RESEARCH PAPER

Plant Growth Promoting Rhizobacteria as Natural Biostimulants: Antioxidant Enzyme Profile of Onion (*Allium cepa L*) Seedlings

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ABSTRACT

The onion (Allium cepa) evaluates the impact of isolates of Plant Growth-Promoting Rhizobacteria (PGPR), namely *Pseudomonas fluorescens* (PGPR 3) and *Bacillus cereus* (PGPR 7), on growth parameters and antioxidant enzyme activities in onions (Allium cepa). PGPR inoculation significantly raised the activity of important antioxidant enzymes in roots and shoots, including ascorbate peroxidase, catalase, superoxide dismutase and peroxidase, which improved resistance to oxidative stress, because of its capacity to produce phytohormones and solubilize nutrients. *Bacillus cereus* showed significant increases in root and shoot development and biomass. *Pseudomonas fluorescens* demonstrated effective biocontrol ability by secreting secondary metabolites and producing siderophores, which aided in the intake of nutrients and the suppression of illness. Additionally, the two strains increased germination, plant height, yield and chlorophyll content while also causing plants to develop systemic resistance. These results show that these PGPR strains have the potential to improve soil health and onion crop yield as sustainable biofertilizers, requiring for additional field research before commercial use.

Keywords: Onion, Peroxidase, Superoxide dismutase, PGPR, ADR, Catalase

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INTRODUCTION

In the *Alliaceae* family, the onion (Allium cepa) is a popular vegetable plant. In the genus *Allium*, the most widely cultivated species is the onion, sometimes referred to as the bulb onion or common onion (1). The onion is generally treated as an annual and harvested in its first growing season, despite being a biannual or perennial plant.The term "PGPR" is used to refer to all bacteria living in the rhizosphere that increase plant growth through direct and indirect mechanisms. A broad range of species belonging to the genera *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* were stated as PGPR. The PGPR effects depend on ecological and soil factors such as plant species, plant age,development phase and soil type (2).

By strengthening the plant's antioxidant defence system, among other processes, Plant Growth-Promoting Rhizobacteria (PGPR) significantly contribute to increased plant growth and tolerance to a range of environmental challenges (3). The activity of important antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) has been shown to be enhanced by PGPR inoculation. In order to prevent oxidative damage and preserve cellular redox homeostasisboth of which are critical for the best possible plant development and survival under stressthese enzymes work together to scavenge reactive oxygen species (ROS). SOD, CAT, and APX are the first line of defence against ROS among the enzymatic antioxidants (4). The extremely reactive superoxide radicals (O_2^-) are converted into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) by superoxide dismutase (SOD). Despite being less harmful than superoxide, H_2O_2 can nonetheless endanger the integrity of cells if it is not effectively eliminated (5). Ascorbate peroxidase (APX) and catalase (CAT)both are also contribute. Catalase breaks down H_2O_2 into oxygen and water, primarily in peroxisomes, where a lot of H_2O_2 is produced. On the other hand, Ascorbate peroxidase (APX), utilizes ascorbate as an electron donor to reduce H_2O_2 to water, operating primarily in chloroplasts, cytosol, and mitochondria. The coordinated action of these enzymes ensures that ROS levels are tightly regulated and kept within non-toxic limits (6).

Table 1. Effect of PGPR cultures in antioxidant enzyme activity (POD and CAT) in roots and shoots in *Allium cepa*

	Treatments	Peroxidase (roots) (ΔOD/min./g FW)	Peroxidase (shoots) (ΔOD/min./g FW)	Catalase (roots) (µmole/min/ mg FW)	Catalase (shoots) (µmole/min/ mg FW)
PRO-6	Control	0.008	0.007	0.26	0.16
	T1	0.028	0.03	0.41	0.18
	T2	0.016	0.013	0.76	0.21
	T3	0.06	0.063	1.46	0.34
	T4	0.012	0.02	0.5	0.2
	T5	0.021	0.018	0.45	0.03
	T6	0.012	0.011	0.16	0.23
	T7	0.064	0.033	1.27	0.51
	T8	0.01	0.012	1.004	0.13
	T9	0.016	0.015	0.62	0.26
	T10	0.012	0.008	0.51	0.11
CD5%		0.17	0.61	0.35	0.23
ADR	Control	0.007	0.009	0.13	0.17
	T1	0.018	0.15	0.4	0.1
	T2	0.011	0.013	0.34	0.22
	T3	0.023	0.11	1.77	0.29
	T4	0.011	0.062	0.2	0.2
	T5	0.02	0.055	0.26	0.29
	T6	0.012	0.009	0.27	0.13
	T7	0.028	0.14	0.62	0.34
	T8	0.016	0.057	0.25	0.14
	T9	0.012	0.087	0.33	0.25
	T10	0.012	0.056	0.44	0.14
CD5%		0.26	0.46	0.18	0.57

METHODOLOGY

Raising of crop and procurement of samples

Seeds were treated with different PGPR cultures procured from the microbiology laboratory of the Department of Plant Breeding and Genetics, PAU, Ludhiana. The crop of onion was raised at the Vegetable Research Farm, Department of Vegetable Science, PAU, Ludhiana.

Collection of samples

In the first trial, onion seeds were sown in Petri dishes. The samples were drawn out at the interval of 7 days and 14 days.

Estimation of Peroxidase (EC 1.11.1.7)

The reaction was initiated by adding 3 ml of 50 mM guaiacol freshly prepared in 0.1 M potassium phosphate buffer of pH 6.5, 0.1 ml of enzyme extract and 0.1 ml of 800 mM H_2O_2. The reaction mixture in the absence of H_2O_2 was taken as a blank. The increase in OD value was measured at an interval of 30 up to 3 min. Specific activity of this enzyme was represented as change in absorbance $min^{-1}mg^{-1}$ of protein (7).

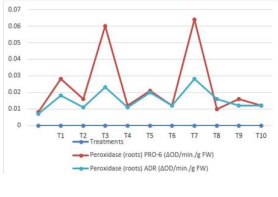

Figure 1. Graphical representation of root peroxidase activity in PRO-6 and ADR across treatments T1–T10

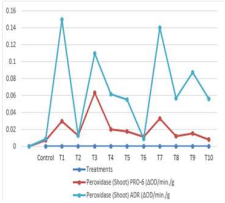

Figure 2. Shoot peroxidase activity in PRO-6 and ADR across treatments T1–T10

Estimation of catalase (EC 1.11.1.6)

The spectrophotometric cuvette was filled with 0.2 ml of enzyme extract and 1.8 ml of 50 mm sodium phosphate buffer (pH 7.5). One milliliter of H_2O_2 was added to start the reaction, and the absorbance at 240 nm was measured at 30-second intervals for three minutes to track the consumption of H_2O_2 0.0394 mM/cm is the value of the H_2O_2 extinction coefficient. The specific activity was measured in µmoles of H_2O_2 decomposed min^{-1} mg^{-1} of protein.

Estimation of superoxide dismutase (EC 1.15.1.1)

The reaction mixture comprised 1.5 ml of 100 mM Tris HCl buffer, 0.5 ml of 6 mM EDTA, 1 ml of 6 mM pyrogallol, and 0.1 ml of the extract. The decline in OD value was measured at an interval of 30 s up to 3 min. Specific activity is expressed as unit's min^{-1} mg^{-1} of protein (9).

Estimation of Ascorbate peroxidase

The reaction mixture consisted of 1.1 ml of 50 mM phosphate buffer (pH 7.0), 0.8 ml of 0.5 mM ascorbate, 0.1

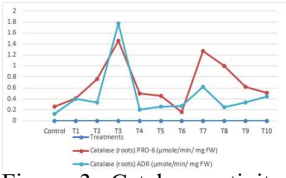

Figure 3. Catalase activity in roots of PRO-6 and ADR across treatments T1–T10

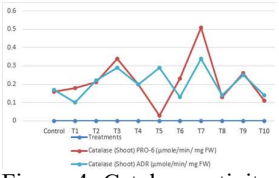

Figure 4. Catalase activity in shoots of PRO-6 and ADR across treatments T1 to T10

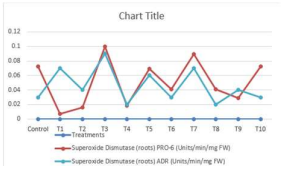

Figure 5. Superoxide dismutase root activity of PRO-6 & ADR across treatments T1–T10 represents in a graphically way

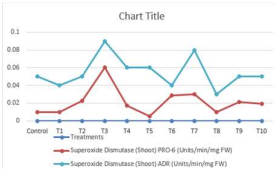

Figure 6. Superoxide dismutase Shoot activity of PRO-6 & ADR across treatments T1–T10

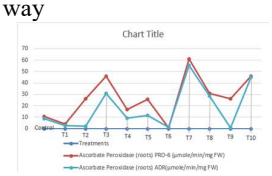

Figure 7. Ascorbate peroxidase root activity of PRO-6 & ADR across treatments T1–T10

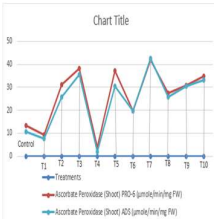

Figure 8. Ascorbate peroxidase shoot activity of PRO-6 & ADR across treatments T1–T10 represents in graph

Table 2. Effect of PGPR cultures in antioxidant enzyme activity (SOD and APX) in roots and shoots in *Allium cepa*

	Treatments	Superoxide Dismutase (roots) (Units/ min/mg FW)	Superoxide Dismutase (shoots) (Units/ min/mg FW)	Ascorbate Peroxidase (roots) (µmole/ min/mg FW)	Ascorbate peroxidase (shoots) (µmole/ min/mg FW)
PRO-6	Control	0.072	0.01	10.9	13.2
	T1	0.007	0.01	3.94	9.38
	T2	0.016	0.023	26.05	31.2
	T3	0.1	0.06	46.1	38.2
	T4	0.018	0.017	16.73	4
	T5	0.069	0.005	25.6	37.1
	T6	0.041	0.029	1.04	19.8
	T7	0.089	0.03	60.88	42
	T8	0.041	0.01	31.04	27.4
	T9	0.029	0.021	26.2	30.8
	T10	0.072	0.019	46	34.8
CD 5%		0.21	0.22	0.25	0.38
ADR	Control	0.03	0.05	8.77	10.8
	T1	0.07	0.04	2.63	7.54
	T2	0.04	0.05	2.09	25.95
	T3	0.09	0.09	30.93	35.65
	T4	0.02	0.06	9.15	2.08
	T5	0.06	0.06	11.45	30.56
	T6	0.03	0.04	1.17	19.57
	T7	0.07	0.08	55.5	42.66
	T8	0.02	0.03	28.48	25.79
	T9	0.04	0.05	0.85	30.6
	T10	0.03	0.05	44.81	33.29
CD 5%		0.20	0.22	0.75	0.45

ml of enzyme extract and 1 ml of H_2O_2 solution. Decrease in OD values were measured in a spectrophotometer at 290 nm after an interval of 30 s up to 3 min. For monodehydroascorbate (MDHA), value for extinction coefficient is 2.8 mM-1cm-1. Specific activity of APX was depicted as µmoles of Mon dehydroascorbic acid produced min- 1 mg-1 of protein (10).

Statistical analysis

Growth data and biochemical parameters have been presented as mean ± S.D. of three replicates. Data was statistically analysed by applying one- way analysis of variance (ANOVA) followed by post hoc analysis, the CD test.

RESULTS AND DISCUSSION

Effect of plant growth promoting rhizobacteria on the activities of antioxidant enzymes and biochemical parameters in onion

Plant growth promoting rhizobacteria are beneficial bacteria found on plant roots. These induce growth by a wide variety of mechanisms. PGPR are used as inoculants for biofertilization, Phyto stimulation and biocontrol (3). Ten isolates of *Pseudomonas* and *Bacillus* were isolated and seedlings of onion were treated with these isolates. The present study was conducted to analyse the effect of these isolates on the antioxidant enzymes in onion (*Allium cepa*).

Peroxidase

Peroxidases are heme containing enzymes that catalyse the one electron oxidation of several substrates at the expense of hydrogen peroxide. Peroxidase uses hydrogen peroxide as an electron acceptor to catalyse a number of oxidation reactions. Peroxidase classes are located in walls and vacuoles(11). **Table 1** indicates that roots have the highest value which was observed in PGPR 7 with the value of 0.064 ΔOD/min/g FW in PRO-6 and 0.028 ΔOD/min/gFWinADR as shown in **figure 1**.BothPRO-6andADRexhibitedhighestvaluesinshootsi.e.0.033 and 0.14 ΔOD/min/g FW, respectively as **representing in figure 2** followed by PGPR 3, while control had the least value as compared to all the treatments in peroxidase.

The recent study showed that inoculation with Mycorrhizal fungi of onion plants significantly increased the specific activity of POD and CAT that ranged between 536.38±4.47 and 12.77±0.38 U/mg protein, respectively (12). It was observed in one such study that peroxidase activity in roots and leaves of onion increased when analyzed after being introduced with the rhizobacteria and inoculated with pathogenic bacteria (13). Peroxidase activity increased from 0 dpi (days post inoculation) to 10 dpi as compared to controls. The activity of peroxidase in roots was higher than that in the leaves. Rhizobacteria isolate PK2RP3 exhibited highest peroxidase activity (0.058 µg/ mL^{-1}) in roots and (0.053 µg/ mL^{-1}) in the leaves at 15dpi (14).

Catalase

Catalases are tetrameric heme-containing enzymes that directly dismutase H_2O_2 into H_2O and oxygen (15). Catalases play an important role in the removal of H_2O_2 generated in peroxisomes and glyoxysomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism. Various PGPR cultures caused significant increase in CAT activity. In **figure 3,** Seedlings treated with PGPR 3 showed maximum catalase activity in roots i.e. 1.46 µmole/min/mg FW and 0.67 µmole/min./mg FW in PRO-6 and ADR, respectively, followed by PGPR 7. The catalase activity of PRO-6 in shoots was increased with the treatment of PGPR 7 by 0.51 µmole min/mg/FW and ADR showed 0.34 µmole/min/mg FW followed by PGPR 3 as shown in figure No 4. The untreated control showed least activity in both roots and shoots with all treatments ofPGPR.

The inhibition of catalase activity with many treatments is shown in **table 1**. It can be due to inhibition of enzyme synthesis or flux of superoxide radicals (16). In contrast to the present study, it indicates that the plants inoculated with PGPR in non- saline conditions showed decrease of 36 to 4% in catalase activity (17). Moreover, it was previously observed by researcher that the CAT enzyme was significantly increased by 3.7 folds as compared to control at 660µmole/mg (18).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is a metal-containing enzyme

that catalyses the dismutation of superoxide radicals to oxygen and hydrogen peroxide. The enzyme is found in all aerobic organisms examined, where it plays a major role in defence against toxic reduced oxygen species, which are generated as by-products of various biological oxidations (19). Various PGPR cultures caused significant increase in the SOD activity. As shown in table **2**, PGPR 3 exhibited maximum superoxide dismutase activity in roots in PRO-6 and ADR (0.10 units/min./mg FW and 0.09 Units/min./mg FW) respectively (shown in figure 5). In shoots, they had maximum activity in the treatment of PGPR 3 (0.09 units/min./mg FW and 0.08 units/min/mg FW), respectively, followed by PGPR 7. On the other hand, PGPR 1 and PGPR 4 indicated the least amount of SODactivity (**fig 6).**

With the treatment of microbial fertilizer, antioxidant enzyme activity increased to 403.39% in comparison to control treatment (20). The study showed that SOD activity decreased by cadmium (Cd) and escalated by 119% with the treatment of *Pseudomonas aeruginosa* (21). The study conducted by researchers and they reported that in welsh onion treated with KNO_3 priming, the SOD activity was improved (22).

Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) is a hydrogen peroxide-scavenging enzyme that is specific to plants and algae and is responsible to protect chloroplasts and other cell constituents from damage done by hydrogen peroxide and hydroxyl radicals produced from APX. In table 2, the results indicated that in roots, PRO-6 showed highest activity for PGPR 7 i.e. 60.88 µmole/min/mg FW and in ADR, maximum activity was 1.93 µmole/min/mg FW for PGPR 3 followed by PGPR 7. However, in shoots, PGPR 7 showed the highest activity in both PRO-6 and ADR (42.0 µmole/min/mg FW and 1.66 µmole/min/mg FW respectively), while the untreated control had the lowest activity for bothtreatments (As showing in figure 7 & 8).

In one study, it stated that the ascorbate peroxidase activity in different varieties of onion (Fepagro and Madrugada) was proportional to the increase in the salt concentration (23). In contrast to the present study, one such researcher observed that with the increase of salt concentration at different interval of time, APXdecresed (24).

CONCLUSION

Beneficial soil microorganisms referred as Plant Growth-Promoting Rhizobacteria (PGPR) populate the rhizosphere and promote plant growth via a number of direct and indirect processes. Bacillus cereus (PGPR 7) and Pseudomonas fluorescens (PGPR 3) were determined to be the most successful plant growth promoters among the different bacterial isolates assessed in the study. Their potent plant growth-promoting characteristics, including phytohormone production, phosphate solubilization, nitrogen fixation, siderophore generation, and biocontrol action against plant diseases, are responsible for their exceptional performance.

The ability of Bacillus cereus (PGPR 7) to improve plant growth indices such root length, shoot length, and total biomass was impressive. This species is well-known for generating growth-stimulating phytohormones that encourage cell division and elongation, including as cytokinins, gibberellins, and indole-3-acetic acid (IAA). Insoluble phosphates in the soil can also be dissolved by B. cereus, which makes phosphorus easily accessible to plants. A vital nutrient for photosynthesis and energy transmission, phosphorus enhances plant vigor and output. Furthermore, B. cereus produces endospores that allow it to endure in hostile environments and sustain long-term rhizosphere colonization, guaranteeing continuous growth promotion throughout the plant's life cycle.

However, Pseudomonas fluorescens (PGPR 3) showed exceptional plant growth-promoting and biocontrol capabilities. This bacterium is well-known for creating siderophores, which chelate iron and make it available to plants while depriving phytopathogens of this important component, so suppressing their growth. Additionally, P. fluorescens secretes a variety of secondary metabolites that suppress soil-borne pathogens, lowering disease incidence and enhancing general plant health. These metabolites include hydrogen cyanide (HCN) and antibiotics. Additionally, by promoting root growth and expanding root surface area, this bacterium improves nutrient uptake, giving plants greater access to water and minerals from the soil.

These two bacterial strains have superior growth-promoting potential due to the synergistic effects of phytohormone production, nutrient solubilization, and pathogen suppression. Pseudomonas fluorescens (PGPR 3) and Bacillus cereus (PGPR 7) considerably enhanced parameters like germination rate, plant height, chlorophyll content, and yield attributes in comparison to other treatments. Additionally, their beneficial effects on soil microbial activity suggest a role in enhancing soil fertility and health.

By causing systemic resistance (ISR) in plants, both of these PGPRs also demonstrate indirect plant growth-promoting mechanisms. By strengthening their natural defense mechanisms, plants become more resistant to both biotic and abiotic stressors. These characteristics make these bacteria sustainable substitutes for chemical pesticides and fertilizers in addition to being efficient growth promoters. The potential use of Pseudomonas fluorescens and Bacillus cereus in environmentally friendly and sustainable agriculture is highlighted by their dual functions.

In conclusion, of all the treatments examined, Bacillus cereus (PGPR 7) and Pseudomonas fluorescens (PGPR 3) are the most effective rhizobacteria for promoting plant growth. They are powerful biofertilizers for increasing crop productivity in a sustainable way because of their many different mechanisms, which include phytohormone synthesis, nutrient solubilization, and pathogen inhibition. To fully utilize their potential for commercial agricultural applications, more extensive field testing and formulation studies could be necessary.

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