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The Importance of Zinc-Mobilizing Rhizosphere Bacteria to the Enhancement of Physiology and Growth Parameters for Paddy under Salt-Stress Conditions

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Abstract

Rhizosphere bacteria are a group of metal-mobilizing and plant growth-promoting bacteria having the ability to solubilize minerals such as zinc. This plant growth-promoting bacterium, which lives symbiotically in/on the root surface, helps directly or indirectly in promoting plant growth. Zinc is one of the essential micronutrients required for optimum plant growth, and plays a vital role in metabolism. It is necessary in low concentrations, and is critically required for the functioning of several plant physiological processes. Zinc deficiency is the most widespread micronutrient disorder in rice (*Oryza sativa*). Out of the twenty-five isolates used in this study, two selected ones, namely, *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes*, were evaluated for the ability to solubilize zinc and for their growth-promotion efficiency on rice in the greenhouse. Zinc- mobilizing bacteria protect plants from salinity injuries by enhancing their growth-related physiology, such as chlorophyll, carotenoid, and antioxidant enzymes catalase (CAT), peroxidase (PO). Plants inoculated with Zn-mobilizing bacteria (ZMB) also accumulate soluble carbohydrates in leaves under salinity, which helps plants overcome osmotic stress.

KeyWords- Zinc mobilizing bacteria, Paddy, Salinity, Chlorophyll, Carotenoid, Catalase (CAT), Peroxidase (PO).

1. Introduction

Salt stress is an increasingly global problem which affects major parts of the global agricultural lands. Some states of India, such as the Gujarat state, have a total coastal length of about 1600 Km, as many districts of the Gujarat state including Valsad, Navsari, Surat and Bharuch have their western boundaries on the Arabian Sea (Garg and Patel, 2007). A high amount of salinity may affect the plant in several ways such as water stress, reduction and expansion of cell division, oxidative stress, and nutritional disorders (Zhu, 2007). The long-term exposure of plants to salinity makes plants experience ionic stress, which may lead to a premature deterioration of adult leaves, affecting the photosynthetic area available to support the continued growth. In fact, excess sodium and more importantly chloride have the potential to affect plant enzymes and cause cell swelling, resulting in reduced energy production and other physiological changes (Larcher, 1980). Hence, arid and semiarid areas of Indian agro ecosystems are often deficient in important minerals including phosphorus, potassium, and zinc. For a proper growth and development, plants need several macro- and micronutrients. The macronutrients including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and the micronutrients including iron (Fe), boron (B), chlorine (Cl), manganese (Mn), zinc (Zn), and copper (Cu) are supplemented through inorganic or organic forms when taken up by the plant roots along with water.

Zinc (Zn) is one of the eight essential micronutrients required for the healthy growth and reproduction of crop plants. For an optimum plant growth, Zinc is required relatively in small concentrations (5–100mg kg^{-1}), and plays an important role in metabolism. Similar to nitrogen, phosphorus and potassium, zinc deficiency has been found widespread and responsible for yield reduction in rice (Fageria *et al.* 2002). In plants, zinc also acts as a regulator being a constituent of more than sixty-five different enzyme systems of drought tolerance and water-use efficiency (Assunção *et al*. 2010). Cereal species greatly differ in their zinc efficiency which is defined as the ability of a plant to grow and yield well under Zn deficiency. All crops require Zn, especially high carbohydrate plants such as rice, potatoes, etc. Although Zn is not an essential component of cell structure, it regulates many biochemical processes essential for growth, development and seed production. Zn solubility is highly dependent upon soil pH and moisture. Bacteria play an important role in mobilizing nutrients' requisites by the plants to some extent. Microbes are a potential alternate which can provide plant zinc requirements by solubilizing the complex zinc in the soil. Microbes solubilize the metal forms by protons, chelated ligands, and oxido-reductive systems that are present on the cell surface and membranes (Hughes and Poole, 1991). Rhizobacteria genera belonging to *Pseudomonas* spp. and *Bacillus* spp. are reported to

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solubilize zinc. Thus, the identification of efficient microbial strains capable of solubilizing minerals rapidly can conserve the existing resources, and avoid environmental pollution hazards caused by the excessive usage of chemical fertilizers.

Salt stress adversely affects plant nutrient acquisition, especially in the root, resulting in a significant decrease in shoots dry biomass (Jha and Subramanian, 2016). The collaboration of plant growth-promoting bacteria, especially Zn-mobilizing bacteria (ZMB), and their effect on the biological growth response of plants under soil salinity are complex. The effect of the inoculation of ZMB in plants, alone or in groups in conferring tolerance to plants against adverse environmental conditions has been analysed. Its effect in improving other nutrient availability to help the plant overcome osmotic stress by the accumulation of soluble sugar has also been analysed. Such change has been correlated with variation in the antioxidant enzymes such as catalase and peroxidase activity, photosynthesis rate, leaf greenness, and other growth-promotion parameters including plant height, dry weight, etc.

2. Materials and Methods

2.1. Isolation, Identification and Zinc Solubilization Assay

Rhizobacteria were isolated and identified by 16S rDNA analysis from the rice field as per the researchers' published method (Jha, 2017). The growth-promotion efficiency of the isolates was analyzed in terms of their ability to solubilize zinc by inoculating it on to the modified Pikovskaya medium (Pikovskaya,1948), containing 1 % insoluble zinc compounds (a) ZnO, (b) $ZnCO_3$, and (c) Zn $(PO_3)^4$. A loopful of a forty-eight-hour bacterial culture was inoculated on the prepared plates. All the plates were incubated for forty-eight hours at 28°C for five days. The halo zone around the colony and the colony growth were measured, and the Zn solubilization efficiency was tested (Gontia-Mishra *et al*., 2017).

Solubilization efficiency = Solubilization diameter/ Diameter of colony growth X 100.

They were subjected to further experimental studies such as quantitative estimation (broth assay), the influence of the isolates on the pH of the medium, and the production of gluconic acid and auxin.

2.2. Quantitative Estimation of Zinc Mobilization

A loopful of a forty-eight- hour bacterial culture was inoculated into 25 mL modified Pikovskaya broth in a 50 mL capacity flask and was incubated at 28±2°C for ten days. The growth suspension was centrifuged at 7,000 g for ten minutes to separate the supernatant from the cell growth and the insoluble Zn. One mL of the supernatant was taken in a 50 mL volumetric flask, and the volume was made to 50 mL with distilled water and was mixed thoroughly. The solution was fed to an atomic absorption spectrometer to determine the Zn content. A standard curve was prepared using various concentrations of a 10 ppm ZnCl solution. The amount of zinc solubilized by the bacterial isolates was calculated from the standard curve.

2.3. Influence of Zinc Solubilizing Organisms on pH of the Growth Medium

The selected strains were inoculated in the flasks containing 50 mL of sterilized modified Pikovskaya medium containing 0.1% of ZnO, $ZnCO_3$ and $Zn(PO_3)^4$ as insoluble sources. An un-inoculated sample was also maintained. The samples were analyzed on the sixth, eighth, and tenth day after incubation. The bacterial cultures were centrifuged at 15,000 rpm for ten minutes and were filtered using Whatman No. 42 filter paper. The pH of the ZMB culture filtrates was measured using a pH meter (Elico).

2.4. Quantitative Estimation of IAA by Zinc Solubilization

The selected bacterial isolates were tested for their ability to produce IAA by inoculation in the flasks containing 50 mL of sterilized modified Pikovskaya medium supplemented with 0.1 % ZnO. Another set devoid of Zn material was also inoculated. All the treatments were amended with 0.1 % tryptophan and were incubated for seven days. The quantity of IAA produced by the organisms was estimated by the method of Brick *et al*. (1991).

2.5. Rice Cultivation and Inoculation

Seeds of rice, variety GJ17, were germinated, and the seedling was inoculated with isolates as per the published method of the researchers (Jha and Subramanian, 2014a). Seven-day-old ZMB inoculated rice plants were carefully removed from different test tubes inoculated with the strain of bacterium, and were planted in a pot. Similarly, the control plants (un-inoculated) were also transferred to a fresh pot. Soil samples were collected from wet rice fields possessing the following physio-chemical properties, pH: 7.79, electrical conductivity 1063 µS/cm, CEC:3 cmol, organic carbon: 5500 mg per kg, available nitrogen 200 mg per square decimeter, available Ca: 12.1cmol, available P 205 : 9.5 mg per square decimeter, available K 20 : 265 mg per kg, Fe: 3.1 mg per kg , Zn: 285 mg per kg, Mn: 3.7 mg per kg, and Cu : 2.2 mg per kg. All the seedlings were grown for four weeks without any fertilizer treatment. The experiment was conducted in a greenhouse at 20 to 25 °C with a relative humidity of 70 to 80 % according to the published method of (Jha and Subramanian, 2014b)

2.6. Effect of Isolates on Chlorophyll and Carotenoid Content under Salinity

Pigments were extracted from the leaves of the seedlings treated with 13.0 dS m^{-1} salt for fourteen days. The extraction of the leaf pigments was performed with 80 % acetone, and the absorbance at 663 and 645 nm was measured with a Hitachi U-2000 dual length spectrophotometer. The chlorophyll a, chlorophyll b, and total chlorophyll quantities were calculated according to the method of Arnon (1949). The total carotenoid content was measured at 470 nm. The pigment concentrations were expressed as µg g^{-1} fresh weight (FW).

2.7. Enzyme Extraction and Enzyme Assay

Leaves (2 g) were homogenized with a mortar and pestle in 4 ml of ice-cold 50 mM Tris-acetate buffer pH 6.0, containing 0.1mM of ethylene diamine tetra-acetic acid (EDTA), 5 mM of cysteine, 2 % (w/v)

polyvinylpyrrolidone (PVP), 0.1mM of phenyl methyl sulphonyl fluoride (PMSF) and 0.2 % (v/v) Triton X 100. The homogenate was centrifuged at 12,000 g for twenty minutes, and the supernatant was filtered through Sephadex G-25 column equilibrated with the same buffer used for homogenization. The column elution was used as the enzyme source for the determination of enzyme activity. All operations were performed at 4°C. Protein concentration was determined by taking OD at 595 nm according to Bradford, (1976) using bovine serum albumin as a standard.

2.8. Measurement of Soluble Sugar Contents

Soluble sugars were determined based on the method of phenolsulfuric acid (Dubois *et al*. 1956). A half gram (0.5g) fresh weight of the roots and shoots was homogenized with deionized water. The extract was filtered and treated with 5 % phenol and 98 % sulfuric acid. The mixture remained for one hour, and the absorbance was determined at 485 nm by a spectrophotometer (Biochrom S 2100). Contents of soluble sugar were expressed as mg g−1 FW.

2.9. Estimation of Catalase (CAT) Activity

CAT activity was assayed by measuring the initial rate of disappearance of H_2O_2 (Bergmeyer, 1970). The reaction mixture consisted of 3 % H_2O_2 and 0.1 m mol L^{-1} EDTA in a 0.05 mol L^{-1} Na-phosphate buffer (pH 7) and 0.1mL enzyme from the plant source. The decrease in H_2O_2 is followed as the decline in optical density at 240 nm, and the activity is calculated as mmol H_2O_2 consumed per minute. All tests were carried out in triplicate.

2.10. Estimation of Peroxidase (PO)

A leaf sample (1g) was homogenized in 1 mL of 0.1 M phosphate buffer, pH 7.0 at 4 ^{0}C. The homogenate was centrifuged at 12000 g at 4 ^{0}C for fifteen minutes, and the supernatant was used as the enzyme source. The reaction mixture consisted of 1.5 mL of 0.05 M pyrogallol, 0.5 mL of the enzyme extract and 0.5 ml of 1 % H_2O_2. The reaction mixture was incubated at room temperature. The change in O.D was recorded at 420 nm at thirty-second intervals for three minutes. The enzyme activity was expressed as changes in the absorbance $min^{-1}g^{-1}$protein (Hammerschmidt *et al*; 1982). All tests were carried out in triplicate.

2.11. Statistical Analysis

Each pot was considered as replicate, and all of the treatments were repeated three times. A two-way analysis of variance (ANOVA) was performed using STATISTICA program. The means and the calculated standard errors are reported. Significance was tested at a 5 % level.

3. Results

Out of twenty-five isolates, the *P. pseudoalcaligenes* and *B. pumilus* strong Zn solubilizers were identified and inoculated in the modified Pikovskaya medium containing different insoluble sources (ZnO, $ZnCO_3$ and $Zn(PO_3)^4$) of Zn at 0.1 % and area of zone of inhibition was maximum 23.6 mm^2 by *P. pseudoalcaligenes.* The solubilization efficiency of the isolates was calculated by measuring the diameter of the colony growth and the solubilization zone. The zinc- solubilizing potential varied from one isolate to another, and the solubilization efficiency ranged between 312 % and 341% in ZnO, indicating its dependence on the zinc sources used (Table 1).

In the quantitative assay, the bacterial isolates were tested after being grown in a modified Pikovskaya liquid medium supplemented with 0.1 % of ZnO, $ZnCO_3$ and Zn $(PO_3)^4$. The bacterial cultures were withdrawn after the sixth, eighth, and tenth day of incubation at 30°C for the estimation of the soluble Zn in the broth using atomic absorption spectrophotometric. The amount of Zn solubilized by both isolates varied, and *P. pseudoalcaligenes* recorded maximum solubilization of Zn in all of the three insoluble sources. The maximum solubilization of Zn was in $Zn(PO_3)^4$ (33.26 mgl^{-1}) at the tenth day of incubation (Table1).

Zinc forms an important metalloprotein which is responsible for the synthesis of tryptophan, which in turn acts as a precursor for the production of IAA. The results show that both isolates produce IAA in the medium supplemented with tryptophan, and that there is further enhancement in the IAA production by the isolates due to the addition of Zn sources. This may be attributed to the induction of high Zn solubilizing efficiency of the isolates, which results in the stimulation of IAA synthesis. Moreover, *P. pseudoalcaligenes* was found to produce more IAA (33.20 mgl^{-1}) in the presence of ZnO (Table 2). Both bacteria were able to solubilize zinc with the production of organic acid resulting in a change in the pH of the medium; with time it became more acidic by both isolates and in all of the three insoluble sources (Table 2).

The plants inoculated with ZMB reduced the effect of salinity, on growth suppression of paddy under salinity. The plant inoculated with ZMB showed a 52 % greater plant height under normal conditions, and 32 % higher under salinity conditions. Similarly, the dry weight increased by 26 % under normal conditions, and by 30 % under salinity (Table 3). Plants inoculated with *P. pseudoalcaligenes* and *B. pumilus* also showed higher chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content at normal conditions as well as under salinity (Table 3).

It has been shown that soluble sugars increase under osmotic stress for osmotic adjustment in paddy under salinity. So the accumulation of soluble sugars significantly increased in the ZMB inoculated plant at normal conditions, but there was no significant change observed in the *P. pseudoalcaligenes* and *B. pumilus* inoculated paddy under salinity as shown in Figure 1.

CAT scavenges H_2O_2 by breaking it down directly to form water and oxygen, and an increase in its activity is related to the increase in stress tolerance. In paddy plants grown in soils devoid of NaCl, catalase activity increased with the inoculation of ZMB. The CAT in non-stressed control plants varied from 40 (plant non treated) to 62 (Plants treated with ZMB) mmol $min^{-1}g^{-1}$FW, while under salinity stress, it ranged between 63 and 69 mmol $min^{-1}g^{-1}$ FW as shown in Figure 2.

The peroxidase (PO) activity was significantly high in the control paddy plant due to salinity. There is no significant change in the PO activity with the inoculation of ZMB, but the saline stress increased the PO activity in the control plants and plants treated with *P. pseudoalcaligenes* as shown in Figure 3.

Table 1. *In vitro* zinc-solubilizing potential of the bacterial isolates.

Zinc-mobilizing bacteria	Zinc source	Area of halo zone (mm^2)	Solubilization efficiency	Zn Solubilization on Day 6	Zn Solubilization on Day 8	Zn Solubilization on Day 10
P. pseudoalcaligenes	ZnO	23.6	341%	17.02 ± 0.12	13.09±0.16	11.22±0.24
	$ZnCO_3$	19.7	276%	11.29 ± 0.14	10.01±0.11	8.62 ±0.12
	$Zn(PO_3)^4$	18.2	121%	23.02 ±1.13	27.23±2.01	33.26±0.28
B. pumilus	ZnO	19.5	312%	19.42 ± 0.23	15.06±0.11	10.34±0.41
	$ZnCO_3$	19.1	266%	12.36 ±0.28	11.02±0.34	8.35 ±0.47
	$Zn(PO_3)^4$	17.2	147%	11.02 ±0.11	09.25±1.06	7.22±0.21

Values are mean ± SD of three samples in each group, and are significantly different at $P < 0.05$

Table 2. Influence of zinc-solubilizing organisms on the pH of the growth medium and the IAA production.

Zinc-mobilizing bacteria	Zinc source	pH of medium on Day 6	pH of medium on Day 8	pH of medium on Day 10	IAA production (mgl^{-1})
Control	Nil				12.11± 0.51
P. pseudoalcaligenes	ZnO	5.13 ± 0.21	4.34 ± 0.24	3.23 ± 0.31	33.20 ± 1.58
	$ZnCO_3$	4.45 ± 0.17	4.01 ± 0.21	3.11 ± 0.25	25.15 ± 1.72
	$Zn(PO_3)^4$	3.24 ± 0.41	3.01 ± 0.31	2.77 ± 0.26	19.87 ± 0.51
B. pumilus	ZnO	5.71 ± 0.20	4.55 ± 0.10	3.33 ± 0.50	28.74 ± 2.41
	$ZnCO_3$	4.61± 0.52	6.24± 0.25	4.21± 0.23	22.62 ± 5.57
	$Zn(PO_3)^4$	4.10 ± 0.30	3.93 ± 0.31	3.37 ± 0.32	15.80 ± 0.40

Values are mean ± SD of three samples in each group, and are significantly different at $P < 0.05$

Table 3. Effect of ZMB on chlorophyll content and other growth parameters.

Zinc-mobilizing bacteria	Plant Height (m)	Dry weight (kg)	chlorophyll a, (mg g^{-1} FW)	chlorophyll b, (mg g^{-1} FW)	total chlorophyll (mg g^{-1} FW)	Total carotenoid (mg g^{-1} FW)
Control						
Non-inoculated Control	0.167^{c}	0.39^{bc}	1.271^{c}	0.542^{c}	0.386^{c}	0.418^{bc}
P. pseudoalcaligenes	0.192^{b}	0.46^{ab}	1.321^{a}	0.633^{a}	0.452^{a}	0.535^{a}
B. pumilus	0.254^{a}	0.49^{a}	1.082^{b}	0.612^{ab}	0.412^{b}	0.517^{ab}
Stressed						
Non-inoculated Control	0.141^{c}	0.34^{bc}	0.561^{c}	0.348^{bc}	0.296^{c}	0.272^{abc}
P. pseudoalcaligenes	0.161^{b}	0.42^{ab}	0.691^{a}	0.411^{a}	0.382^{a}	0.354^{a}
B. pumilus	0.187^{a}	0.43^{a}	0.678^{b}	0.376^{b}	0.341^{ab}	0.316^{ab}

Values are the means of replicates. Values with different letters are significantly different at $P < 0.05$ (Duncan's Test).

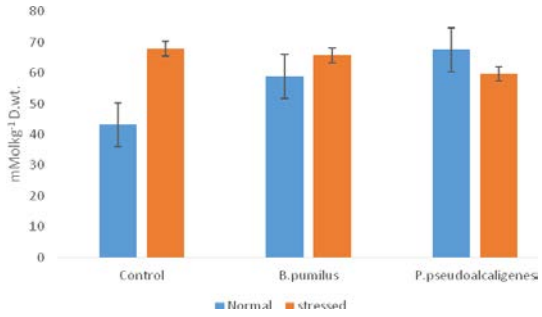

Figure 1. Effect of *B.pumilus* and *P. pseudoalcaligenes* on the accumulation of soluble sugars in paddy under salinity stress. Values are means from five replications. Vertical bars indicate±S.D**.**

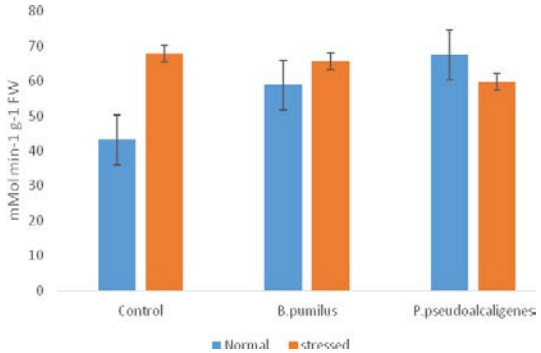

Figure 2. Effect of *B.pumilus* and *P. pseudoalcaligenes* on catalase activity in paddy under salinity stress. Values are means from five replications. Vertical bars indicate±S.D

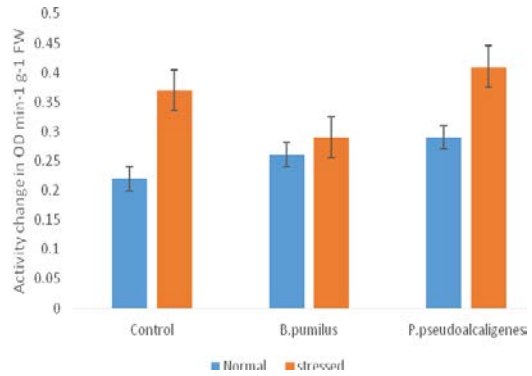

Figure 3. Effect of *B. pumilus* and *P. pseudoalcaligenes* on peroxidase activity in paddy under salinity stress. Values are means from five replications. Vertical bars indicate ±S.D.

4. Discussion

India is considered as one of the main regions for rice cultivation, covering forty-four million hectares of land. In India, rice accounts for 40 % of the nation's food production, and is a stable food for around 65 % of the total population. Crop production in the arid and semi-arid environments is highly unstable and unsustainable due to uncongenial climates (Sharma *et al*. 2011). Low soil fertility is one of the most important factors which not only seriously affect the rice production, but also reduce the quality of the rice. Chaudhary *et al*. (2007) reported Zn deficiency as a key factor in determining the rice production in several parts of India. Zinc plays an important role in maintaining the structure and function of a large number of macromolecules, and is also found responsible for controlling over 300 enzymatic reactions (Tapiero and Tew, 2003). Graham (2008) stated that zinc deficiency is the highest priority among micronutrients for agriculture experts to address. The use of chemical fertilizers to enhance soil fertility and crop productivity is an option, but the continuous application of chemical fertilizers as well as their low use efficiency cause leaching and runoff of nutrients, leading to environmental degradation (Gyaneshwar *et al*. 2002). One of the possible methods to increase crop productivity and food quality without creating environmental hazards is the use of zinc-mobilizing bacteria (ZMB). Several efforts have been made to identify the zinc solubilizers with their varying abilities. Bacterial genera such as *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported to be the most significant mineral solubilizing bacteria (Bhattacharyya and Jha, 2012).

In the present study, two bacterial isolates, *P. pseudoalcaligenes* and *B. pumilus* were found to be efficient with reference to their zinc mineralization capability. Zaidi *et al*. (2009) reported that the mineral solubilization by the bacteria may be attributed to the secretion of organic acids. So in the present study, change in the pH of the medium has been studied, and the results showed that the medium had become acidic with time. The production of organic acids for the solubilization of minerals, such as zinc, and potassium, is a well-known mechanism. In the present study, the reason for the reduction in pH may also be attributed to the production of organic acids by the isolates. Auxin may function as an important signal molecule in the regulation of plants' development. This hormone influences many cellular functions, the orientation of root and shoot growth in response to light and gravity, the differentiation of vascular tissue, apical dominance, the initiation of lateral and adventitious roots, and the stimulation of cell division and elongation of stems and roots. In this study, both of the bacterial isolates were positive for auxin production, and *P. pseudoalcaligenes* was found to be better than *B. pumilus* in presence of Zn in the medium compared to the control. Patten and Glick, (2002) also reported that auxin producing *P. putida* increased the length of canola seedling roots.

Salinity affects the crop productivity and yield as salt stress affects plant growth negatively (Parida and Das, 2005). In the present study, the physiological and biochemical changes in a paddy cultivar were studied in the presence of ZMB. However, when the plants were inoculated with ZMB, the extent of growth suppression was ameliorated and the treated plants showed growth in terms of dry weight compared to non-inoculated control plants as also was reported by Vaid *et al.* (2014) and Jha and Subramanian, (2018a). The zinc application increased the plant height significantly. This may be attributed to the adequate supply of zinc which contributes to acceleration of the enzymatic activity and auxin metabolism in plants. These results are also in agreement with the findings of Khan *et al* (2009).

Different stress situations directly result in the accumulation of ROS and are associated with soluble sugar accumulation, which has generally been considered to be an adaptive response to the stress condition. Carbohydrates such as soluble sugars (glucose, fructose, sucrose, fructans) accumulate under salt stress to accommodate the ionic balance in the plant (Ivan Couee *et al*. 2006). Their major functions include osmoprotection, osmotic adjustment, carbon storage, radical scavenging and the stabilization of the structure of proteins (Jha and Subramanian, 2018b). In the present study, the contribution of total accumulation of soluble sugars to osmotic adjustment was significant, since the total soluble sugars content increased with the increase of salinity in both the ZMB inoculated and non-inoculated plants. Similar results were obtained by Rejsiková *et al.* (2007), who reported that the concentrations of sugars change in response to salt stress in plants. Soluble sugars accumulation may be attributed to the further transformation of starch to sugars, or to the less consumption of carbohydrates by the tissues under saline conditions.

In order to allow the adjustment of the cellular redox state and to reduce the toxic effects of salinity, plant antioxidant system, peroxidase (POX), and catalase (CAT), are common and important indices for evaluating the redox status of plants. Increasing salinity stress affects the CAT and POX activity in the ZMB inoculated and non-inoculated plants. Hafeez *et al.* (2013) reported that under salinity non-inoculated plants had an increased antioxidant activity compared to the ZMB inoculated plants. These antioxidant enzymes are involved in eliminating H_2O_2 from salt–stressed plants. In the present

study, CAT and POX activities were higher in the plants inoculated with the isolates under normal conditions, but under salinity, CAT activity decreased in the plants inoculated with *P. pseudoalcaligenes*. The POX activity decreased in the plants inoculated with *B. pumilus*. This may be attributed to the free radical scavenging activities of these isolates, due to the decreased H_2O_2 levels (Jha and Subramanian, 2015). The salt stress results in the induction of CAT and POX activities in the plants, but the activities of these enzymes were significantly higher in the presence of ZMB under normal conditions. The further induction of CAT and POX activities are attributed to the ZMB inoculation, pointing to its signaling role in the generation of H_2O_2 and the detoxifying activity of enzymes in rice leaves, similar to other abiotic stresses as reported by Sairam *et al.* (2005). The present study shows that *P. pseudoalcaligenes* and *B. pumilus* have zinc solubilizing abilities, and are able to induce stress-related proteins and enzymes and protect the paddy plant under salinity. The results suggested that the inoculation of salt-stressed plants with ZMB strains reduced the negative effects of salinity stress, improved tolerance, and enhanced plant growth.

5. Conclusions

Crop productivity is decreasing due to climatic changes. Moreover, human populations are increasing daily, which results in starvation problems in the developing countries. Nowadays, research is more focused on enhancing crop yields in spite of various unfavorable environmental conditions. Plants inoculated with ZMB have an enhanced growth and acquired a better capacity for salt tolerance, correlated with the regulation of ion concentrations. To grow food for all, the use of such biofertilizers, especially ZMB, may be a beneficial means for the enhancement of plant growth and yield for the growing populations.

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