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Induction of Bacterial Leaf Blight (*Xanthomonas oryzae pv. oryzae*) Resistance in Rice by Potential Endophytic Bacterial Consortium

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The world's most important food crop and the most important staple food for the expanding Indian population is rice (*Oryza sativa* L.). In many regions of the world, the disease has resulted in epidemic causing crop losses up to 50 per cent. Biocontrol has emerged as a useful tool in utilizing hostile microorganisms for a sustainable and friendly form of agriculture to protect crops from pathogens. Endophytes are microorganisms that reside inside the host plant and are known to be able to fight plant infections without creating any disease signs or negative effects. Since BLB of rice poses significant damage to rice farming and chemical control strategies are not viable, alternate fertilizers are being used to combat this problem by maintaining soil fertility and by increasing productivity. Isolated 52 bacterial endophytes from rice based on antagonistic activities against *Xoo*, based on biochemical characterization, plant growth promoting activities, nutrient

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solubilization and molecular characterization and compatibility four potential isolates EBP26, EBP39, EBP47 and EBP52 identified as *B.subtilis, Pseudomonas fluorescence, Pseudomonas* sp. and *Pantoea dispersa* developed as bacterial consortium. Application of microbial consortium under glass house and induction of defence enzymes (PAL, PO and PPO) and PR proteins (chitinase and glucanase) were recorded highest in T_7 (Seed treatment with promising endophytic bacterial consortium @ 4g/kg + seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen.

Keywords: Chitinase; consortium; endophytic bacteria; peroxidase; phenylammonia lyase; polyphenol oxidase.

1. INTRODUCTION

For the ever-growing Indian population, rice (*Oryza sativa* L.) is the essential staple food, and it is the leading food crop in the world [1]. It contains more starch (72.8g/100g) than all other cereals [2]. India has the most significant area that grows rice (45.7 M ha) followed by China and Indonesia [3]. They are susceptible to many pests and diseases like Blast, Bacterial blight, Sheath blight, Brown spot, Tungro virus and Others. Among these Bacterial leaf blight of rice (BLB) is the most economically important. Despite the fact that BLB affects rice all over the world, it is most economically important in Asia and some parts of Western Africa, where rice is grown in irrigated and low-land habitats [4]. In many regions of the world, the disease has resulted in epidemic causing crop losses up to 50 per cent [5].

The severity and scope of BLB damage have led to the development of disease management techniques for rice. The majority of high-yielding varieties introduced for cultivation have been found to be susceptible to the BLB to variable degrees and efforts are being made to develop resistant types. Further, exploring antibiotics against *Xanthomonas oryzae* pv. *oryzae* could be a viable technique for disease suppression through biological management. Endophytes are microorganisms that reside inside the host plant and are known to be able to fight plant infections without creating any disease signs or negative effects [6]. Endophytic microorganisms improve the acquisition of plant nutrients from the environment, such as nitrogen, iron, and phosphorus, through various processes like biological nitrogen fixation [7], phosphate solubilization [8] and siderophore production [9]. Endophytes take nutrients from plants made available in the form of photosynthates [10] in exchange. Additionally, they support the production of phytohormones such as auxins, gibberellins, and cytokinins [11]. By creating antifungal and antibacterial substances [12] as well as 1-aminocyclopropane-1-carboxylate (ACC), which reduces the ethylene level of the plant caused by biotic or abiotic stress [13], they help shield the plant against pathogenic diseases. Induced systemic resistance (ISR), which offers a better level of protection to a variety of phytopathogens [9], has also been observed to be activated by endophytic bacteria. As a result, it was considered necessary to explore tissue-specific bacterial endophytes that may be used as possible endophytic biocontrol agents for the management of BLB.

2. MATERIALS AND METHODS

The pathogen was isolated from infected rice leaves. Plant samples were taken from different treatments at various intervals, including before inoculation and every 24 hours up to one-week following *Xoo* challenge inoculation. Plant samples were crushed in a pre-chilled mortar and pestle homogenized with liquid nitrogen. The activity of defence-related enzymes phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and PR proteins like Chitinase, and β-1,3 glucanase was estimated using a spectrophotometer [14,15,16] with slight modifications.

2.1 Surface Sterilization of Rice Samples

Rice plant leaves, stem and root samples were rinsed in flowing tap water before being sliced into one-inch pieces. One gram of each sample was placed in a tube containing 0.1 per cent Tween 20. [17]. To eliminate bacteria clinging to the surface of leaves, stem and roots, the tubes were sonicated for 3 to 5 minutes. After that, the leaves, stem and roots were dipped in 70% ethanol for 3 minutes before being rinsed with sterile distilled water. The leaves, stem and roots were then immersed in 1 percent sodium

hypochlorite for 2 minutes before being rinsed 5 to 6 times with sterile distilled water. To assess the success of surface sterilization, an aliquot of 0.1 ml from the final rinse was plated on Nutrient agar [18]. Using a sterile mortar and pestle, surface sterilised rice root tissues were aseptically crushed in one ml of sterilized water. Root homogenates were used for serial dilutions and plating on various medium [19].

Root homogenates were serially diluted (10-fold) were spread over four different media, including Nutrient agar (NA; Hi media), Triptic soy agar (TSA; Hi media), Pseudomonas isolation agar (PIA; Hi media) and Yeast extract mannitol agar (YEMA; Hi media). After two days of incubation at 30°C, bacterial isolates were obtained. After purification, isolates were grown on NA plates and used for further studies.

2.2 Isolation of the Pathogen

The pathogen was isolated from infected rice leaves collected from different farmer's field. The samples that showed characteristic leaf blight and exuded bacterial ooze from the cut portion were used for isolation. The bits were placed on a microscopic slide and cut into smaller pieces using a sterile blade. The bacterial suspension was made by dropping sterile distilled water onto the chopped pieces on the slide. With the aid of a sterilized inoculation loop, this suspension was streaked on a modified Wakimoto's agar medium [20]. The inoculated plates were incubated for 48 hrs at room temperature (27 ± 2°C). On the MWA plate, Typical pin head sized, mucoid, convex colonies of *Xoo* were observed. The pure culture, thus obtained was preserved in the refrigerator at 4°C for further investigations.

2.3 Collection of Samples

Fresh leaves from each treatment were taken at random before and after pathogen inoculation for one week. The activities of Phenylalanine ammonia-lyase (PAL), Polyphenol oxidase (PPO), Peroxidase, Chitinase, and β-1,3 Glucanase were estimated in rice samples.

2.4 Phenylalanine Ammonia-lyase (PAL) Assay

The activity of phenylalanine ammonia-lyase (PAL) was estimated using [21,22]. Extracting 0.5 g of fresh leaves in 4 ml of 0.2 M borate buffer yielded an enzyme extract (pH 8.7). 1 ml enzyme extract, 2 ml 0.2 M borate buffer (pH 8.7), and 0.2 ml 0.1 M L-phenylalanine were added and incubated for 30 minutes at 32± 2^{o}C. After that, 0.5 ml of 1 M trichloroacetic acid was added to halt the reaction. After incubation, the absorbance at 290 nm of the whole reaction mixture was measured in a spectrophotometer to estimate the amount of cinnamic acid generated [23]. The cinnamic acid standard curve was created by measuring the absorbance of produced standard quantities of cinnamic acid at 290 nm.

2.5 Polyphenol Oxidase (PPO) Assay

The activity of polyphenol oxidase (PPO) was measured using [24,25]. Fresh leaf samples (0.5 g) were homogenized in 4 ml of 0.1 M phosphate buffer (pH 7.0), then centrifuged for 15 mins at 4°C for 10000 rpm. For the assay 500 µl of the supernatant was added to 2 ml of 0.1 M phosphate buffer (pH 7.0), and 500 µl of 0.01 M catechol was added for starting the reaction. The changes in the absorbance were recorded at 30 second intervals for 2 minutes at 495 nm. PPO activity was expressed as a change in absorbance of the reaction mixture min^{-1} g^{-1} of fresh weight.

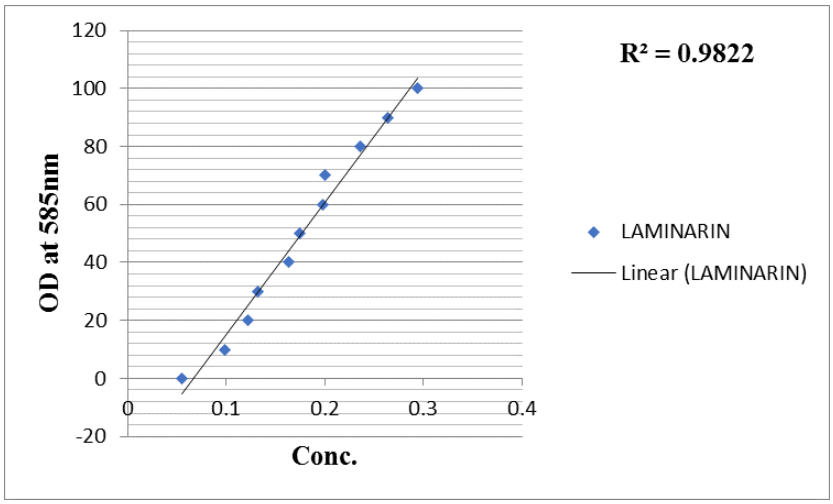
2.6 Peroxidase Assay

The reaction mixture for the peroxidase assay contained 100 µl of the enzyme extract, 2 ml of 0.1 M potassium phosphate buffer (pH 7.0), and 100 µl of 0.4 mM guaicol. 30 µl of 2 mM H_2O_2 were added to this mixture, and the subsequent change in absorbance at 436 nm was measured for two minutes at intervals of 30 seconds.

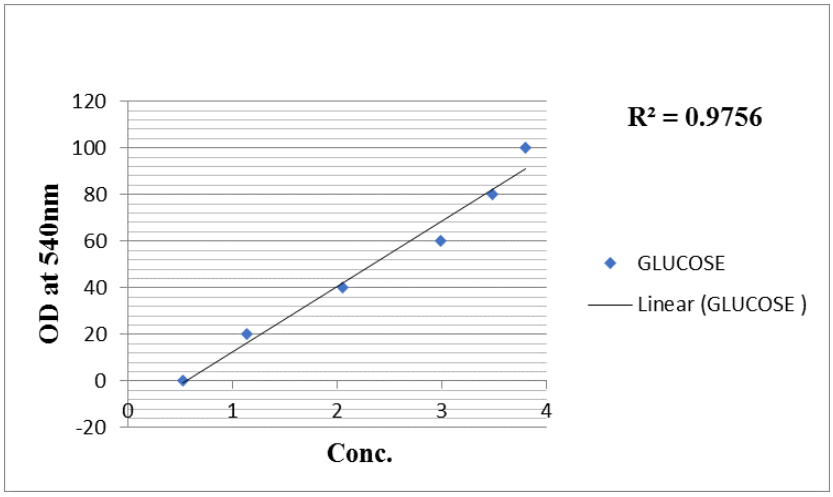
2.7 PR Proteins

2.7.1 Chitinase assay

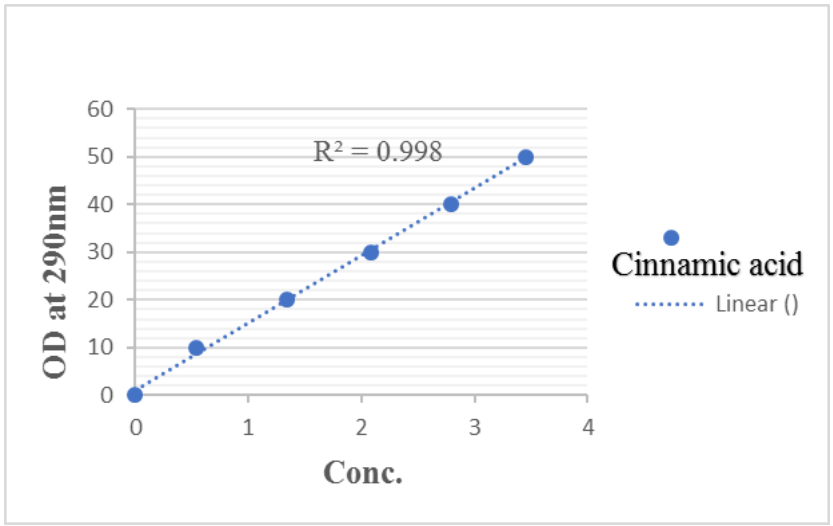
For the chitinase assay [26] 300 µl of crude enzyme extract, an equal volume of substrate (300 µl colloidal chitin) was added and incubated at 37°C for 60 mins. The reaction was terminated by centrifugation @ 5000 rpm for 5 mins. The supernatant 500 µl was collected. To this 0.1ml of borate buffer was added and incubated in boiling water for 3 mins. After cooling to room temperature 1 ml of dimethyl amino benzaldehyde (DMAB) was added. The mixture was incubated at 37±2°C for 20 mins, and absorbance were measured at 585 nm. One enzyme unit was expressed as the amount of enzyme that released one ml of N-acetyl glucosamine (NAG) from colloidal chitin in 1 hr, under the assay conditions.



Graph 1. Standard curve of laminarin at 585nm



Graph 2. Standard curve of glucose at 540nm



Graph 3. Standard curve of cinnamic acid at 290nm

2.7.2 ß 1,3- glucanase assay

In order to measure the amount of ß-1,3-glucanase activity, 62.5 µl of enzyme extract, 4% w/v laminarin and 200 µl of sodium acetate buffer, pH 5.2 were combined and incubated for 10 min at 40°C. With glucose as a standard, the amount of reducing groups released from laminarin was measured using the dinitro salicylic acid reagent [27]. At 540 nm, the absorbance of the reaction mixture was assessed.

3. RESULTS AND DISCUSSION

Isolated 52 endophytic bacterial isolates, based on antagonistic activity against *Xoo*, biochemical characterization, plant growth promoting traits, nutrient solubilization, molecular characterization and compatibility test, four isolates EBP26, EBP39, EBP47 and EBP52 (*B. subtilis, Pseudomonas fluorescence, Pseudomo*nas sp. and *Pantoea dispersa*) were used and developed potential endophytic bacterial consortium and screened in glass house condition under pathogen pressure.

3.1 PR Proteins

3.1.1 Chitinase assay

Chitinase activity was significantly higher in treatments (T_1-T_8) before and after pathogen inoculation compared to pathogen inoculated control (T_9) and uninoculated control (T_{10}). The highest chitinase was recorded on 6th day in treatment T_7 (Seed treatment with promising endophytic bacterial consortium @ 4g/kg + Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 59.037 µmoles/min/gm followed by T_6, T_5 and T_8 with 52.415 µmoles/min/gm, 49.104 µmoles/min/gm and 42.481 µmoles/min/gm and lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 22.614 µmoles/min/gm and 32.548 µmoles/min/gm. Peak activity of PPO was up to 6th day and declined on 7th day onwards. On 7th day highest enzyme activity was recorded in T_7 with 56.830 µmoles/min/gm and the lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 20.407 µmoles/min/gm and 28.685 µmoles/min/gm. All treatments are significantly different from each other.

3.1.2 β-1,3 glucanase assay

β-1,3 glucanase activity was significantly higher in treatments (T_1-T_8) before and after pathogen inoculation compared to pathogen inoculated (T_9) and uninoculated control (T_{10}). Highest β-1,3 glucanase was recorded on 6th day in treatment T_7 (Seed treatment with promising endophytic bacterial consortium @ 4g/kg + Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application

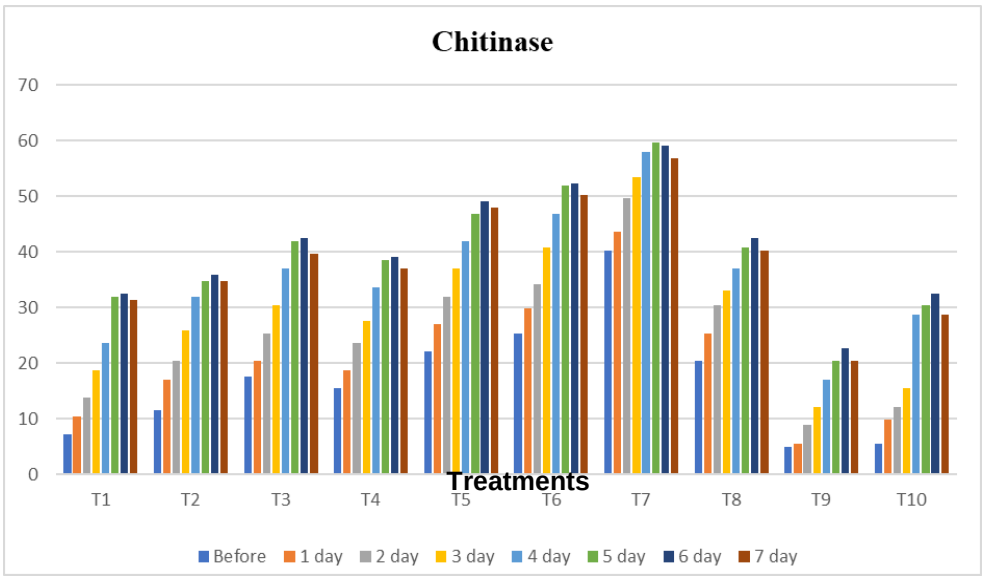


Fig. 1. Activity of chitinase (µmoles/min/gm) in different treatments of rice cv. BPT-5204 in glass house experiment

Table 1. Activity of chitinase (µmoles/min/gm) in different treatments of rice cv. BPT- 5204 in glass house

Treatments	Before Pathogen inoculation	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
T_1 - ST with promising endophytic bacterial consortium @ 4g/kg	7.163^{ab}	10.474^{b}	13.785^{b}	18.751^{c}	23.718^{b}	31.996^{bc}	32.548^{b}	31.444^{c}
T_2 - SD with promising endophytic bacterial consortium @ 4g/kg at 15DAS	11.5778^{c}	17.096^{c}	20.407^{c}	25.926^{d}	31.996^{d}	34.755^{c}	35.859^{c}	34.755^{d}
T_3 - FA with promising endophytic bacterial consortium 250g/ha at 30DAT	17.648^{de}	20.407^{d}	25.374^{d}	30.340^{ef}	36.963^{e}	41.929^{d}	42.481^{e}	39.722^{e}
T_4 - T_1 + T_2	15.440^{d}	18.751^{cd}	23.718^{d}	27.581^{de}	33.652^{d}	38.618^{d}	39.170^{d}	36.963^{d}
T_5 - T_1 + T_3	22.063^{fg}	27.029^{e}	31.996^{ef}	36.963^{g}	41.929^{f}	46.896^{e}	49.104^{f}	48.000^{f}
T_6 - T_2+ T_3	25.374^{g}	29.789^{f}	34.203^{f}	40.826^{h}	46.896^{g}	51.863^{f}	52.415^{g}	50.207^{f}
T_7 - T_1 + T_2+ T_3	40.274^{h}	43.585^{g}	49.655^{g}	53.518^{i}	57.933^{h}	59.589^{g}	59.037^{h}	56.830^{g}
T_8 - Chemical check- FS with Streptomycin sulphate @ 200 ppm + COC @ 0.3%	20.407^{ef}	25.3742^{e}	30.340^{e}	33.100^{f}	36.963^{e}	40.826^{d}	42.481^{e}	40.274^{e}
T_9 - Inoculated control	4.955^{a}	5.507^{a}	8.8185^{a}	12.129^{a}	17.096^{a}	20.407^{a}	22.614^{a}	20.407^{a}
T_{10} - Uninoculated control	5.507^{bc}	9.922^{b}	12.129^{b}	15.440^{b}	28.685^{c}	30.340^{b}	32.548^{b}	28.685^{b}
CD(p=0.05)	0.023	0.015	0.02	0.02	0.017	0.02	0.01	0.015
SE(m) ±	0.008	0.005	0.007	0.007	0.006	0.007	0.003	0.005
C.V	5.09	4.52	5.36	4.72	3.43	3.85	1.84	2.84

Numerical values with different letters are significantly different at 0.05 % level by DMRT; DMRT, Duncan's multiple range test
ST- Seed treatment; SD- Seedling dip

with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 441.47 µmoles/min/gm which was on par with T_6 (Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 393.56 µmoles/min/gm and lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 182.72 µmoles/min/gm and 245.01 µmoles/min/gm. Peak enzyme activity of β-1,3 glucanase was up to 6th day and declined on 7th day onwards. On 7th day highest enzyme activity was recorded in T_7 with 403.14 µmoles/min/gm and the lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 158.76 µmoles/min/gm and 201.88 µmoles/min/gm. All treatments are significantly different from each other

3.2 Induced Systemic Resistance

The experiment was conducted under greenhouse conditions. Phenylalanine ammonia-lyase (PAL), Polyphenol oxidase (PPO) and Peroxidase (PO) were analysed in fresh leaf samples collected before and after BLB inoculation randomly for 7 days from each of the treatments. Treatments involved was different methods of application of potential endophytic bacterial consortium (T_1-T_7) along with chemical control (T_8), pathogen inoculated (T_9) and uninoculated control (T_{10}).

It is well known that the proper stimuli or signals are required to trigger defence genes and that is why the accumulation of plant defense-related enzymes (PO, PPO and PAL) in plant tissues are associated to the plant defence response and induced resistance by endophytic bacteria [28,29].

3.3 Phenylalanine Ammonia-lyase (PAL)

Phenylalanine ammonia-lyase (PAL) activity was significantly higher in treatments (T_1-T_8) before and after BLB pathogen inoculation compared to pathogen inoculated (T_9) and uninoculated control (T_{10}). The highest PAL was recorded on 6th day in T_7 (Seed treatment with promising endophytic bacterial consortium @ 4g/kg + Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 10.647 µmoles/min/gm which was on par with T6 (Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 9.348 µmoles/min/gm and lowest was recorded in T_9 (BLB inoculated pot) and T_{10} (Uninoculated pot) with 4.2 µmoles/min/gm and 5.24 µmoles/min/gm. From 7th day onwards peak activity was declined. Among the ten treatments, highest PAL was recorded in T_7 with 9.418 µmoles/min/gm and lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 3.898 µmoles/min/gm and 5.035 µmoles/min/gm. All treatments that are significantly different from each other were presented in the Table 3 and Fig. 3.

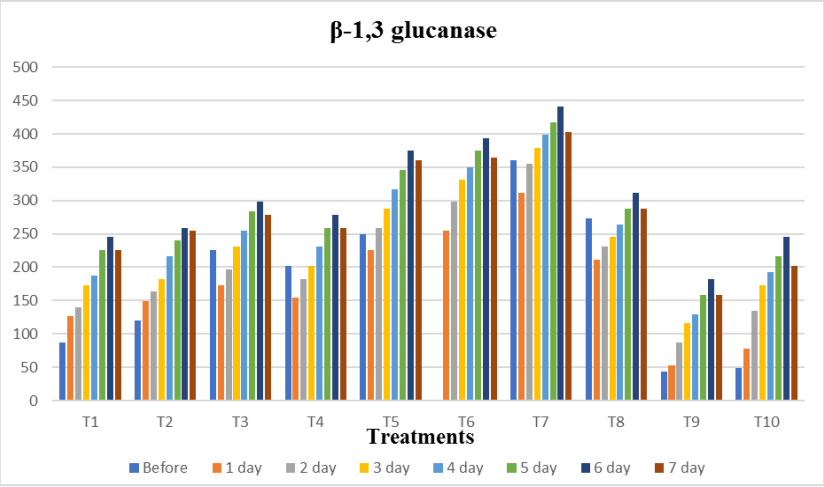


Fig. 2. Activity of β-1,3 glucanase (µmoles/min/gm FW) in different treatments of rice cv BPT- 5204 in glass house experiment

Table 2. Activity of β-1,3 glucanase (µmoles/min/gm) in different treatments of rice cv. BPT- 5204 in glass house

Treatments	Before Pathogen inoculation	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
T_1 - ST with promising endophytic bacterial consortium @ 4g/kg	86.88^{ab}	127.13^{b}	139.59^{b}	173.13^{b}	187.51^{b}	225.84^{b}	245.01^{b}	225.84^{b}
T_2 - SD with promising endophytic bacterial consortium @ 4g/kg at 15DAS	120.42^{b}	149.17^{bc}	163.55^{bc}	182.72^{bc}	216.26^{c}	240.22^{bc}	259.38^{b}	254.59^{c}
T_3 - FA with promising endophytic bacterial consortium 250g/ha at 30DAT	225.84^{cd}	173.13^{cd}	197.09^{d}	230.63^{d}	254.59^{d}	283.34^{de}	297.72^{d}	278.55^{cd}
T_4 - T_1 + T_2	201.88^{c}	153.97^{bc}	182.72^{cd}	201.88^{c}	230.63^{c}	259.38^{cd}	278.55^{c}	259.38^{cd}
T_5 - T_1 + T_3	249.80^{ef}	225.84^{ef}	259.38^{e}	288.14^{e}	316.89^{e}	345.64^{f}	374.39^{e}	360.01^{e}
T_6 - T_2+ T_3	316.89^{gh}	254.59^{f}	297.72^{f}	331.26^{f}	350.43^{f}	374.39^{g}	393.56^{f}	364.80^{e}
T_7 - T_1 + T_2+ T_3	360.01^{h}	312.09^{g}	355.22^{g}	379.18^{g}	398.35^{g}	417.51^{h}	441.47^{g}	403.14^{f}
T_8 - Chemical check- FS with Streptomycin sulphate @ 200 ppm + COC @ 0.3%	273.76^{fg}	211.47^{de}	230.63^{e}	245.01^{d}	264.18^{d}	288.14^{e}	312.09^{d}	288.14^{ed}
T_9 - Inoculated control	43.75^{a}	53.34^{a}	86.88^{a}	115.63^{a}	130.01^{a}	158.76^{a}	182.72^{a}	158.76^{a}
T_{10} - Uninoculated control	48.55^{a}	77.30^{a}	134.80^{b}	173.13^{b}	192.30^{b}	216.26^{b}	245.01^{b}	201.88^{b}
CD(p=0.05)	0.03	0.028	0.02	0.01	0.014	0.017	0.012	0.02
SE(m) ±	0.01	0.009	0.007	0.006	0.005	0.006	0.004	0.007
C.V	2.58	2.45	1.84	1.45	1.12	1.34	0.93	1.55

*Numerical values with different letters are significantly different at 0.05 % level by **DMRT**; DMRT, Duncan's multiple range test*

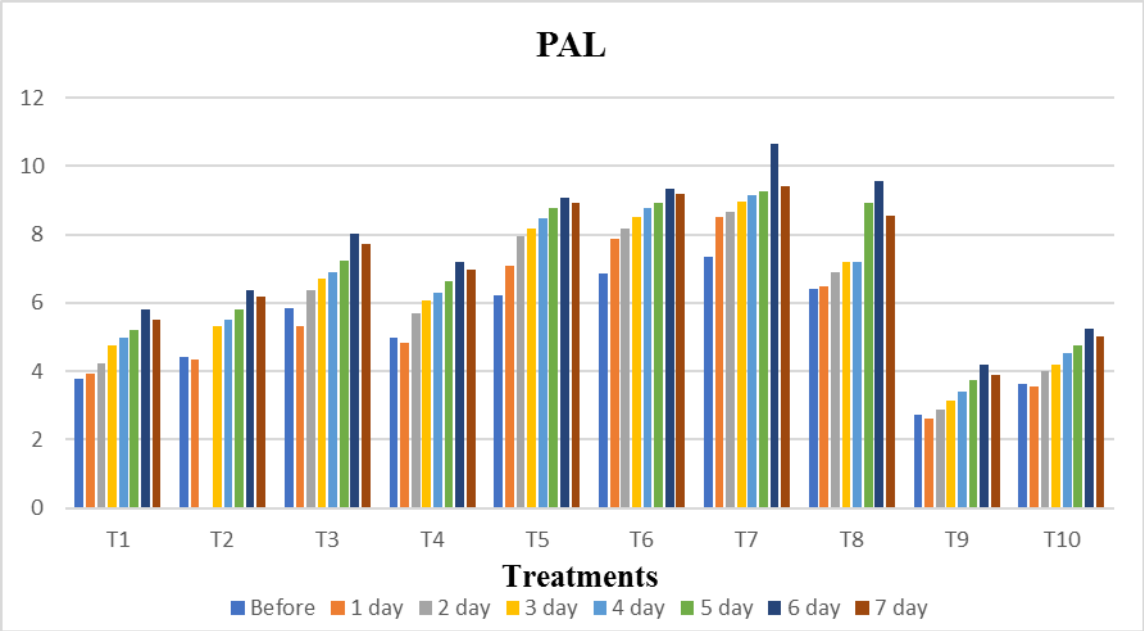


Fig. 3. Activity of Phenylalanine ammonia lyase (µmoles/min/gm) in different treatments of rice cv. BPT- 5204 in glass house experiment

In response to pathogen infection, PAL the first enzyme of phenylpropanoid metabolism is crucial in controlling phenol production in plants [30]. In order to create the precursors for lignins, furanocoumarin phytoalexins and flavonoid pigments PAL converts phenylalanine to trans-cinnamic acid [31].

3.4 Peroxidase

Peroxidase activity was significantly higher in treatments (T_1-T_8) before and after pathogen inoculation compared to inoculated (T_9) and uninoculated control (T_{10}). The highest peroxidase was recorded on 5th day in T_7 (Seed treatment with promising endophytic bacterial consortium @ 4g/kg + Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 1.133 mmoles/min/gm which was on par with T_6 (Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 0.952 mmoles/min/gm and the lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 0.273 mmoles/min/gm and 0.3 mmoles/min/gm. Peak activity of peroxidase was up to 5th day and declined on 6th day onwards. On 7th day highest enzyme activity was recorded in T_7 with 0.694 mmoles/min/gm and lowest was recorded in T_9 (BLB inoculated) and T_{10} (uninoculated) with 0.247 mmoles/min/gm and 0.250 mmoles/min/gm. All treatments are significantly different from each other were presented in the Table 4 and Fig. 4.

For plants to defend themselves against pathogen infections, peroxidases are required. It is necessary for the final polymerization of phenolic derivatives into lignin, the enzyme peroxidase is also involved in suberization, or the healing of wounds [32]. When rice leaves were inoculated with microbial consortia and challenge inoculated with *Xoo,* there was an almost 100% increase in peroxidase activity as compared to control leaves. The increased peroxidase activity in rice leaves may have contributed to the formation of lignin.

3.5 Polyphenol Oxidase

Polyphenol oxidase activity was significantly higher in treatments (T_1-T_8) before and after pathogen inoculation compared to inoculated (T_9) and uninoculated control (T_{10}). Highest PPO was recorded on 6th day in T_7 (Seed treatment with promising endophytic bacterial consortium @ 4g/kg + Seedling dip with promising endophytic bacterial consortium @ 4g/l at 15DAS + Foliar

application with promising endophytic bacterial consortium @ 250g/ha at 30DAT) and challenge inoculated with BLB pathogen having enzyme activity of 10.305 nmoles/min/gm followed by T_6, T_5, T_8, T_3, T_4, T_2 and T_1 with 9.317 nmoles/min/gm, 8.047 nmoles/min/gm,7.764 nmoles/min/gm,7.623 nmoles/min/gm, 6.917 nmoles/min/gm, 6.211 nmoles/min/gm and 6.070 nmoles/min/gm. Lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 4.517 nmoles/min/gm and 5.505 nmoles/min/gm. Peak activity of PPO was up to 6^{th} day and declined on 7^{th} day onwards. On 7^{th} day highest enzyme activity was recorded in T_7 with 10.023 nmoles/min/gm and the lowest was recorded in T_9 (pathogen inoculated) and T_{10} (uninoculated) with 4.235 nmoles/min/gm and 5.223 nmoles/min/gm. All treatments are significantly different from each other were presented in the Table 5 and Fig. 5.

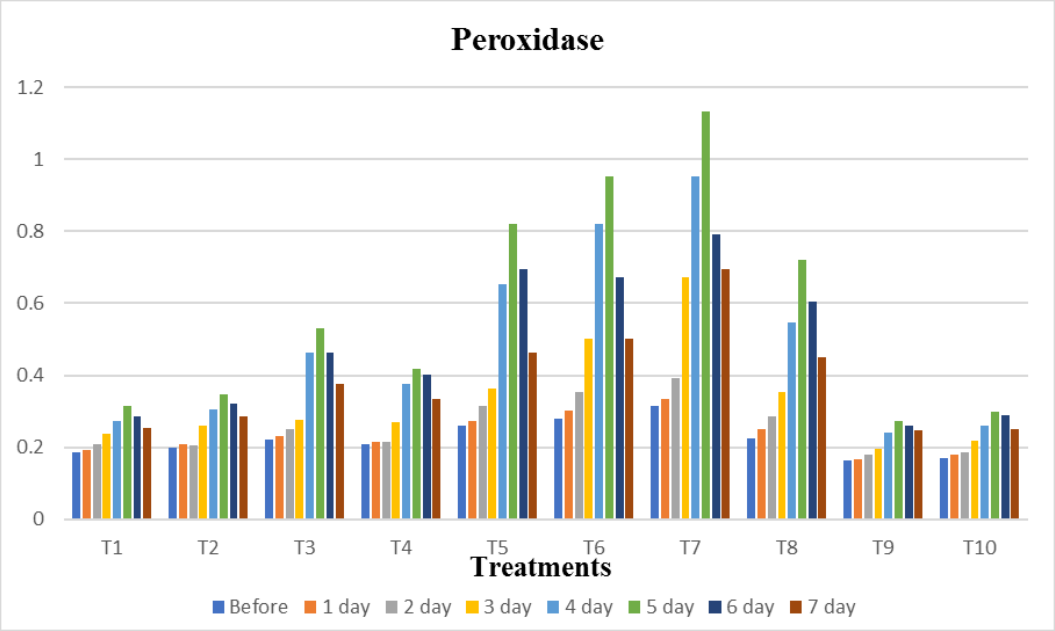


Fig. 4. Activity of Peroxidase(µmoles/min/gm) in different treatments of rice cv. BPT-5204 in glass house experiment

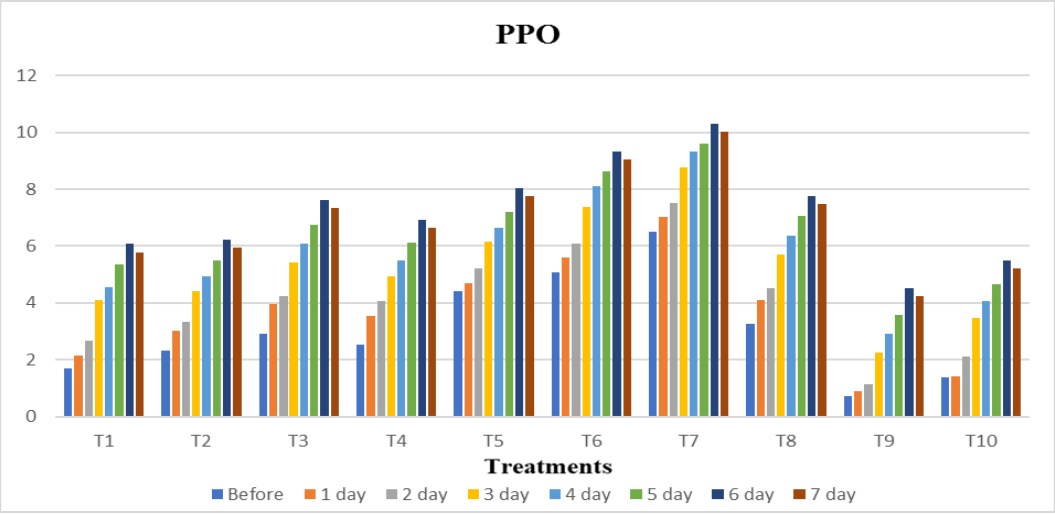


Fig. 5. Activity of Polyphenol oxidase(nmoles/min/gm) in different treatments of rice cv BPT- 5204 in glass house experiment

Table 3. Activity of phenylalanine ammonia lyase (µmoles/min/gm) in different treatments of rice cv. BPT- 5204 in glass house

Treatments	Before Pathogen inoculation	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
T_1 - ST with promising endophytic bacterial consortium @ 4g/kg	3.782^{b}	3.922^{bc}	4.246^{bc}	4.756^{b}	4.965^{c}	5.197^{c}	5.800^{c}	5.499^{bc}
T_2 - SD with promising endophytic bacterial consortium @ 4g/kg at 15DAS	4.409^{c}	4.339^{bc}	5.012b^{c}	5.313^{c}	5.522^{d}	5.823^{d}	6.357^{d}	6.171^{c}
T_3 - FA with promising endophytic bacterial consortium 250g/ha at 30DAT	5.846^{e}	5.336^{d}	6.357^{d}	6.704^{e}	6.890^{f}	7.238^{f}	8.026^{f}	7.725^{d}
T_4 - T_1 + T_2	4.965^{d}	4.826^{cd}	5.684^{cd}	6.078^{d}	6.310^{e}	6.635^{e}	7.191^{e}	6.960^{d}
T_5 - T_1 + T_3	6.241^{f}	7.099^{fg}	7.957^{ef}	8.189^{g}	8.490^{h}	8.768^{h}	9.070^{g}	8.931^{e}
T_6 - T_2+ T_3	6.867^{g}	7.864^{gh}	8.165^{fg}	8.513^{g}	8.792^{i}	8.931^{i}	9.348^{h}	9.186^{e}
T_7 - T_1 + T_2+ T_3	7.331^{h}	8.513^{h}	8.652^{g}	8.954^{h}	9.139^{j}	9.279^{j}	10.647^{i}	9.418^{e}
T_8 - Chemical check- FS with Streptomycin sulphate @ 200 ppm + COC @ 0.3%	6.403^{f}	6.496^{e}	6.890^{e}	7.191^{f}	7.191^{g}	8.931^{g}	9.557^{j}	8.560^{e}
T_9 - Inoculated control	2.716^{a}	2.623^{a}	2.878^{a}	3.156^{a}	3.388^{a}	3.736^{a}	4.200^{a}	3.898^{a}
T_{10} - Uninoculated control	3.620^{b}	3.574b	3.991^{b}	4.200^{b}	4.525^{b}	4.756^{b}	5.243^{b}	5.035^{b}
CD(p=0.05)	0.03	0.13	0.04	0.02	0.036	0.093	0.25	0.032
SE(m) ±	0.011	0.044	0.016	0.008	0.012	0.031	0.086	0.011
C.V	2.80	10.65	3.53	1.64	2.41	5.86	5.76	1.94

*Numerical values with different letters are significantly different at 0.05 % level by **DMRT**; DMRT, Duncan's multiple range test*

Table 4. Activity of Peroxidase (µmoles/min/gm) in different treatments of rice cv. BPT- 5204 in glass house

Treatments	Before Pathogen inoculation	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
T_1 - ST with promising endophytic bacterial consortium @ 4g/kg	0.185^{e}	0.191^{g}	0.207^{e}	0.237^{d}	0.273^{e}	0.316^{e}	0.286^{f}	0.253^{f}
T_2 - SD with promising endophytic bacterial consortium @ 4g/l at 15DAS	0.2^{d}	0.209^{f}	0.206^{e}	0.261^{e}	0.306^{f}	0.346^{f}	0.321^{e}	0.286^{e}
T_3 - FA with promising endophytic bacterial consortium @ 250g/ha at 30DAT	0.222^{c}	0.230^{de}	0.25^{d}	0.277^{d}	0.463^{c}	0.53^{c}	0.463^{c}	0.375^{c}
T_4 - $T_1 + T_2$	0.207^{d}	0.216^{ef}	0.214^{e}	0.27^{d}	0.375^{d}	0.419^{d}	0.401^{d}	0.334^{d}
T_5 - $T_1 + T_3$	0.261^{b}	0.274^{bc}	0.316^{b}	0.362^{c}	0.654^{b}	0.821^{bc}	0.694^{ab}	0.463^{b}
T_6 - $T_2 + T_3$	0.281^{b}	0.301^{ab}	0.353^{a}	0.502^{b}	0.821^{a}	0.952^{ab}	0.672^{ab}	0.502^{b}
T_7 - $T_1 + T_2 + T_3$	0.316^{a}	0.334^{a}	0.393^{a}	0.672^{a}	0.952^{a}	1.133^{a}	0.793^{a}	0.694^{a}
T_8 - Chemical check- FS with Streptomycin sulphate @ 200 ppm + COC @ 0.3%	0.225^{c}	0.25^{cd}	0.286^{c}	0.353^{c}	0.548^{b}	0.722^{c}	0.604^{b}	0.45^{b}
T_9 - Inoculated control	0.162^{f}	0.166^{h}	0.181^{f}	0.197^{g}	0.24^{g}	0.273^{h}	0.261^{g}	0.247^{f}
T_{10} - Uninoculated control	0.171^{g}	0.181^{g}	0.187^{f}	0.217^{f}	0.259^{f}	0.3^{f}	0.29^{f}	0.25^{f}
CD(p=0.05)	0.15	0.169	0.165	0.192	0.15	0.104	0.138	0.145
SE(m) ±	0.05	0.057	0.056	0.065	0.05	0.035	0.046	0.049
C.V	3.74	4.43	4.66	6.54	6.86	5.56	6.58	5.84

*Numerical values with different letters are significantly different at 0.05 % level by **DMRT**; DMRT, Duncan's multiple range test*

Table 5. Activity of Polyphenol oxidase(nmoles/min/gm) in different treatments of rice cv. BPT- 5204 in glass house

Treatments	Before Pathogen inoculation	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
T_1 - ST with promising endophytic bacterial consortium @ 4g/kg	1.694^{c}	2.164^{c}	2.682^{c}	4.094^{c}	4.564^{c}	5.364^{c}	6.070^{c}	5.788^{c}
T_2 - SD with promising endophytic bacterial consortium @ 4g/l at 15DAS	2.305^{d}	3.011^{d}	3.341^{d}	4.423^{c}	4.941^{c}	5.505^{c}	6.211^{c}	5.929^{c}
T_3 - FA with promising endophytic bacterial consortium @ 250g/ha at 30DAT	2.917^{e}	3.952^{e}	4.235^{et}	5.411^{e}	6.070^{e}	6.729^{e}	7.623^{e}	7.341^{e}
T_4 - T_1 + T_2	2.541^{d}	3.529^{d}	4.047^{e}	4.941^{d}	5.505^{d}	6.117^{d}	6.917^{d}	6.635^{d}
T_5 - T_1 + T_3	4.423^{g}	4.705^{g}	5.223^{g}	6.164^{f}	6.635^{f}	7.2^{f}	8.047^{f}	7.764^{f}
T_6 - T_2+ T_3	5.082^{h}	5.6^{h}	6.070^{h}	7.388^{g}	8.094^{g}	8.611^{g}	9.317^{g}	9.035^{g}
T_7 - T_1 + T_2+ T_3	6.494^{i}	7.011^{i}	7.529^{i}	8.752^{h}	9.317^{h}	9.6^{h}	10.305^{h}	10.023^{h}
T_8 - Chemical check- FS with Streptomycin sulphate @ 200 ppm + COC @ 0.3%	3.247^{f}	4.094^{f}	4.517^{f}	5.694^{e}	6.352^{et}	7.058^{et}	7.764^{e}	7.482^{e}
T_9 - Inoculated control	0.705^{a}	0.894^{a}	1.129^{a}	2.258^{a}	2.917^{a}	3.576^{a}	4.517^{a}	4.235^{a}
T_{10} - Uninoculated control	1.364^{b}	1.411^{b}	2.117^{b}	3.482^{b}	4.047^{b}	4.658^{b}	5.505^{b}	5.223^{b}
CD(p=0.05)	0.023	0.025	0.023	0.024	0.03	0.025	0.02	0.017
SE(m) ±	0.05	0.057	0.056	0.065	0.05	0.035	0.069	0.046
C.V	6.21	5.62	4.63	4.30	4.20	3.25	2.22	2.03

*Numerical values with different letters are significantly different at 0.05 % level by **DMRT**; DMRT, Duncan's multiple range test*

In comparison to untreated plants in the control, [33] found increased PO and PPO activity in the combined treatment of seed treatment at 4g/kg + seedling dip at 4g/l + soil application at 500g/ha + foliar spray at 500g/ha with *B. Subtilis* (FZB 24). The increased activity of PO and PPO was observed only upto the third day of *Xoo* inoculation in untreated control plants, and thereafter there was a dramatic decline. Our results showed a similar tendency. Similar to this, plants inoculated with Pseudomonas sp. Rh323 and P-solubilizing bacteria showed enhanced activity of defense-related enzymes such as Phenylalanine ammonia-lyase, Polyphenol oxidase, and Peroxidase in response to *Xoo* [34,35].

Results showed that, plants inoculated with efficient antagonistic endophytic bacterial consortium after challenge inoculation with the *Xoo* pathogen produced more defensive enzymes such PO, PPO, and PAL. The rice plants treated with endophytic bacteria have elevated defense-related enzyme activity, which either directly or indirectly inhibit pathogen growth.

4. CONCLUSION

Plants with systemic acquired resistance (SAR) have an accumulation of PR-proteins and defense enzymes. The findings of this study showed that increased chitinase and ß-1,3-glucanase activity and accumulation in response to microbial consortia inoculation under pathogen pressure have boosted resistance to bacterial leaf blight disease in rice. These hydrolytic enzymes are thought to contribute resistance to bacterial leaf blight.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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