of spinach leaves under saline stress

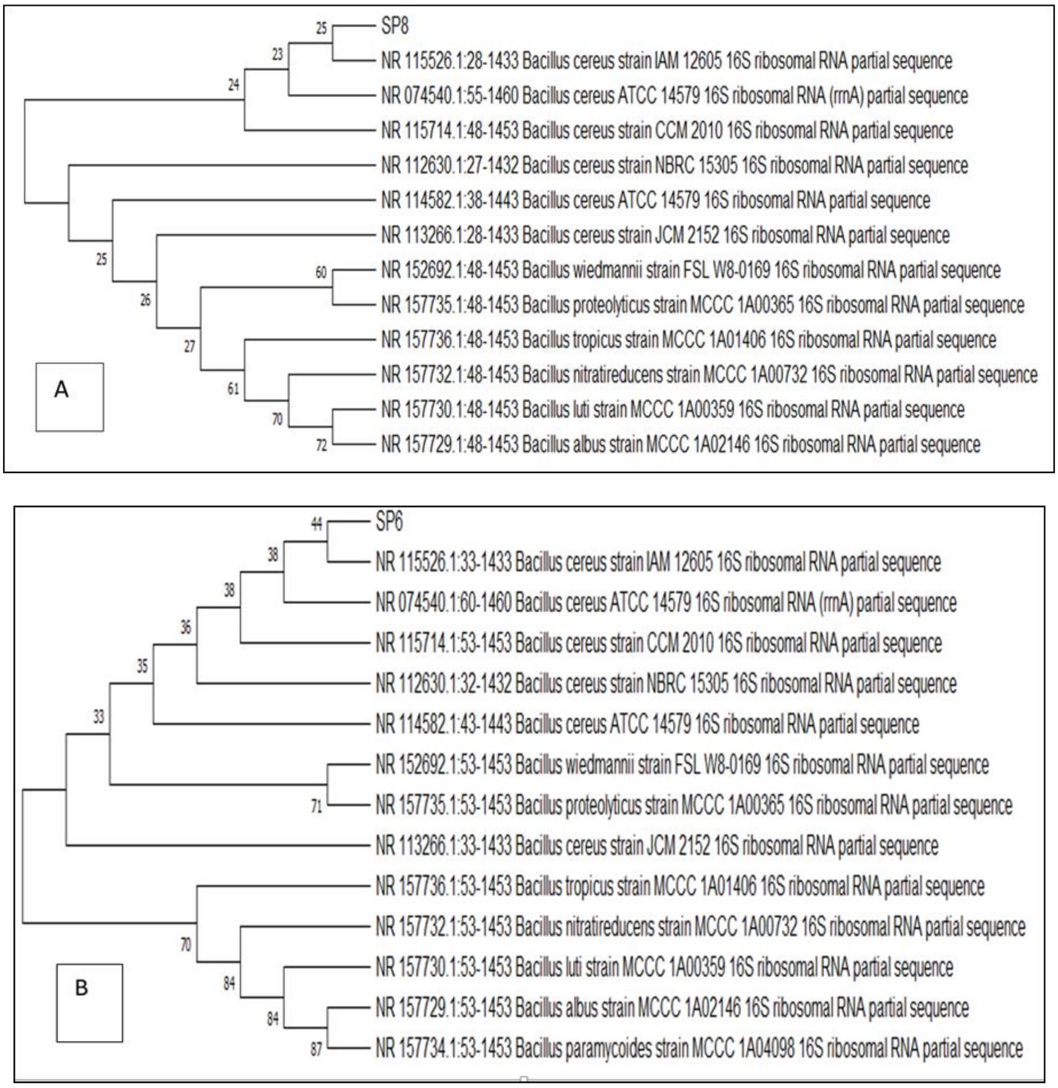

Fig 8: Phylogenetic tree showing evolutionary relationship between the isolated strain SP8 (A) and SP6 (B) with the matching strains from NCBI database. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches

Conclusion

The pot-based study revealed that *Bacillus cereus* SP8 and SP6 strains could restore spinach growth under saline stress by overcoming the deleterious effect of salt injury through accumulating proline as osmolyte and by preventing ROS induced membrane damage. However, the study needed to be performed under actual field condition and competitive colonisation ability of the strains must be verified.

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