



# Beneficial Soil Bacterium *Pseudomonas frederiksbergensis* OS261 Augments Salt Tolerance and Promotes Red Pepper Plant Growth

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Soil salinity, being a part of natural ecosystems, is an increasing problem in agricultural soils throughout the world. *Pseudomonas frederiksbergensis* OS261 has already been proved to be an effective bio-inoculant for enhancing cold stress tolerance in plants, however, its effect on salt stress tolerance is unknown. The main aim of the present study was to elucidate *P. frederiksbergensis* OS261 mediated salt stress tolerance in red pepper. The plants were exposed to a salt stress using NaCl at the concentrations of 50, 100, and 150 mM after 12 days of transplantation, while plant growth and enzyme activity were estimated 50 days after sowing. The height in *P. frederiksbergensis* OS261 inoculated plants was significantly increased by 19.05, 34.35, 57.25, and 61.07% compared to un-inoculated controls at 0, 50, 100, and 150 mM of NaCl concentrations, respectively, under greenhouse conditions. The dry biomass of the plants increased by 31.97, 37.47, 62.67, and 67.84% under 0, 50, 100, and 150 mM of NaCl concentrations, respectively. A high emission of ethylene was observed in un-inoculated red pepper plants under salinity stress. *P. frederiksbergensis* OS261 inoculation significantly reduced ethylene emission by 20.03, 18.01, and 20.07% at 50, 100, and 150 mM of NaCl concentrations, respectively. Furthermore, the activity of antioxidant enzymes (ascorbate peroxidase, superoxide dismutase, and catalase) also varied in the inoculated red pepper plants. Salt stress resistance in the bacterized plants was evident from the improved antioxidant activity in leaf tissues and the decreased hydrogen ion concentration. Thus, we conclude that *P. frederiksbergensis* OS261 possesses stress mitigating property which can enhance plant growth under high soil salinity by reducing the emission of ethylene and regulating antioxidant enzymes.

**Keywords:** plant growth promotion, salt stress, *Pseudomonas frederiksbergensis*, antioxidant enzyme, bio-fertilizer

## INTRODUCTION

Abiotic stresses exert a serious impact on crop productivity throughout the world. Soil salinity is an alarming stress, which limits plant growth and affects crop production to a large extent (Allakhverdiev et al., 2000; Bano and Fatima, 2009; Bacilio et al., 2016). The high amount of salt in soil disturbs the ionic balance in plant cells and decreases water uptake capacity in plants (Cheng et al., 2007; Singh and Jha, 2016; Stavridou et al., 2016; Yaish et al., 2016). In principle, high amounts of potassium or calcium ions are needed for the proper functioning of plant metabolism, whereas increased uptake of sodium ion due to high salinity reduces the ionic uptake of potassium and calcium ions (Cheng et al., 2007), thereby negatively affecting the growth of plants. It disturbs plant cell division and elongation and reduces the rate of photosynthesis (Munns and Tester, 2008; Forieri et al., 2016). This finally induces the generation of reactive oxygen species (ROS). The generated ROS negatively affect plant growth and development (Bojórquez-Quintal et al., 2014). Thus developing salt stress tolerant plants has been a highly desirable goal but there has been a little success to date; as only a few major genetic determinants of salt stress have been identified (Munns and Tester, 2008; Schubert et al., 2009). The alternate strategy is to introduce salt-tolerant microbes into plants under saline conditions. Beneficial microbes, such as plant growth promoting rhizobacteria (PGPR), which live in and around the root zone (rhizosphere), have been investigated as the means to overcome salinity stress. Most of the bacterial genera, such as *Agrobacterium*, *Azospirillum*, *Bacillus*, and *Rhizobium*, have already been reported to increase salt tolerance in plants (Hamdia et al., 2004; Ahmad et al., 2012; Bharti et al., 2013). They are known to stimulate plant growth through a variety of mechanisms such as the production of phytohormones, ACC deaminase (Siddikee et al., 2015), exopolysaccharide (EPS) (Siddikee et al., 2011), fixation of atmospheric nitrogen (Islam et al., 2013), and by solubilizing phosphate (Tank and Saraf, 2010). The stress usually induces ethylene emission (Morgan and Drew, 1997) and PGPR play a major role in reducing ethylene in plants via the action of ACC deaminase that cleaves ACC to  $\alpha$ -ketobutyrate and ammonia (Glick et al., 1998). An abiotic stress induces ROS as a result of the stress signal within the plant. Mostly,  $H_2O_2$ ,  $O_2^{\cdot-}$ , and  $OH^{\cdot-}$  are generated, which damage biological macromolecules: DNA, RNA, and proteins. ROS not only affect plants at a molecular level but also hamper main physiological activities. There are some antioxidant enzymes that can control the production of ROS under stressful conditions (Munne-Bosch and Pinto-Marijuan, 2016). Superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) are the main enzymes that play a major role in the self-defense in plants (Chojak-Koźniewska et al., 2017). SOD primarily converts superoxide radicals into hydrogen peroxide, thus defending the plants from oxidative damage. APX mainly works in peroxisomes where it helps in the breakdown of  $H_2O_2$ . CAT lowers the hydrogen peroxide level by converting it into water and oxygen (Habib et al., 2016). The microbes provide plants resistance to stress by enhancing the activity of

the antioxidant enzymes and other non-enzymatic antioxidants (Gururani et al., 2013).

Pepper (*Capsicum annuum* L. family Solanaceae) is widely grown in the different parts of the world and is one of the most popular vegetables in South Korea. It is an important ingredient of many traditional Korean foods, including Kimchi (Sang et al., 2008; Patra et al., 2016). Pepper is being cultivated in greenhouses as well as in fields since a long time. The repeated cultivation of pepper can increase soil salinity through the accumulation of organic fertilizers and pesticides, and as a result, an agricultural land can become highly saline, unsuitable for agriculture. High soil salinity causes major economic losses by reducing plant yield. In South Korea, a low temperature which is unfavorable for plant growth dominates 6 months of the year making productive agriculture possible only in summer. In addition to that, the crop production in the coastal regions of South Korea is also limited due to high salinity. This leads to an economically unfavorable situation. Recently, various approaches have been suggested to control salinity stress, one of them is the use of beneficial PGPR, which can enhance plant growth and productivity by eliciting salt-stress tolerance to host plants (Yang et al., 2009; Siddikee et al., 2010; Dodd and Pérez-Alfocea, 2012). In order to maximize the crop yield and make it economically favorable, it would be convenient to use a bacterial strain capable of mitigating both low temperature and salinity stress. *P. frederiksbergensis* OS261, a PGPR, and member of the group Gammaproteobacteria, has been proved to increase the fitness of tomato plants under cold stress conditions (Subramanian et al., 2016) and also displayed its ability to colonize in plant parts of red pepper (Sandipan et al., 2016) and tomato (Subramanian et al., 2015). Even though *P. frederiksbergensis* OS261 has been proved to be an effective bio-inoculant for enhancing cold stress tolerance in plants (Subramanian et al., 2016), its effect on increasing salt stress tolerance still remains largely unknown. This work was aimed to evaluate *P. frederiksbergensis* OS261 as an effective inoculant for enhancing salt stress tolerance in plants through the production of ACC deaminase, reduction of stress ethylene level, and induction of antioxidant enzymes.

## MATERIALS AND METHODS

### Bacterial Strain and Culture Conditions

*Pseudomonas frederiksbergensis* OS261 (GenBank Accession no. KF424313, 16S ribosomal RNA gene) was previously isolated from the experimental fields of Chungbuk Agricultural Research and Extension Services, Ochang-eup, South Korea (36°43' N; 127°27' E). This strain was selected on the basis of plant growth promoting effects and ACC deaminase activity (Subramanian et al., 2016). For inoculum preparation, a single colony of *P. frederiksbergensis* OS261 grown on a nutrient agar (NA) plate was transferred to 5 mL nutrient broth (NB) and incubated at 30°C on a shaker (150 rpm) for 24 h. After the incubation, 1.0 mL of the culture ( $\sim 1.0 \times 10^8$  CFU mL<sup>-1</sup>) was transferred to 50 mL fresh NB and allowed to grow for further 24 h. The culture was centrifuged at 5000 rpm for 5 min at 4°C, and the pellet was washed twice with 30 mM MgSO<sub>4</sub>. The pellet

was resuspended in 30 mM MgSO<sub>4</sub> to an optical density of 0.8 at 600 nm (cell count  $\sim 1.0 \times 10^8$  CFU mL<sup>-1</sup>).

## Plant Experiments

### Seed Sterilization and Bacterization

Red pepper (*C. annuum* L. cv. Bulmat) seeds (Syngenta seeds, Seoul, Republic of Korea) were surface sterilized with 70% ethanol for 30 s and 2% sodium hypochlorite for 1 min, followed by rinsing for several times with sterile deionized water. The bacterial cultures were grown in NB media at 30°C for 24 h with 150 rpm. The grown culture was harvested by centrifuging at 5000 rpm for 5 min at 4°C and the cell pellets were resuspended in 30 mM MgSO<sub>4</sub> to obtain an optical density of 0.8 at 600 nm (cell count  $\sim 1.0 \times 10^8$  CFU mL<sup>-1</sup>). The bacterization was performed by soaking the seeds in bacterial suspension for 4 h whereas the control seeds were soaked only in MgSO<sub>4</sub>.

### Gnotobiotic Pouch Experiments

The red pepper seeds were surface sterilized and bacterized as mentioned above. After decanting the suspension, the seeds were transferred to growth pouches (six seeds per pouch), with six replications (i.e., six pouches) per treatment. The pouches were then kept in a growth chamber (DS 54 GLP, DASOL Scientific Co., Ltd., Korea) at 25°C with 12 h day-night photoperiod for 7 days (relative humidity 70% and light intensity 18  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). After 7 days of plant growth, 0, 50, 100, and 150 mM of sodium chloride solutions prepared in 1X Hoagland solution were used to induce salt stress. The effect of inoculation on early growth promotion was determined by measuring the root and shoot length after 14 days of sowing (DAS). Total dry matter accumulation was measured after drying the seedlings in an oven at 70°C for 48 h.

### Determination of Ethylene Emission

The ethylene emission from the red pepper seedlings was measured following the protocol of Mayak et al. (2004) with some modifications. The seed bacterization was performed as mentioned above. Following the bacterization, 30 seeds were imbibed in either a bacterial suspension or 30 mM MgSO<sub>4</sub> for 2 h, the suspension or buffer was drained, the seeds were placed inside 120 mL narrow neck bottles on a piece of filter paper and 2 mL of de-ionized water was added to each bottle. After 8 days, the excess liquid was drained and 2 mL of respective salt solution (50, 100, and 150 mM) was added. The seeds those were imbibed in buffer and were not treated with salt served as un-inoculated control. After 4 h of salt addition, the narrow neck bottles were closed for 2 h with a rubber septum and 1 mL sample from the headspace was injected into a gas chromatograph (dsCHROM 6200, Donam Instruments Inc., Republic of Korea) packed with a Porapak-Q column and equipped with a flame ionization detector. The oven, injection, and detection temperatures were set at 40, 150, and 250°C, respectively. Six replicates were used for each treatment.

### Greenhouse Experiments

The bacterized and non-bacterized red pepper seeds were sown in seedling trays containing 40 g of nursery soil (Nongwoo-Bio Co.,

Ltd., Yeosu-gun, Gyeonggi-do, Republic of Korea; composition: 58.8% coco peat, 17% peat moss, 10% perlite, 10% vermiculate, 4% zeolite, 0.004% pyroligneous acid and 0.01% wetting agent) and incubated in the greenhouse at a temperature range of 25–30°C with 50–70% relative humidity and a 15–9 h day/night period. Seven days old red pepper seedlings were transplanted into plastic pots (16 cm  $\times$  16 cm) containing 500 g of nursery soil. After transplanting, the plants were allowed to grow under normal conditions in the greenhouse. The bacterial inoculum was prepared as mentioned above and the inoculation was carried out in the soil at 14 DAS by adding 10 mL of the bacterial suspension (cell count  $\sim 1.0 \times 10^8$  CFU mL<sup>-1</sup>) near the root zone. Five days after applying the bacterial suspension, salt stress was induced gradually by applying 25 mM of sodium chloride solution to each pot on alternative days to avoid osmotic shock; and the desired salt concentrations of 50, 100, and 150 mM were achieved after 2, 6, and 10 days, respectively. Parallel controls were maintained by irrigating with tap water. The leaching of water from the pots was prevented by retaining the soil water to a level below water holding capacity. The soil electrical conductivity of 0, 50, 100, and 150 mM treatments were  $1.31 \pm 0.04$ ,  $6.23 \pm 0.07$ ,  $10.23 \pm 0.35$ , and  $16.23 \pm 0.21$  dS/m, respectively, at the time of harvest. Fifty days after the sowing, the plants were uprooted and the length of the plants was measured. Additionally, the number of leaves per plant was also recorded. The total dry biomass of the plants was determined after drying the plants in an oven at 70°C for 72 h.

### Determination of Antioxidant Enzymatic Activity

Antioxidant enzymes were measured from a crude enzyme extract derived from red pepper leaves (approximately  $\sim 1$  g) obtained from the pot culture plants that were harvested at 50 DAS. Briefly, the fresh leaves were ground using liquid nitrogen and the ground leaf samples were stored at  $-80^\circ\text{C}$ . The ground leaf samples (0.5 g) were homogenized in ice using 10 mL of 50 mM of potassium phosphate buffer, 1% (w/v) polyvinylpyrrolidone (pH 7.8) then incubated for 10 min at 4°C. The homogenate was filtered using Advantech Qualitative Filter Papers (110 mm) and centrifuged (VS-24SMTi, High Speed Refrigerated Centrifuge, Vision Scientific Co. Ltd., Korea) at  $4000 \times g$  for 15 min at 4°C. The supernatant was considered as the enzyme extract and was used for the determination of enzyme activities. The CAT activity (EC 1.11.1.6) was determined by a hydrogen peroxide assay based on the formation of its stable complex with ammonium molybdate (Goth, 1991). Briefly, 0.2 mL of the enzyme extract was incubated in 1 mL reaction mixture containing 65 mM hydrogen peroxide in 60 mM sodium phosphate buffer at room temperature for 4 min. The reaction was terminated using 1 mL of 32.4 mM of ammonium molybdate and the concentration of the yellow complex formed during the reaction was measured at 405 nm. SOD (EC 1.15.1.1) and APX (EC 1.11.1.11) activities were measured according to Ding et al. (2011). In the case of SOD, the assay mixture contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57  $\mu\text{M}$  NBT, 0.025% (w/v) Triton X-100, 0.0044% (w/v) riboflavin and the enzyme extract. The SOD activity was measured at 560nm by following the decrease in the absorbance due to



the photochemical reduction of nitro-blue tetrazolium (NBT). The APX activity was evaluated by the oxidative conversion of ascorbic acid to dehydroascorbate with regard to the decrease in the absorbance. It was calculated by the means of extinction coefficient  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$  at 290 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.3 mM ascorbic acid, 0.1 mM  $\text{H}_2\text{O}_2$ , 0.1 mM EDTA and 50  $\mu\text{L}$  of the enzyme extract. Hydrogen peroxide content in the leaves was evaluated according to the protocol of Theocharis et al. (2012).

### Statistical Analysis

In this study, a completely randomized block design was employed for the early growth and greenhouse experiments. The data from experimental results were subjected to a two-way analysis of variance (ANOVA). The percentage data and the significant differences between the means were determined by Duncan's Multiple Range Test (DMRT) at  $P < 0.05$  using SAS package, Version 9.4.

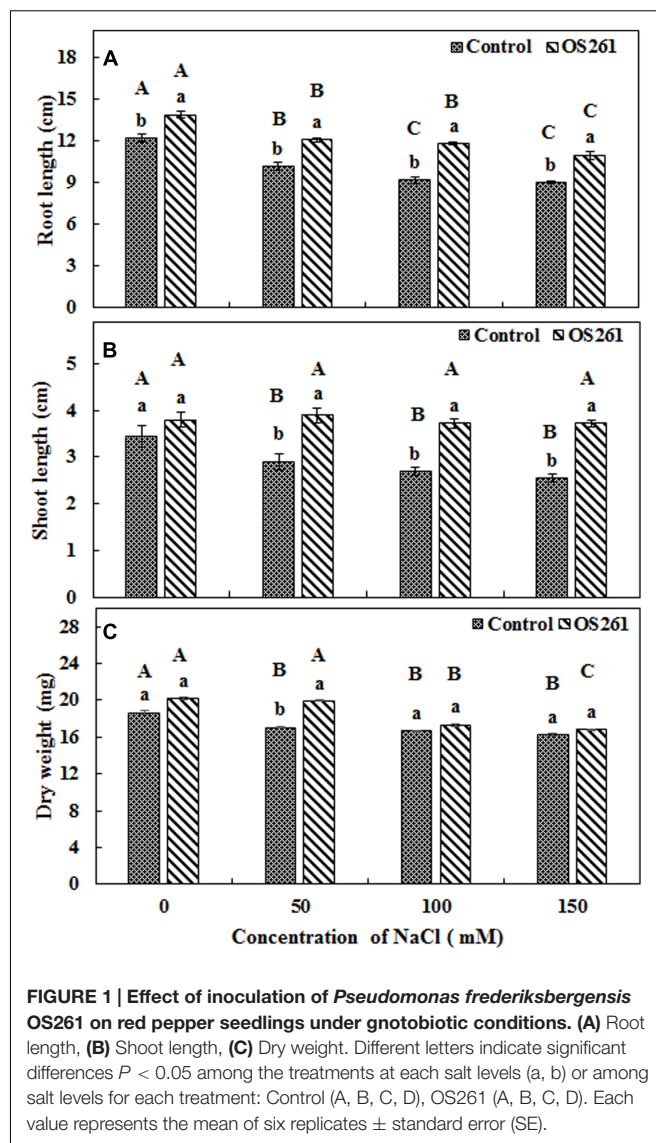
## RESULTS

### Effect of *P. frederiksbergensis* OS261 on the Growth of Red Pepper Seedlings under Gnotobiotic Conditions

The effects of inoculation of *P. frederiksbergensis* OS261 on growth of red pepper under gnotobiotic conditions are shown in Figure 1. The plants inoculated with the bacterium developed longer roots (Figure 1A) compared to the un-inoculated controls after a period of 14 days under salinity stress. The inoculation increased the root length by 13.93, 18.62, 28.26, and 22.22% under 0, 50, 100, and 150 mM of NaCl treatment, respectively, compared to the controls. On the other hand, the changes in shoot length (Figure 1B) were also noticeable. The bacterial inoculation increased the shoot length by 11.76, 34.48, 37.03, and 45.09% under 0, 50, 100, and 150 mM of salt treatment, respectively, compared to the controls. Similarly, the inoculated red pepper plants accumulated higher plant biomass (Figure 1C) compared to the un-inoculated controls under salt-stressed conditions with a significant increase in the salt stress induced by 50 mM NaCl.

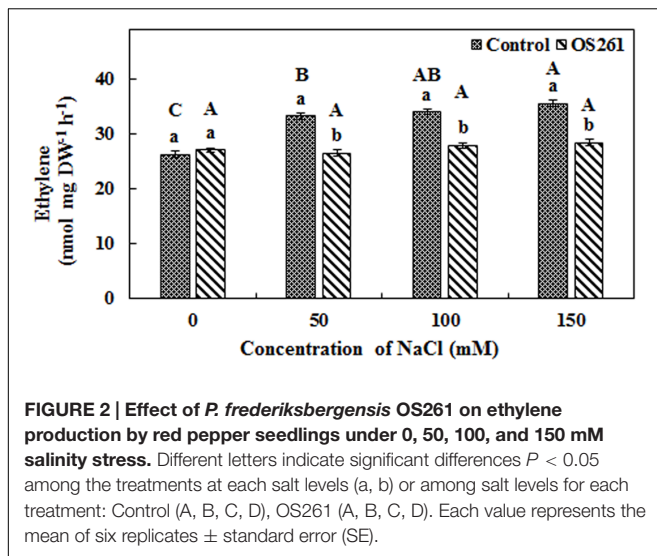
### Determination of Ethylene Emission from Red Pepper Seedlings with and without Inoculation

A higher amount of ethylene emission was observed from un-inoculated red pepper seedlings under 50, 100, and 150 mM of salt stress (Figure 2), but inoculation of *P. frederiksbergensis* OS261 reduced ethylene emission. It was observed that ethylene production increased by 20, 18, and 20.07% in un-inoculated red pepper seedlings at 50, 100, and 150 mM NaCl, respectively, compared to the inoculated red pepper seedlings.



### Effects of Inoculation with *P. frederiksbergensis* OS261 on the Growth of Red Pepper Plants under Salt Stress Conditions in the Greenhouse

In order to validate the results obtained in gnotobiotic conditions, similar experiments were performed under greenhouse conditions and the effect of inoculation on plant growth was checked by the means of plant height, dry biomass, and the number of leaves (Figure 3). The inoculated plants showed 37.79% increase in root length, 19.05% increase in shoot length and 31.97% increase in dry weight under normal conditions. Among the different levels of salt stress (50, 100, and 150 mM), the trends were similar, where inoculation increased root length (Figure 3A) by 36.44, 84.44, and 86.63%, respectively, as well shoot length (Figure 3B) by 34.35, 57.25, and 61.07%, respectively, compared to the un-inoculated controls. The number of leaves in each plant (Figure 3C) also increased



significantly, contributing to the increase in dry biomass (Figure 3D), by 37.47, 62.67, and 67.84% under 50, 100, 150 mM of NaCl treatments in comparison to the un-inoculated plants. Overall, the inoculation resulted in a significant improvement in plant growth under both normal and salt-stressed conditions.

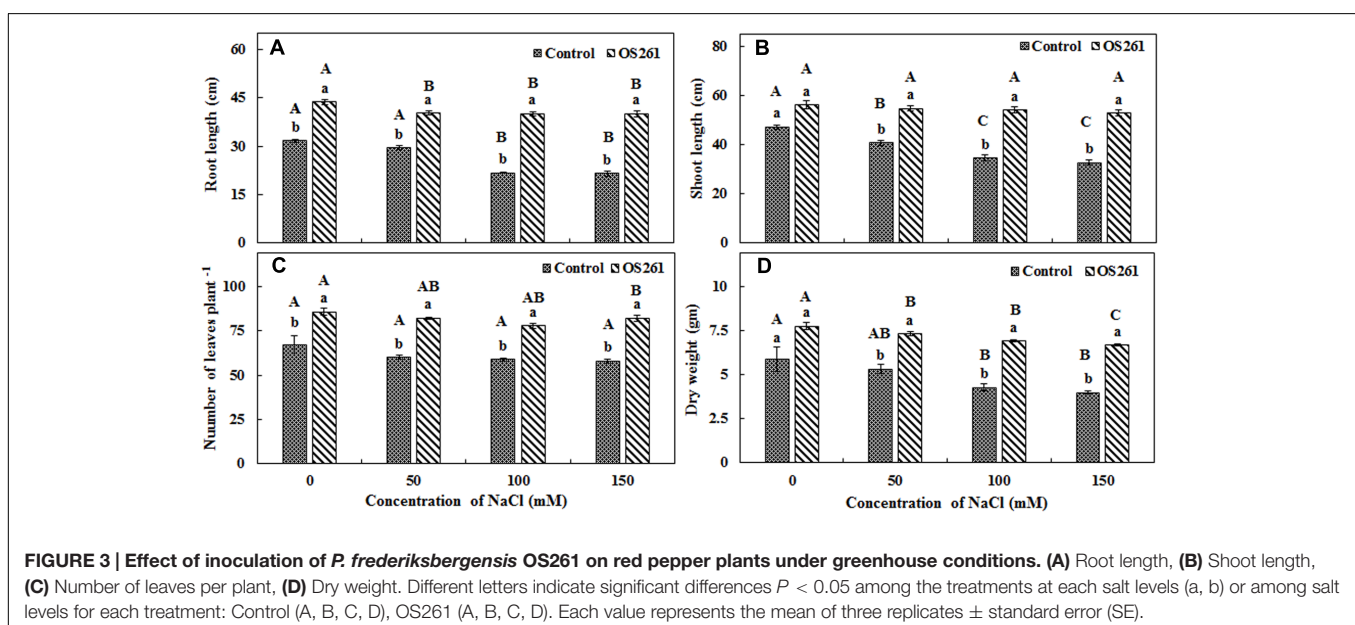
### Antioxidant Enzyme Activities and Hydrogen Peroxide Content in Red Pepper Plants Inoculated with *P. frederiksbergensis* OS261

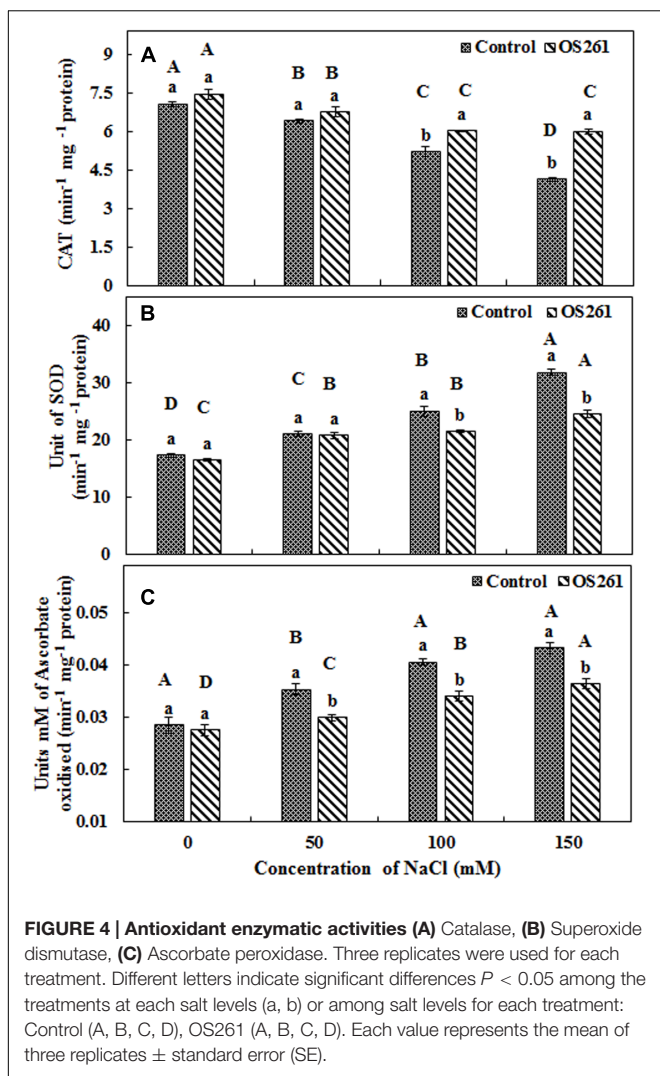
The activity of three antioxidant enzymes (CAT, SOD, and APX) varied under salt stress condition compared to the normal conditions. The CAT activity in the plants seemed to increase significantly after the treatment with the bacterium under the

different levels of salt concentrations (Figure 4A). The inoculated plants showed 5.37, 5.62, 15.96, and 45.45% increase in CAT activity at 0, 50, 100, and 150 mM of the salt treatments. In the inoculated plants, the SOD activity showed no significant changes upon the inoculation at 0 and 50 mM salt concentration but was significantly reduced at 100 and 150 mM salt concentrations in comparison to the un-inoculated control plants (Figure 4B). Similarly, APX activity (Figure 4C) showed a similar pattern with SOD where bacterial inoculation significantly reduced the activity under 50, 100, and 150 mM of salt concentrations with no significant changes at 0 mM compared to the un-inoculated plants. Hydrogen peroxide content increased considerably in the un-inoculated control plants with an increase in the soil salinity (Figure 5). Interestingly, the bacterial inoculation reduced the peroxide content significantly, which may help the plant cope up with the effect of stress.

## DISCUSSION

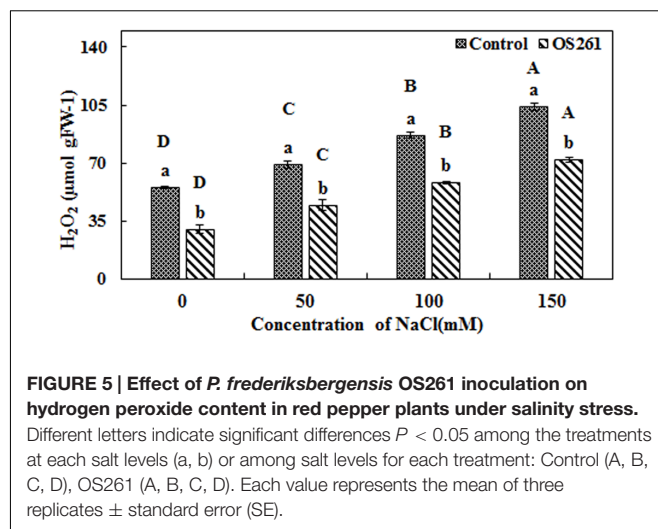
The use of ACC deaminase producing PGPR in soil resulted in an enhanced growth of red pepper plants under salt stress. These positive effects were exerted by maintaining the low levels of ethylene and modulating the expression of ROS-scavenging enzymes. Ethylene is a plant growth regulator, which is involved in several physiological responses (Abeles et al., 1992). In addition to that, it is also a stress hormone as it is synthesized rapidly under stress (Stearns and Glick, 2003). Several studies (Belimov et al., 2001; Saravanakumar and Samiyappan, 2007) have reported that ethylene reduces seed germination rate and root development during stress, which eventually affects plant growth. Microorganisms synthesizing the ACC deaminase enzyme can cleave ACC to  $\alpha$ -ketobutyrate and ammonia, thereby decreasing ethylene stress in plants (Glick, 2005; Sun et al., 2009; Siddikee et al., 2011; Rashid et al., 2012). In this study, a





PGPR strain *P. frederiksbergensis* OS261 which possesses ACC deaminase activity demonstrated its effectiveness in inducing salt tolerance and consequent improvement in the growth of pepper plants under salt stress.

Pepper (*Capsicum* spp.) along with tomato and potato is an economically important genus of Solanaceae family. Owing to its ability to adapt to different climates and being available in varieties of shapes, sizes and color, pepper plants are cultivated all over the world (Qin et al., 2014). Various biotic stresses such as viruses and abiotic stresses especially drought and salinity are known to affect this economically important family. Aktas et al. (2006) reported that pepper plants are highly susceptible to salinity stress. In plant growth experiments, pepper plants exhibited a decrease in plant height with an increase in the salinity in the un-inoculated controls. Salinity stress is one of the main obstacles that limit higher crop production. It increases the osmotic potential of the growth medium resulting the seeds to require more energy for water absorption, which leads to poor germination (Jamil and Rha, 2004). It decreases the rate of photosynthesis by restricting the photosynthetic electron



transport, which in turn paralyzes the whole plant mechanism for survival and development (Hameed et al., 2015). In the present study, in contrast to the un-inoculated plants, the inoculated or bacterized plants showed enhancement in the growth despite increasing salinity. It indicates the ACC deaminase producing isolate *P. frederiksbergensis* OS261 is able to ameliorate the effect of salt on growth medium. Our results were consistent with the other findings (Mayak et al., 2004; Madhaiyan et al., 2006; Zahir et al., 2009; Ahmad et al., 2012; Cheng et al., 2012), where ACC deaminase-producing bacteria enhanced the growth of red pepper plants compared to un-inoculated control. Additionally, the stimulation of different crops by PGPR has been verified in both laboratory and field trials. *P. putida* and *P. fluorescens* have been found to play a considerable role in root and shoot elongation in canola, lettuce, and tomato (Kloepper et al., 1988; Hall et al., 1996) as well as crop yields in potato, radish, rice, sugar beet, tomato, lettuce, apple, citrus, beans, some ornamental plants, and wheat (Kloepper et al., 1988; Lemanceau, 1992; Kloepper, 1994). Interestingly, a study by Li et al. (2000) and Madhaiyan et al. (2006) observed that PGPR with ACC deaminase mutants could not modulate plant growth whereas ACC deaminase transgenic canola plants showed improved growth under salinity stress (Sergeeva et al., 2006). On the basis of the earlier studies, it can be suggested that ACC deaminase producing bacteria can promote plant growth and increase dry mass under salinity stress by cleaving ACC, which consequently reduces ethylene levels.

Abiotic stress such as salinity leads to ROS formation especially superoxide, hydroxyl ion, singlet oxygen and hydrogen peroxide, which results in the oxidation of cell membranes thereby causing damage to membrane and cell structures. These processes are called as oxidative stress (Apel and Hirt, 2004; Hussain et al., 2013). In plants, a defensive system consisting of ROS scavenging enzymes namely SOD, peroxidase (POD), glutathione reductase (GR), mono-hydroascorbate reductase (MDHAR), APX, CAT and redox ions work in a concerted way to carry out ROS detoxification (Mittler, 2002; Abogadallah, 2011; Abbas et al., 2013).



The pepper plants inoculated with ACC deaminase-containing PGPR exhibited a significant elevation of the antioxidant enzyme CAT compared to the un-inoculated plants suggesting that they were adapted to saline conditions by eliminating ROS. Our results were consistent with Gururani et al. (2013), where the CAT activity was enhanced in the inoculated potato plants under salt stress and also Subramanian et al. (2015), where the enzyme activity was improved in the inoculated tomato plants under cold stress. In the bacterium inoculated plants, the SOD activity showed a significant decrease in higher salinity levels suggesting neutralization of potentially harmful molecules leading to alleviation of salt stress (Cavalcanti et al., 2004). Similarly, the APX activity showed a pattern similar to SOD, where the bacterial inoculation significantly reduced the enzyme activity under salt stress (Han and Lee, 2005; Upadhyay et al., 2012). The reason behind the decrease may be the lack of hydrogen peroxide production in the plants inoculated with bacteria, where PGPR played a major role in maintaining the normal homeostasis of plant system (Gururani et al., 2013). The antioxidant enzyme activities have been studied extensively, but still the significance of enzymes in salt tolerance is not clear, as high antioxidant activities are linked to both salt tolerance and sensitivity (Abogadallah, 2010). Many studies (Shalata and Tal, 1998; Shalata et al., 2001; Mittova et al., 2003; Sumithra et al., 2006; Hediye Sekmen et al., 2007) have related the salt tolerance to an increase in the activity of antioxidant enzymes, whereas on the contrary, few studies (Cavalcanti et al., 2004; Kim et al., 2004) have shown that a higher salt tolerance does not depend always on a higher antioxidant activity.

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## CONCLUSION

The present study illustrated that *P. frederiksbergensis* OS261 can promote salt stress tolerance in red pepper by regulating ethylene biosynthesis and increasing CAT enzyme activity. A further investigation on the genes responsible for CAT enzyme may reveal the exact mechanism by which *P. frederiksbergensis* OS261 enhances salt stress tolerance. This strain has already been well studied and proved to enhance plant cold stress tolerance. The use of this strain as a bio-inoculant can also enhance plant growth under other stress conditions. Therefore, it may be recommended to the farmer as a stress mitigator for the regions with high soil salinity after assessing its field level performance.

## AUTHOR CONTRIBUTIONS

PC, SS, and TS: conception and design of the work. PC, SS, and YK: performed the work. PC, SS, YK and KK: acquisition of data. PC, SS, GS and KK: analyzed the data. PC, SS, GS, RA and TS: critical revision of manuscript. PC, SS, RA and TS: wrote the paper.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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