

## Research Article

## Effect of some *Pseudomonas fluorescens* and *Bacillus subtilis* strains on osmolytes and antioxidants of cucumber under salinity stress

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**Abstract:** One critical environmental stress that limits plant production and development is salinity stress. Recently it has been shown that application of plant growth-promoting rhizobacteria (PGPR) can alleviate the deleterious effects of environmental stresses. Present study aimed to evaluate the effects of some bacterial strains on proline, sugar, total phenolic compounds (TPC), Phenylalanine ammonia lyase (PAL), photosynthetic pigments and antioxidant activities (guaiacol peroxidase, polyphenol oxidase and superoxide dismutase) of cucumber plants under salinity stress. A completely randomized design was applied with a factorial arrangement of two factors: salinity at three levels (0, 50 and 100 mM) and *Pseudomonas fluorescens* and *Bacillus subtilis* strains, with three replications. The results showed that cucumber plants that were inoculated with *Pseudomonas* and *Bacillus* strains possessed noticeable variations in proline, sugar, TPC, PAL and enzymes activity compared to uninoculated control. These results suggest that use of these bacterial strains overcame harmful effect of salinity by accumulation of proline, TPC, sugar, PAL activity and enzymes activity that can be considered as a suitable method to manage salinity stress.

**Keywords:** Cucumber, Enzymes activity, Phenolic compounds, PGPR, Salinity stress

### Introduction

Cucumber *Cucumis sativus* belongs to the Cucurbitaceae family and is widely cultivated plant in the world (Korkmaz *et al.*, 2007). Cucumber plant is considered moderately sensitive to salt stress and salinity is one of the deleterious environmental factors causing decreased cucumber yields (Colla *et al.*, 2012).

Water and soil salinity are the main problems in agricultural sector, which in recent years have significantly reduced the quality and quantity of agricultural products (Cramer *et al.*, 1994). Soil salinity decreases the soil water potential and consequently water and mineral absorption by plant roots (Wang *et al.*, 2018). Salinity also changes  $\text{Na}^+/\text{K}^+$  ratio and osmotic stress, inhibits many biochemical and physiological processes of plant growth and development (Yordanov *et al.*, 2003). High salinity stress can lead to an increased production of reactive oxygen species (ROS) in plants, due to the disruption of cellular homeostasis, Protein decomposition, DNA, lipids, programmed cell death (PCD) pathway

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activation and finally tissues death (de Cássia Alves *et al.*, 2018).

Under salinity stress conditions plants use several mechanisms to decrease deleterious effects of stress. To confront harmful effects of reactive oxygen species and the cascade of uncontrolled oxidation reactions by them, plants use different types of antioxidants like enzymatic and non-enzymatic antioxidants (Alves *et al.*, 2017). High antioxidant enzymes activity such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) is the primary defense response of plant under stress condition and scavenge reactive oxygen species (Farooq *et al.*, 2009; Foyer and Noctor, 2013). Also accumulation of low-molecular-weight organic solutes such as soluble sugars, proline, glycine-betaine, polyols and others compounds is another strategy that result in increased osmotic potential, preservation of cellular turgor pressure and protect cells from damaging effects of stress (Sharma *et al.*, 2012).

In recent years plant growth promoting rhizobacteria (PGPR) are used to reduce the effect of salinity stress. PGPR improve plant growth by various direct and indirect mechanisms such as facilitating uptake of mineral nutrients like iron and phosphorous, nitrogen fixation, phytohormone production and stimulating the expression of salinity-response genes (Valencia-Cantero *et al.*, 2007; Yang *et al.*, 2008). Li *et al.* (2016) reported that PGPR are an important group of microbial community that exert beneficial impacts on plant growth and development. Use of *Pseudomonas fluorescens* as PGPR also increased tolerance of barley to salinity (Kasim *et al.*, 2016). Kumar *et al.* (2017) also reported that some PGPR such as *Bacillus*, *Agrobacterium*, *Alcaligenes*, *Lysinibacillus* and *Pseudomonas* enhanced the growth of paddy seedlings under salinity stress.

The aim of the present study was to evaluate the role of some strains of *Pseudomonas fluorescens* and *Bacillus subtilis* to increase salinity tolerance in cucumber plants.

## Materials and Methods

### Salt tolerance test

Salinity stress was done at four levels including 0.5, 5, 7.5 and 10% NaCl in nutrient agar. Bacterial strains were cultured in these conditions. Petri dishes were incubated at 28 °C for 96 h and bacterial growth was measured (Maria *et al.*, 1995). Bacterial strains grown under saline condition, were evaluated as tolerant and completely tolerant. Strains that did not grow under saline condition were introduced as incapable strains.

### Plant material

Cucumber seeds *Cucumis sativus* L. after germination were sown in a 4-liter plastic pots (top diameter 20 cm and 17 cm height) containing sandy soil (pH 7.6 and EC 1.2 ds.m<sup>-1</sup>). Seedlings were grown under greenhouse conditions at temperature of 25-27 °C. Twenty days after planting, salinity treatments were applied as follow:

### Salinity stress

In order to evaluate the effects of PGPR strains on cucumber plant under salinity stress a factorial experiment was done based on completely randomized design with two factors; salinity at three levels (0, 50 and 100 mM) and *Pseudomonas fluorescens* and *Bacillus subtilis* strains, with three replications. The salinity treatment was applied along with irrigation water.

### Inoculation of bacterial inoculum

*Pseudomonas fluorescens* strains (VUPF5, CHA0, T17-4) and *Bacillus subtilis* strains (Bs96, BsVRU, BsVRU1) were retrieved from the Collection of Plant Protection Group of Vali-e-Asr University of Rafsanjan, Iran (These strains were chosen based on previous studies that showed suitable biocontrol ability and plant growth promotion (Lagzian *et al.*, 2013; Baradar *et al.*, 2015)). The bacterial suspensions were diluted with distilled water and the concentration adjusted to  $1 \times 10^{10}$  CFU/mL (OD 0.5 at 540 nm =  $10^{10}$ ) using a spectrophotometer (U-2000,

Hitachi Instruments, Tokyo, Japan). Ten ml of these suspensions were added to each pot to drench the soil.

#### Determination of Chlorophyll-A, B and Total

0.25 g of fresh plant leaf was homogenized with 10 ml of 80% acetone and kept in refrigerator for 24 h. The extract was centrifuged for 10 min at 3500 rpm. The optical density (O. D.) of centrifuged extracts was measured at 645 and 663 nm using spectrophotometer (U-2000, Hitachi Instruments, Tokyo, Japan) (Lichtenthaler, 1987).

The chlorophyll content was measured using the formulae below:

$$\text{Chlorophyll a (mg.g}^{-1}\text{fw)} = [(12.7 \times \text{OD } 663) - (2.69 \times \text{OD } 645) \times V] \div [1000 \times V]$$

$$\text{Chlorophyll b (mg.g}^{-1}\text{fw)} = [(22.9 \times \text{OD } 645) - (4.68 \times \text{OD } 663) \times V] \div [1000 \times V]$$

$$\text{Total Chlorophyll (mg.g}^{-1}\text{fw)} = [(8.02 \times \text{OD } 663) + (20.2 \times \text{OD } 645)] \div [1000 \times V]$$

#### Proline and total soluble sugar content

To determine proline concentration, 0.5 g leaf fresh tissue was homogenized with 10 mL of 95% ethanol. The homogenate extract was centrifuged at 3500 g for 10 min at 4 °C and the supernatant was recovered and kept at 4 °C. One ml of alcoholic extract was diluted with 10 ml of distilled water and 5 ml ninhydrin (ninhydrin, phosphoric acid 6 M and glacial acetic acid at 99%) and 5 ml of glacial acetic acid were added, then incubated for 45 min at 100 °C. After cooling, the samples were mixed with 10 ml benzene and absorbance was determined at 515 nm. The proline concentration was determined using a standard curve and was expressed as  $\mu\text{g.g}^{-1}\text{fw}$  of leaves (Paquin and Lechasseur, 1979).

To determine soluble sugars content, 0.1 ml of alcoholic extract was mixed with 3 ml antron (150 mg antron plus 100 ml of 72% sulphuric acid). The tubes were placed in a bath at 100 °C for 10 min. After cooling, the absorption was measured at 625 nm. The concentration of soluble sugars was determined using the standard curve and expressed as  $\text{mg.g}^{-1}\text{leaves}$  (Irigoyen *et al.*, 1992).

#### Enzyme extraction and activity determination

0.5 g fresh leaves were homogenized with 50 mM potassium phosphate buffer (pH 7.2) containing 1 mM EDTA and 1% (w/v) soluble PVP. The extracts were centrifuged at 20000 g for 20 min at 4 °C. The supernatant was used for enzyme activity.

#### Guaiacol peroxidase (GPX)

The GPX activity was determined as described by Plewa *et al.* (1999). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1% hydrogen peroxide, 4% guaiacol and enzyme extract. The increase in absorbance due to formation of tetraguaiacol was measured by the absorbance at 470 nm and using an extinction coefficient of  $25.5 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### Polyphenol oxidase (PPO)

The PPO activity was determined by the method of Nicoli *et al.* (1991) with some modifications. The assay mixture containing 50 mM potassium phosphate buffer at (pH 7.0), 0.02 M pyrogallol and enzyme extract, the enzyme activity was expressed as changes in absorbance  $\text{min}^{-1} \text{ mg}^{-1}$  of protein. The activity was expressed as change in absorbance at 420 nm.

#### Phenylalanine ammonia-lyase (PAL)

PAL activity was measured by following method described by D'cunha *et al.* (1996). The reaction mixture consisting of extraction buffer, 10 mM L-phenylalanine, deionized water and enzyme extract was incubated at 37 °C for 1 h. The reaction was terminated by the addition of 6M HCl. The absorbance at 290 nm was read and the enzyme activity was expressed as 1  $\mu\text{mol}$  cinnamic acid produced in 1 min.

#### Superoxide dismutase (SOD)

Activity of SOD was assayed according to the method of Giannopolitis and Ries (1977) by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.5), 1.5 mM carbonate sodium, 3 mM EDTA, 60 mM riboflavin, 200 mM L-

methionine, 2.25 mM NBT and 50  $\mu$ L enzyme extract. The reaction mixture was placed under fluorescent lamps (30W) for 10 min and then by switching off the light reaction was stopped.

**Total phenolic compounds**

Measurement of total phenolic compounds was carried out following the method by Folin-Ciocalteu (Roland and Laima, 1999). Five hundred grams of the fresh leaf tissue of pistachio seedlings was ground in 5 ml of 95% ethanol and was kept in dark for 48 h. Then 0.5 ml ethanol was added to the 0.5 ml supernatant and topped to 2.5 ml with distilled water. 0.25 ml of 50% folin reagent and 0.5 ml of 5% carbonate of sodium were added to samples and color clenched to black. The samples were placed in the dark for an hour and gallic acid measured at 725 nm. Gallic acid was used for constructing the standard curve. Results were expressed as mg gallic acid (GA) per milligram of the fresh weight.

**Experimental design**

In order to evaluate the effects of PGPR strains on cucumber plants under salinity stress an experiment was arranged as a factorial in the framework of completely randomized design with two factors. Salinity levels were done at

three levels (0, 50 and 100 mM NaCl) as first factor and bacteria strains were done as second factor with three replications. Analysis of variance (ANOVA) was performed using the SAS program, SAS Institute, Cary, NC, USA. Significant differences of the means ( $P < 0.05$  for F-test) were determined by LSD's Multiple Range Test.

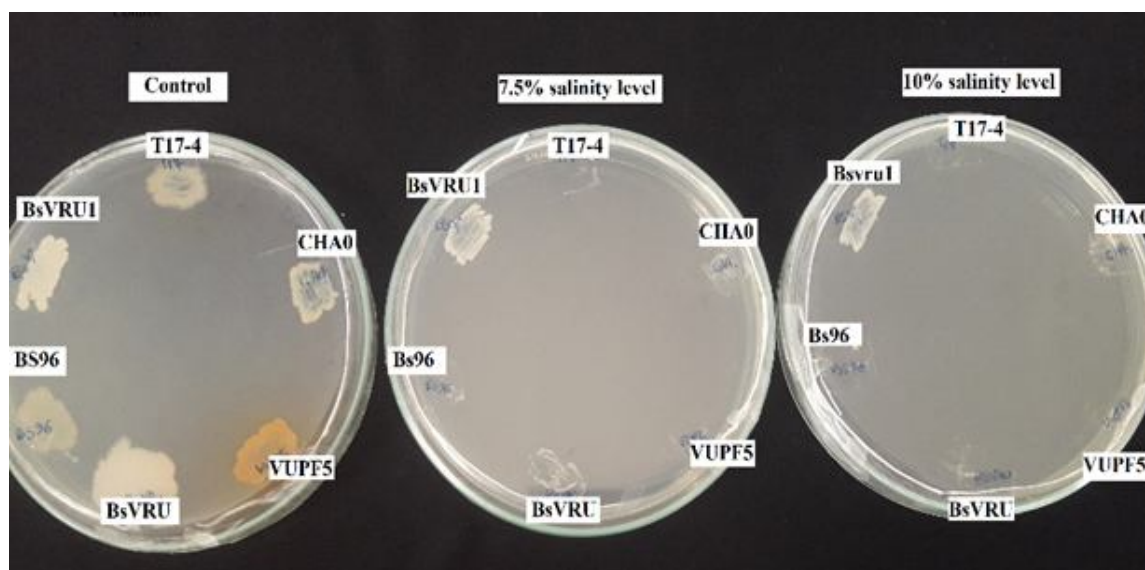
**Results**

**Salinity tolerance of bacterial strains**

The results of salt sensitivity test of bacterial strains showed that all strains could tolerate salinity levels of 0.5%. Three strains (VRU1, VRU and T17-4) could grow well at 5% NaCl and the maximum NaCl tolerance was shown by VRU1 (5-10% NaCl) (Table 1, Fig. 1).

**Table 1** Growth of *Pseudomonas fluorescens* and *Bacillus subtilis* strains in different NaCl concentrations at 28 °C and 96 h incubation.

Salinity stress	Salinity stress levels (%)	Bacterial strains
Sensitive	< 5%	CHA0-VUPF5-Bs96
Tolerant	5%	BsVRU-T17-4, BsVRU1
Completely tolerant	5-10%	BsVRU1



**Figure 1** Growth of *Pseudomonas fluorescens* and *Bacillus subtilis* strains at 7.5 and 10% NaCl concentrations.

**Proline content**

Proline content was also affected by salinity and bacterial strains (Table 2). As shown in Fig. 2, proline concentration was increased by increasing salinity intensity in control and inoculated plants. The highest and the lowest increase of proline were recorded in inoculated plant with T17-4 (70%) and Bs96 (16.72%) strains, respectively. Although proline content in inoculated plant with Bs96 strain had no significant difference with control and BsVRU inoculated plants at 100 mM treatment.

**Sugar content**

Sugar content was significantly affected by salinity, bacterial strains and their interactions (Table 2). High level of salinity increased leaf sugar content in comparison to control plants. While there was no significant difference between 50 and 100 mM NaCl treatments in inoculated plants with CHA0, BsVRU1, BsVRU, Bs96 strains and control plants. As shown in Fig. 3 the maximum sugar content

was recorded in inoculated plants with T17-4 and VUPF5 strains at 100 mM NaCl.

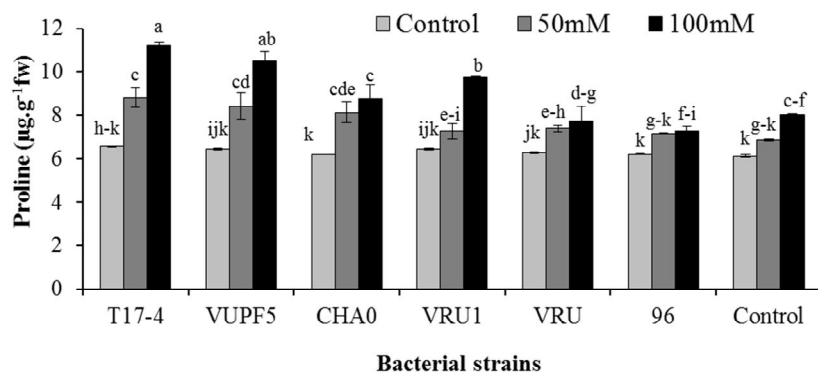
**Photosynthesis pigments**

Salinity and bacterial strains had significant effect on Chl a, Chl b and TChl content (Table 2). Leaf Chl a content decreased by about 19% and 46% in plants treated by 50 and 100 mM NaCl, respectively compared to control plant. All inoculated plants with different bacterial strains had more Chl a content in comparison to control plant (Fig. 4). Salinity stress also decreased Chl b in inoculated and control plants. The maximum reduction of Chl b was observed in plants inoculated with BsVRU1 and the minimum reduction was observed in inoculated plants with CHA0 and 96 strains (Fig. 5). Regardless of bacterial strains, leaf TChl was decreased under saline conditions (50 and 100 mM NaCl). TChl content in inoculated plants was higher than control plants. Among inoculated plants, those inoculated with VUPF5 and CHA0 strains showed the highest and lowest TChl content, respectively (Fig. 6).

**Table 2** ANOVA analysis of the *Pseudomonas fluorescens* and *Bacillus subtilis* strains, effect on proline, sugar, chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TChl) content of cucumber plants under salinity stress.

Source of variations	df	Mean squares				
		Proline	Sugar	Chla	Chlb	TChl
Bacterial strains (B)	6	5.15**	6.49**	0.010**	0.0020**	0.020**
Salt stress (D)	2	38.49**	44.19**	0.100**	0.0300**	0.260**
B × D	12	1.60**	3.38**	0.001 <sup>ns</sup>	0.0010**	0.005 <sup>ns</sup>
Error		0.30	0.79	0.003	0.0003	0.004
CV%		7.12	12.16	14.96	12.82	12.02

\*\* -significant (P < 0.01), ns- not significant.



**Figure 2** Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on leaf proline content of cucumber plants under salinity stress.

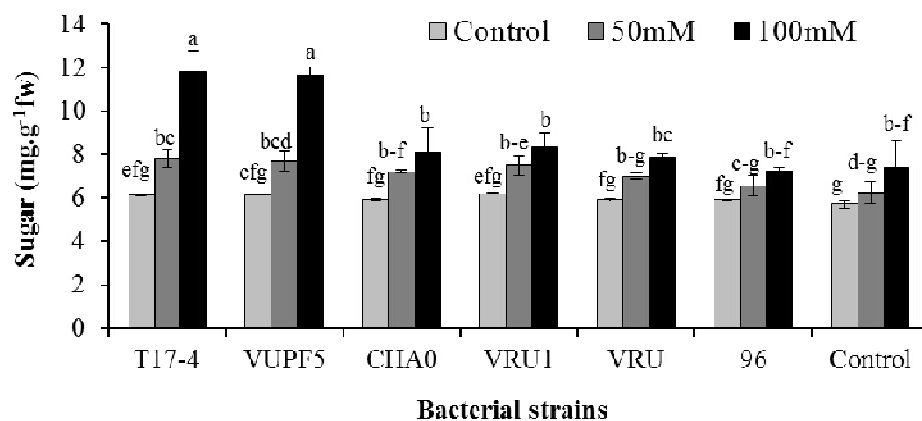


Figure 3 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on leaf sugar content of cucumber plants under salinity stress.

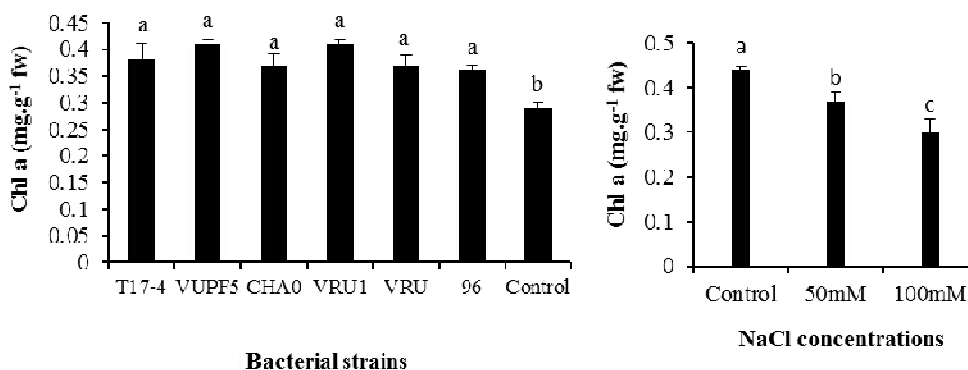


Figure 4 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on leaf chlorophyll a content of cucumber plants under salinity stress.

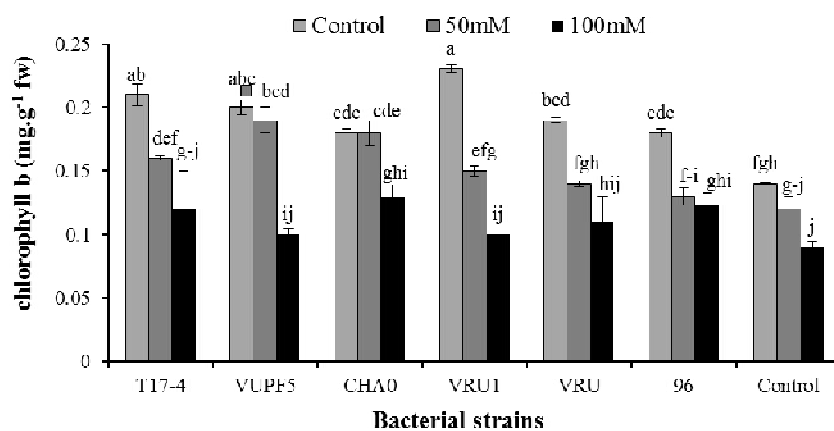


Figure 5 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on leaf chlorophyll b content of cucumber plants under salinity stress.

**Total phenolic compounds**

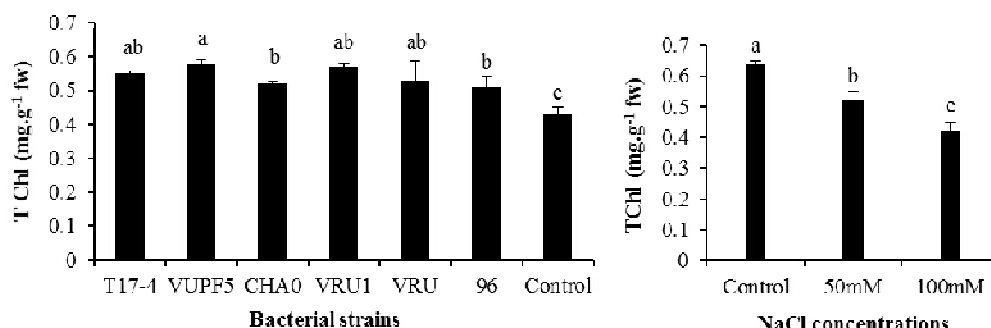
Salinity, bacterial strains and their interactions had significant effects on total phenolic compounds (Table 3). Salinity stress significantly increased total phenolic compounds in control and inoculated plants. The maximum and minimum increase occurred in plants inoculated with T17-4 and Bs96 strains by about 127% and 36% (Fig. 7).

**PAL activity**

As shown in Table 3, salinity and bacterial strains had significant effects on PAL activity but the interaction effects of salinity and bacterial strains had no significant effects on PAL activity (Table 3). Regardless of bacterial strains leaf PA activity was increased by about 29% and 41% at 50 and 100 mM NaCl conditions, respectively. PAL activity in inoculated plants was higher than control plants. Among inoculated plants, those inoculated with BsVRU1 strain showed the highest PAL activity (Fig. 8).

**Antioxidant activity**

According to ANOVA results, Salinity and bacterial strains had significant effect on PPO, GPX and SOD activity (Table 3). PPO activity content increased by about 75% and 98% in plant treated with 50 and 100 mM NaCl, respectively compared to control plant. All inoculated plants with different bacterial strains had more PPO activity in comparison to control plant. Among inoculated plants, those inoculated with BsVRU1 strain showed the highest PPO activity (Fig. 9). Salinity stress also increased GPX activity in inoculated and control plants. The maximum increase of GPX activity was observed in control plants (6.5-fold) and the minimum increase was observed in inoculated plants with VUPF5 (2.67-fold) (Fig. 10). Regardless of bacterial strains leaf SOD activity in plants treated with 50 and 100 mM increased compared to control plants. The maximum and minimum increase of SOD activity was recorded in control and BsVRU1 inoculated plants (87.15% and 25.24%), respectively (Fig. 11).



**Figure 6** Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on leaf total chlorophyll content of cucumber plants under salinity stress.

**Table 3** ANOVA analysis of the *Pseudomonas fluorescens* and *Bacillus subtilis* strains, effect on total phenolic compounds, Phenylalanine ammonia lyase (PAL), poly phenol oxidase (PPO), super oxide dismutase (SOD) and glycol peroxidase (GPX) under salinity stress.

Source of variations	df	Mean squares				F
		GPX	PPO	PAL	SOD	
Bacterial strains (B)	6	2.03**	1.70**	0.36**	247.95**	0.100**
Salt stress (D)	2	22.97**	9.98**	3.47**	4241.63**	0.410**
B × D	12	0.22**	0.24 <sup>ns</sup>	0.01 <sup>ns</sup>	62.71**	0.010**
Error		0.02	0.16	0.02	19.16	0.003
CV%		7.51	19.06	6.29	5.63	10.750

\*\* -significant (P < 0.01), ns- not significant.

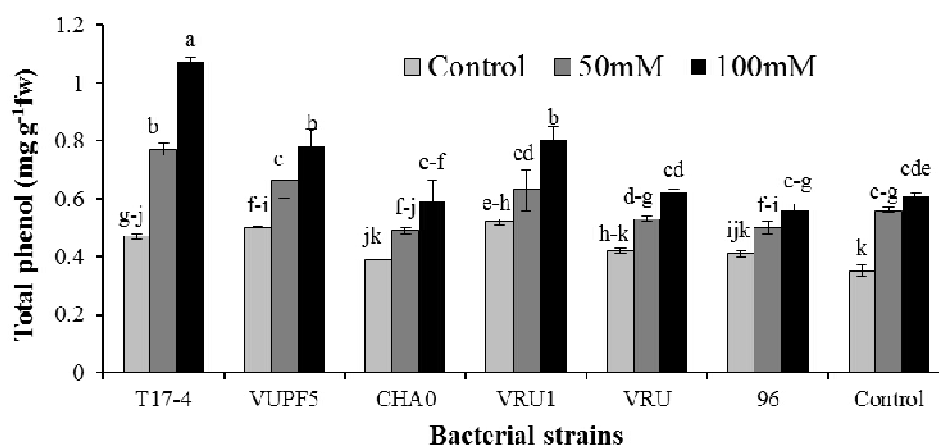


Figure 7 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on total phenolic compounds of cucumber plants under salinity stress.

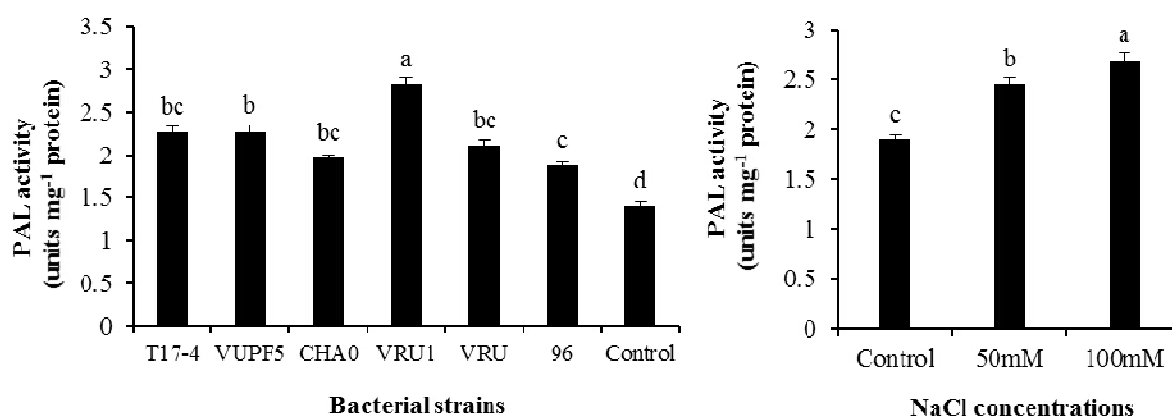


Figure 8 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on PAL activity of cucumber plants under salinity stress.

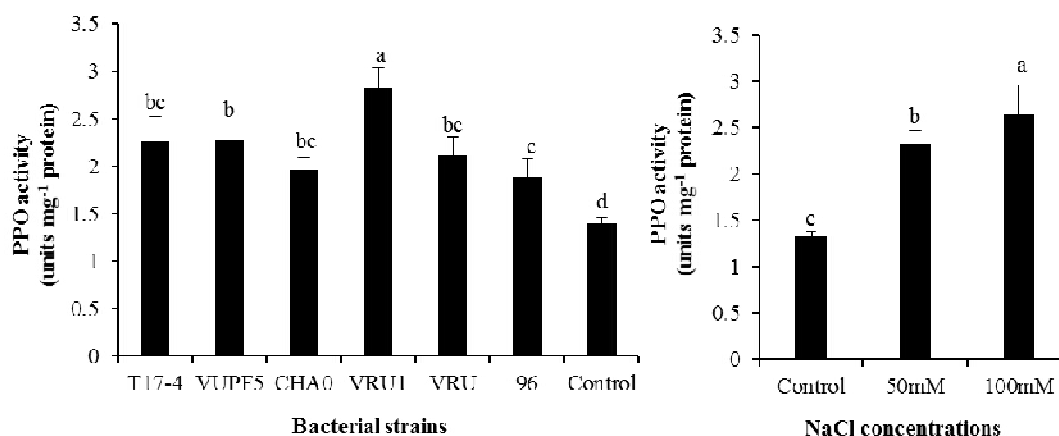


Figure 9 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on PPO activity of cucumber plants under salinity stress.



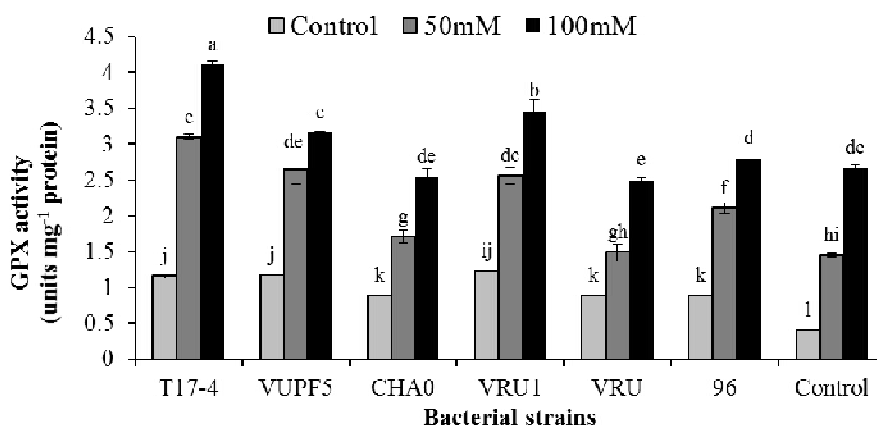


Figure 10 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on GPX activity of cucumber plants under salinity stress.

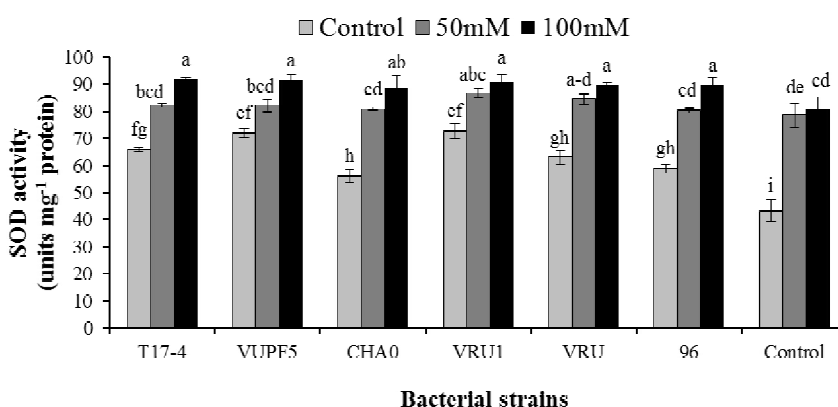


Figure 11 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* strains on SOD activity of cucumber plants under salinity stress.

### Discussion

This study evaluated the effects of PGPR bacteria on the growth, osmoregulators and antioxidant enzymes of cucumber plants under salinity stress. Rhizobacteria have been shown to promote plant growth and alleviate the deleterious effects of various abiotic stresses including salinity. The results indicated that bacterial strains had different response to salinity stress. Salinity stress by decreasing osmotic potential and resource food access competition, decreased the bacterial population (Ngumbi and Kloepper, 2016). Among the tested bacterial strains for stress tolerance, *Bacillus subtilis* strain could grow in culture

medium with up to 10% NaCl. Salt tolerant bacteria develop different defense mechanisms to overcome salinity stress such as: synthesis of some proteins (Paul *et al.*, 2006), salt-tolerant enzymes like glutaminase, amylase, lipase and thermolysin (Deutch, 2002; Weingand-Ziadé *et al.*, 2003), synthesis of betaine, ectoine and hydroxyectoine,  $\beta$ -Glutamate, trehalose and the production of spores (Oren, 2008; Kondepudi and Chandra, 2011) which neutralize detrimental effects of high osmolality.

Salinity decreases soil water potential, therefore water uptake is disturbed due to lack of negative water potential in root tissue compared to soil water potential. In this condition plant accumulate osmotic adjustments

compounds to increase water potential gradient between soil and root tissue and maintain turgor pressure (McCutchan and Shackel, 1992). On the other hand, accumulation of osmoregulators such as proline and soluble sugars under stress conditions protects cell membranes against reactive oxygen species (ROS) (Szabados and Saviouré, 2010). Osmolytes also function as osmoprotectants by preventing desiccation of membranes and stabilize dehydrated enzymes thereby playing role in osmoregulation. They facilitate stabilization of subcellular structures and free radical scavenging and protect plants from osmotic -stress -induced dehydration (Ilangumaran and Smith, 2017). Proline accumulation in plants can serve as an indicator of stress tolerance and as a hydroxyl radical scavenger (Hoque *et al.*, 2007). PGPR have the ability to enhance accumulation of proline in plants under abiotic stress (Choudhary, 2012). Our results also showed that accumulations of proline soluble sugars was increased by increasing salinity intensity, also inoculated plants with different bacterial strains stimulated accumulation of both of them in leaf of cucumber plants under salinity stress. These results are in agreement with results reported by Bano and Fatima (2009) on *Zea mays*, Armada *et al.* (2014) on *Lavandula dentate* and Gharsallah *et al.* (2016) on tomato. It has been suggested that PGPR inoculation up regulates some genes expression in plant leading to accumulation of free proline content (Kumari *et al.*, 2015). Nautiyal *et al.* (2013) reported that inoculation of salt tolerant *Bacillus amyloliquefaciens* SN13 on to rice *Oryza sativa* plants exposed to salinity, (200 mMNaCl) in hydroponic and soil conditions, increased plant salt tolerance by affecting expression of 14 genes. Inoculation of plants by PGPR has been reported to increase soluble sugars under salinity stress that is known as a defense mechanism against stress (Kumar *et al.*, 2010; Shukla *et al.*, 2012).

In present study, decrease in chlorophyll a, chlorophyll b and total chlorophyll with increasing salinity were also observed. The loss of photosynthetic pigments under salinity

stresses could be due to the destruction of chloroplast (Gharsallah *et al.*, 2016) and reduction of chlorophyll content due to chlorophyllase activity (Woodward and Bennett, 2005). Accumulation ROS under salinity stress causes chlorophyll degradation and lipid peroxidation that affects photosynthesis and membrane permeability, respectively (Ilangumaran and Smith, 2017). As expected, photosynthesis pigments were higher in inoculated plants than control plants under salinity stress. The PGPRs by improving water potential and increasing water uptake decreased the effect of salinity stress. Therefore improving water potential under salinity stresses decreased chlorophyllase activity (Ebrahimi *et al.*, 2016). Heidari and Golpayegani (2012) found that leaf Chl content of inoculated and non-inoculated basil plant significantly decreased under salinity stress but the decreasing of Chl content ratio in inoculated plant with different bacteria were lower than non-inoculated plants. Paul and Nair (2008) also reported that PGPR promoted wheat growth under drought stress by increasing proline and Chl content. Also Kumar *et al.* (2017) showed that inoculated plants with *Pseudomonas aeruginosa* (PRR1 and PHL3) and *Lysinibacillus* sp. (BPC2) effectively enhanced photosynthesis pigments under salinity stress. Rojas-Tapias *et al.* (2012) reported that PGPRs by improving ion balance under salinity stress protect membrane cell and chlorophyll content. Our results correspond with these results.

Salinity induces an accumulation of reactive oxygen species (ROS) in plants which leads to oxidative stress (Ngumbi and Kloepper, 2016). Accumulation of free radicals cause major damage to cell macromolecules such as proteins, lipids, nucleic acids and ultimately cell death (Potts, 1999). Plants have developed antioxidant mechanisms involving molecules (carotenoids, flavonoids, and other phenolics) and enzymes (superoxide dismutase, glutathione reductase, catalase, and peroxidases) that prevent tissues from oxidative damages by quenching and detoxifying ROS (Ilangumaran and Smith, 2017).

Polyphenolic compounds such as phenolic acids, flavonoids, anthocyanins and ligninase secondary metabolites play an important role in scavenging reactive oxygen species (Mechri *et al.*, 2015). Phenylalanine ammonia lyase (PAL) is the most important enzyme in the phenylpropanoid pathway which leads to biosynthesis of the polyphenol compounds (Boudet, 2007). This study also confirmed positive correlation between PAL activity and soluble phenolic content in plant tissue. Therefore high PAL activity causes more phenolic compound synthesis. Although we did not investigate the mechanism of phenolic compound stimulation by salinity stress, previous studies have demonstrated that PAL activity is stimulated by biotic and abiotic stress (Lim *et al.*, 2012). In response to stress, plants induce endogenous plant hormones, including jasmonic acid and its methylated derivative (methyl jasmonic acid), which, in turn, induce enzymes involved in the phenylpropanoid pathway, including phenylalanine ammonia lyase (PAL), thereby resulting in the accumulation of phenolic compounds. Similarly, salinity stress also induces the phenylpropanoid pathway via the accumulation of endogenous jasmonic acid (Pedranzani *et al.*, 2007) and the stimulation of PAL activation (Lim *et al.*, 2012). Becerra-Moreno *et al.* (2015) reported high activity of PAL enzymes as a reason of increasing ABA concentration. Our results are in agreement with these studies. Caliskan *et al.* (2017) reported that salt stress especially at elevated doses increased the levels of phenolic compounds in *Hypericum pruinatum* plants, whereas drought stress did not cause a significant change in this compound. Our results also showed that inoculating plants with different bacterial strains resulted in production of higher phenolic compounds compared to control plant. Therefore, plant with more phenolic compounds has more resistance to salinity conditions. These findings are in agreement with the results of Erdogan *et al.* (2016) on strawberry who reported that use of multi-trait bacteria increased leaf TPC of strawberry under stress. Production of more phenolic compounds in inoculated

maize plants under salinity stress has been previously reported by Li and Jiang (2017), who suggested that PGPRs induced PAL activity and antioxidant activity that increased plant tolerance to salinity stress. Accumulation of TPC in inoculated plant may be due to the effect of PGPR on increasing PAL activity.

Under saline stress activity of antioxidant enzymes relieve the oxidative stress. The antioxidant enzymes including superoxide dismutase (SOD), guaiacol peroxidase (GPX) and polyphenol oxidase (PPO) are critical for eliminating or reducing ROS and protect cells from oxidative damage (Kumar *et al.*, 2016). In our study, plants grown under salinity stresses showed more antioxidant enzyme activity which was increased in inoculated plants. Our results are in agreement with finding of Gururari *et al.* (2012). They reported that in potato plants inoculated with PGPR, antioxidant enzymes, such as APX, CAT, GR, and SOD were increased under various stressors (salt, drought, and heavy metals). Seon Hahm *et al.* (2017) showed that activities of APX, CAT, and GPX enzymes in PGPR-inoculated pepper plants were significantly greater than non-inoculated control plants under salt stress.

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## اثر برخی استرین‌های سودوموناس فلورسنت و باسیلوس سوبتیلیس بر میزان اسمولایت‌ها و آنتی‌اکسیدان‌ها در گیاه خیار تحت تنش شوری

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**چکیده:** یکی از تنش‌های مهم محیطی که تولید و رشد گیاه را محدود می‌کند تنش شوری است. امروزه کاربرد باکتری‌های محرک رشد گیاه می‌تواند اثرات زیان‌آور تنش‌های محیطی را کاهش دهد. هدف از این مطالعه بررسی اثرات برخی از استرین‌های باکتریایی بر میزان پرولین، قند، ترکیبات فنلی، آنزیم فنیل آمونیا لیاز، رنگیزه‌های فتوسنتزی و آنزیم‌های آنتی‌اکسیدان در گیاه خیار تحت تنش شوری است. آزمایش در قالب طرح کاملاً تصادفی با دو فاکتور: شوری در سه سطح (۰، ۵۰ و ۱۰۰ میلی‌مولار نمک) و استرین‌های باکتریایی، در سه تکرار انجام گرفت. نتایج نشان داد گیاهچه‌های خیار تلقیح شده با استرین‌های باکتریایی تغییرات قابل توجهی در میزان پرولین، قند، فنل کل، آنزیم فنیل آلانین آمونیا لیاز، رنگیزه‌های فتوسنتزی و آنزیم‌های آنتی‌اکسیدان در مقایسه با گیاهچه‌های شاهد داشتند. نتایج نشان می‌دهد استفاده از این باکتری‌ها اثرات زیان‌آور تنش شوری را از طریق تجمع پرولین، قند، ترکیبات فنلی، فعالیت آنزیم فنیل آلانین آمونیا لیاز و آنزیم‌های آنتی‌اکسیدان بهبود می‌بخشد که می‌تواند به‌عنوان یک روش مناسب برای مدیریت تنش‌های محیطی باشد.

**واژگان کلیدی:** باکتری‌های محرک رشد گیاه، خیار، فعالیت آنزیمی، ترکیبات فنلی، تنش شوری