Journal of Advances in Biology & Biotechnology

Volume 27, Issue 8, Page 239-249, 2024; Article no.JABB.119920
ISSN: 2394-1081

Mechanisms of Cell Wall Degrading Enzymes from *Bacillus methylotrophicus* and *Bacillus subtilis* in Suppressing Foliar Blight Pathogens

Neha Saini [a*], Manvika Sahgal [a] and Ajay Veer Singh [a*]

[a] Department of Microbiology, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.

Authors' contributions

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

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i81136

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119920

Original Research Article

Received: 13/05/2024
Accepted: 15/07/2024
Published: 16/07/2024

ABSTRACT

Bacillus strains are potent Biocontrol agents (BCAs) and have been identified as an effective way to control the growth of phytopathogens of wheat. Through a direct inhibition mechanism which involves the production of cell wall degrading enzymes (proteases, glucanase, and chitinases) and siderophores they suppress the foliar blight disease. Both the strains of *Bacillus* P10 and UP11 shows growth inhibition *Alternaria triticina* (77.56%, 67.83%) and *Bipolaris sorokiniana* (73.97%, 62.16%) through a dual culture assay. These strains were subsequently examined for their ability to produce cell wall-degrading enzymes i.e., chitinase, protease, and β-1,3-glucanases and antifungal metabolites i.e., siderophores. It was found that when the antagonist's bacteria were Co-cultured with fungal pathogens then maximum production of hydrolytic enzymes and siderophore was

**Corresponding author: E-mail: nehasaini141994@gmail.com, ajaygbpuat@gmail.com;*

Cite as: *Saini, Neha, Manvika Sahgal, and Ajay Veer Singh. 2024. "Mechanisms of Cell Wall Degrading Enzymes from Bacillus Methylotrophicus and Bacillus Subtilis in Suppressing Foliar Blight Pathogens". Journal of Advances in Biology & Biotechnology 27 (8):239-49. https://doi.org/10.9734/jabb/2024/v27i81136.*

achieved at 96 hrs, but when both strains P10 and UP11 were alone maximum production was found at 48 hrs i.e., the exponential phase. The current study determined that *Bacillus methylotrophicus* (P10) and *Bacillus subtilis* (UP11) are highly effective strain for controlling Foliar Blight disease. Direct or Antibiosis is the main mechanism involved in this study. Further research on the interaction mechanisms between *Bacillus*-derived compounds and host plants is necessary.

Keywords: Cell wall degrading enzymes; chitinase; β-1,3-glucanse; protease; bacillus subtilis; bacillus methylotrophicus.

1. INTRODUCTION

Fungal diseases in plants pose a major threat to global agriculture, causing significant economic losses. Key soil-borne pathogens of wheat include *Pythium*, *Pyrenophora triticirepentis*, *Fusarium*, *Alternaria triticina*, *Rhizoctonia, Bipolaris sorokiniana*. Majorly *Bipolaris sorokiniana* and *Alternaria triticina* both cause foliar blight disease. It is most destructive wheat disease causing 50% to 85 % yield loss under conducive conditions (relative humidity > 70 % and an average temperature of 20 – 30°C) [1,2]. An eco-friendly alternative to chemical methods for protecting crops from phytopathogenic fungi is the use of Biological control agents (BCAs). Bacteria from the genus *Bacillus* are key microorganisms for biological control of plant diseases across various crops. They are highly effective against phytopathogens and offer advantages like easy culture, storage, and manufacture due to their ability to produce endospores [3].

Recently, *Bacillus*-based biological pesticides have seen increased global use. *B. subtilis* and *Bacillus methylotrophicus* is one of the most utilized species effective in controlling plant diseases through direct and indirect mechanisms [4]. It produces antimicrobial compounds, including cyclic lipopeptides (surfactins, iturins, and fengycins), which antagonize a range of pathogens like bacteria, fungi, and oomycetes. Additionally, they produce cell wall degrading enzymes (CWDEs) or pathogenesis related enzymes such as chitinases, β-1, 3-glucanase, and proteases, which degrade essential components of fungal cell walls of pathogens, limiting their growth and activity [5]. The antifungal activity of extracellular metabolites of bacteria against several fungi has already been investigated and reported by Dahiya et al. [6] and Gajera et al., [7]. Chitin, protease, and β-1,3-glucan are key structural components of the cell walls in fungi, known for their regularly arranged materials. Enzymes like chitinases, proteases and β-1,3-glucanases are crucial in various biological processes and interactions in nature, including plant defense mechanisms, fungal cell wall degradation, and the competitive interactions between organisms like Trichoderma spp. and phytopathogenic fungi [8]. The several study related to *Bacillus subtilis* and *Bacillus methylotrophicus* were also found that they produce secondary metabolites like phytohormones, hydrolytic enzymes, siderophores, and antibiotics, enhancing soil fertility, suppressing phytopathogens, and serving as eco-friendly biocontrol agents [9-12].In this study, we compared the enzymatic characteristics, effects on fungal cell walls, and antifungal activities against *Bipolaris sorokiniana* and *Alternaria triticina* at different time intervals. Therefore, understanding the induction process of these enzymes is crucial for selecting the most effective *Bacillus* strains for biocontrol purposes.

2. MATERIALS AND METHODS

2.1 Biocontrol Agent

The antagonistic wheat rhizobacteria strains (P10, UP11), previously isolated and stored at the Rhizosphere Biology Laboratory, Department of Microbiology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (Table 1) were cultured and maintained on Nutrient Agar (NA).

Table 1. Percent similarity and GenBank accession numbers of the bacterial isolates

Strain code	Description	% Identity	Accession no
P10	*Bacillus methylotrophicus*	94.04%	MN099430.1
UP11	*Bacillus subtilis*	97.43%	MN099431.1

2.1.1 Fungal pathogens

The fungal pathogens *Bipolaris sorokiniana* (ITCC 4869) and *Alternaria triticina* (ITCC 1186) were acquired from the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi, India. These fungal pathogens were cultured and kept on Potato Dextrose Agar (PDA) plates at 25±2°C for 5-7 days to ensure viability.

2.2 Dual Culture Activity of *Bacillus* Strains (P10 & UP11) Against Foliar Pathogens

The antagonistic activity of *Bacillus* strains (P10 & UP11) against phytopathogens (*Bipolaris sorokiniana and Alternaria triticina*) was examined using the dual culture plate method [13]. Fungal discs, 5 mm in diameter, were taken from 5-7day-old cultures and placed in the center of plates containing a medium composed of an equal mix of NA and PDA. The *Bacillus* strains ($2x10^7$) were then inoculated on either side of the fungal disc, each positioned 2.0 cm away from the disc. The plates were incubated at 27±1°C for 5-7 days. Control plates, which only contained the fungal growth without Bacillus inoculation, were also prepared. The percentage inhibition of fungal growth was determined by comparing the growth in the dual culture plates to that in the control plates, using the formula:

$$\% \text{ Inhibition} = [(C - T)/C] \; X \; 100$$

(Where, C= Radius of fungal growth in control plate, T = Radius of fungal growth in dual culture plate)

2.3 Production of Hydrolytic Enzymes

2.3.1 Chitinase production

2.3.1.1 Preparation of colloidal chitin

Chitin flakes obtained from HI Media were finely powdered. Five grams of this chitin powder were slowly added to 90 ml of concentrated HCl with vigorous stirring. The resulting mixture was then poured into 500 ml of ice-cold ethanol while stirring rapidly, and the solution was left overnight at 4°C. The precipitate formed was collected by centrifugation at 8000 rpm for 10 minutes at 4°C, followed by washing with distilled water until neutral pH was achieved. The resulting colloidal chitin, which contained 85-90% moisture, was subsequently air-dried and stored at 4°C for future use [14].

2.3.2 Procedure

The qualitative assay for chitinase production by bacterial isolates was conducted on a minimal agar medium ($MgSO4.7H_2O$, 0.2 g; K_2HPO_4, 0.9 g; KCl, 0.2 g; NH_4NO_3, 1.0 g; $FeSO_4$_7H2O, 0.002 g; $MnSO_4$, 0.002 g; $ZnSO_4$, 0.002 g; distilled water, 1.0 l; pH 6.8); 0.5g of yeast extract, and 15g of agar. Additionally, the medium was supplemented with 0.5% colloidal chitin. The minimal agar plates were spot inoculated with log phase bacterial cultures and plates were incubated at 30^0C for 3-4 days [15]. The appearance of halo zone around the colony indicated positive result.

2.3.3 β-1,3 glucanase production

β-1,3-Glucanase activity was assayed using *Laminaria digitata* laminarin as the substrate [16]. The log phase culture was spot inoculated on minimal agar media plates supplemented with 0.25% laminarin and incubated at 30^0C for 72 hrs. Thereafter the plates were stained with 0.1% Congo red solution. The halo zone observed around bacterial colony indicated positive test.

2.3.4 Proteolytic activity

A skim milk agar medium was created by dissolving 5.15 grams of skim milk powder in 100 milliliters of agar medium. Freshly grown 48-hour-old cultures were inoculated onto these skim milk agar plates and incubated at 28±2°C for two to three days [17]. The formation of a clear zone around *Bacillus* sp. colonies indicated protease enzyme production.

2.4 Siderophore Production

P10 and UP11 was spot inoculated onto chrome-azurol S-agar medium (CAS) agar medium, incubated at 30^0C for 72 hrs after that appearance of zones around the colony shows positive result (Schwyn and Neilands 1987).

2.5 Quantification of Hydrolytic Enzymes and Siderophore in Presence and Absence of Fungal Pathogens

2.5.1 Culture preparation

To quantitatively estimation of bacterial culture and co-cultures with fungal pathogens were cultivated in a 1:1 mixture of Nutrient Broth and Potato Dextrose Broth for 5 days. At various time

intervals (24-120 hours), 10 ml samples were taken from the flasks and centrifuged. The supernatants from both the antagonistic bacteria (control) and co-cultures with fungal pathogens were used for enzyme assays. The experiment was conducted in triplicate.

2.5.2 Enzymatic assay

Chitinase (EC 3.2.1.14) activity was determined using the DNS method [18]. The reaction mixture contained 0.5 ml of 0.5% colloidal chitin in phosphate buffer (pH 5.5), 0.5 ml of crude enzyme, and 1 ml of distilled water, making a total volume of 2 ml. After thorough vortexing, the mixture was incubated in a water bath shaker at 40°C for 1 hour. The reaction was halted by adding 3 ml of DNS reagent, followed by boiling in a water bath for 5 minutes. After cooling to room temperature, enzyme activity was measured at 540 nm. One unit of chitinase activity corresponded to the amount of enzyme needed to release 1 µmol of N-acetyl-β-D-glucosamine under the specified assay conditions [19].

β-1,3 glucanase (EC 3.2.1.39) activity was assessed by measuring the amount of reducing sugars released from laminarin, with glucose serving as a standard [20]. The assay involved combining 250 µl of 0.05 M potassium acetate buffer (pH 5.0) containing 2.5 mg/ml laminarin with 250 µl of culture filtrate. This enzyme-substrate mixture was then incubated at 40°C for 2 hours. After incubation, 0.5 ml of DNS reagent was added, followed by boiling at 100°C for 5 minutes. Upon cooling, 2 ml of deionized water was added, and the absorbance was measured at 595 nm using a spectrophotometer. β-1,3-glucanase activity was quantified as the amount of enzyme required to release 1 µmol of reducing sugar equivalents (measured as glucose) under the specified assay conditions.

Protease activity (EC 3.4.21.4) was determined using casein as the substrate, following the assay method described by Takami et al. [21]. 0.5 ml of the enzyme solution was incubated in a shaker water bath with 1.5 ml of 1.0% casein dissolved in a glycine-NaOH buffer (50 mM, pH 7) at 40°C for 30 minutes. The reaction was stopped by adding 2.5 ml of TCA solution (0.11 M trichloroacetic acid, 0.22 M sodium acetate, and 0.33 M acetic acid). The mixture was then filtered, and 0.5 ml of the filtrate was combined with 2.5 ml of 0.5 M Na_2CO_3 and 0.5 ml of Folin-Ciocalteau reagent. After allowing the reaction mixture to stand for30 minutes, the absorbance measured at 660 nm. One unit of protease activity was defined as the amount of enzyme required to produce 1 µg of tyrosine under the specified assay conditions.

Siderophore production was determined by antagonists and co culture were grown in an iron-free minimal medium incubated for different time intervals 24-120 hr at 25±2°C. Afterwards samples were withdrawn, centrifuged at 3000 rpm for 15 minutes. Culture supernatant (1.5 ml), CAS dye solution (1.5ml) and 30 µl of shuttling solution (sulfo-salicyclic acid) were added, mixed, kept for 20 minutes. The presence of siderophores in the culture supernatant leads to the removal of iron from the dye complex, resulting in a reduction in the intensity of the blue coloration absorbance recorded at 630 nm (Payne 1994). The minimal medium (blank) and % siderophore units were calculated by the following formula:

$$\% \text{ Siderophore units} = [(A_r - A_s)/ A_r] \times 100$$

Where A_r = absorbance of reference; A_s= absorbance of the sample

2.6 Statistical Analysis

All experiments were conducted in triplicates. Statistical analysis of the data was done using Statistical Package for Social Studies (SPSS), version 21.0. software. Values $p < 0.05$ and $p< 0.01$ were considered to indicate statistical significance. All data are expressed as means ± SE.

3. RESULS AND DISCUSSION

3.1 Antagonistic Activity of P10 & UP11 Against Foliar Blight Pathogens

The percent mycelial growth inhibition by biocontrol agents P10 against the *Alternaria triticina* (77.56%), followed by UP11 (67.83%) and *Bipolaris sorokiniana* (73.97%), followed by UP11 (62.16%) at 6 DAI (Table 2 and Fig. 1). P10 shows the maximum inhibition for foliar blight pathogens as compared to UP11. The similar study was also reported by Jaggi et al., [22].

3.2 Production of Hydrolytic Enzymes and Siderophore

Clear zone around the colony of *Bacillus*strains P10 & UP11 indicated positive results for

chitinase, β-1,3-glucanase, protease and as well as siderophore (Fig. 2). These positive results shows that both *Bacillus* strains are potent antagonists to suppress the fungal pathogen growth because they degrade the main components of fungal cell wall. They also show siderophore positive that means they compete for nutrition or iron availability.

3.3 Effect of Different Time on Total Hydrolytic Enzymes and Siderophore of *Bacillus* Strains Alone and Co-Cultured with Fungal Pathogens

Bacillus methylotrophicus and *Bacillus subtilis* both the strains show the increased expression of lytic enzymes, in presence of pathogens (*Alternaria triticina* & *Bipolaris sorokiniana*) such as chitinases, β-1,3-glucanase, and protease. Chitinase activity, expressed as (U/ML), significantly found maximum at 48 hr in alone or without pathogen but in presence of pathogens the chitinase activity was found increased upto 96 hr and decreased at 120 hr so the maximum duration for *Bacillus* strains alone at 48 hr and with pathogen at 96 hr. The similar observation was also found in another cell wall degrading enzymes (β-1,3-glucanase, and protease) and siderophore. So in absence of pathogen P10 and UP11 shows maximum activity of cell wall degrading enzymes i.e., chitinase (7.45 and 6.83) β-1,3-glucanase (4.28 and 3.79), and protease (1.91 and 1.76) as well as siderophore (59.61 and 52.63) at 48 hr [23,24].

Table 2. Bacterial isolates against foliar blight pathogens

Bacterial isolates	Percent inhibition (*Alternaria triticina*)	Percent inhibition (*Bipolaris sorokiniana*)
P10	77.56^{a}± 2.15	73.97^{a} ± 2.53
UP11	67.83^{b} ±2.24	62.16^{b} ±2.76

Values are expressed as the means of three replications ± standard deviation. Means within each column followed by the same letter are not significantly different (p<0.05), according to one-way ANOVA and Duncan's multiple range test (DMRT).

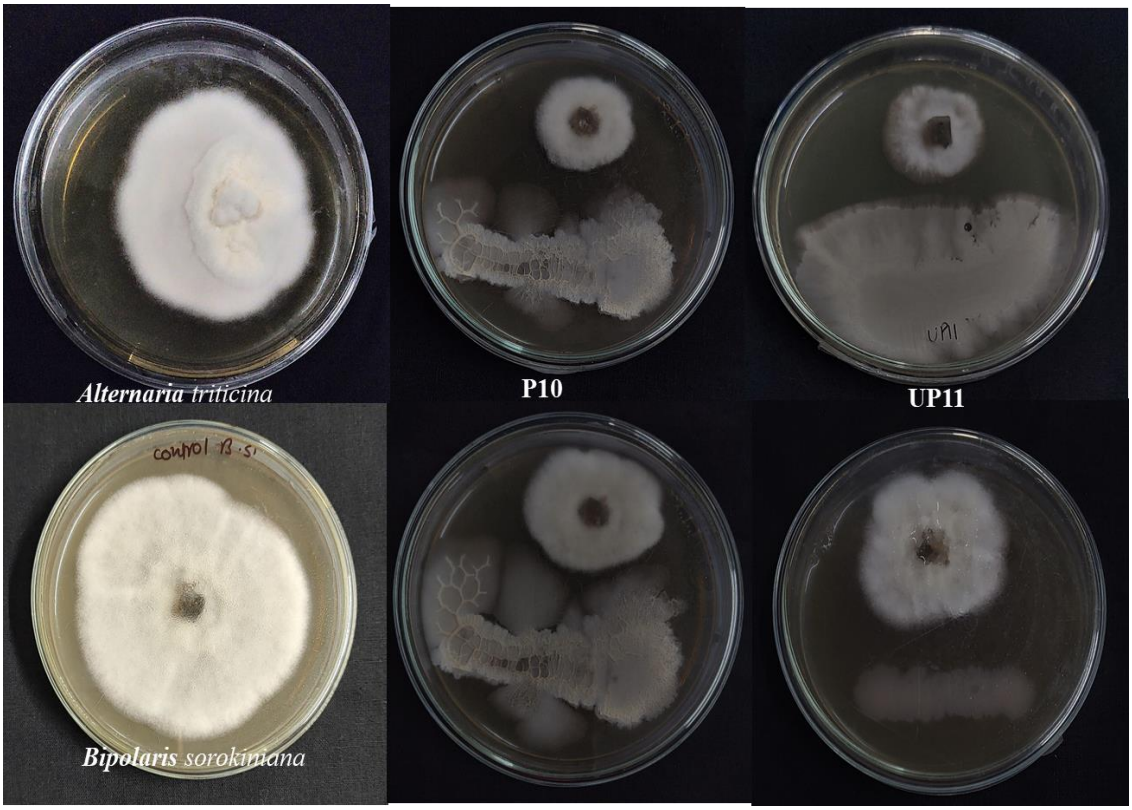


Fig. 1. Antagonistic activity of P10 & UP11 against the *Alternaria triticina* and *Bipolaris sorokiniana* after 6 DAI (Day after infection)

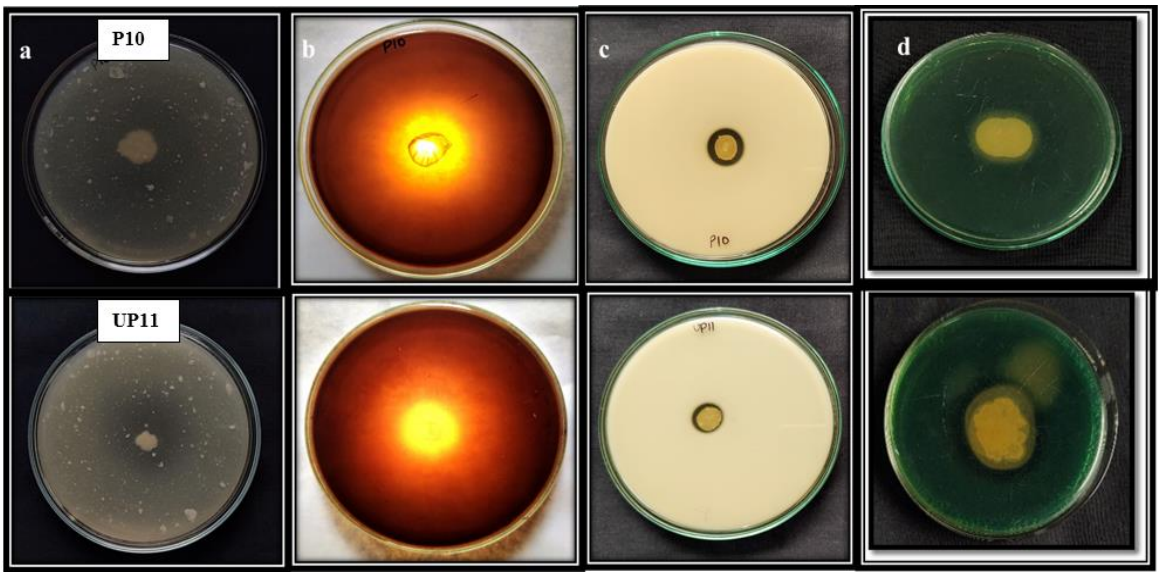
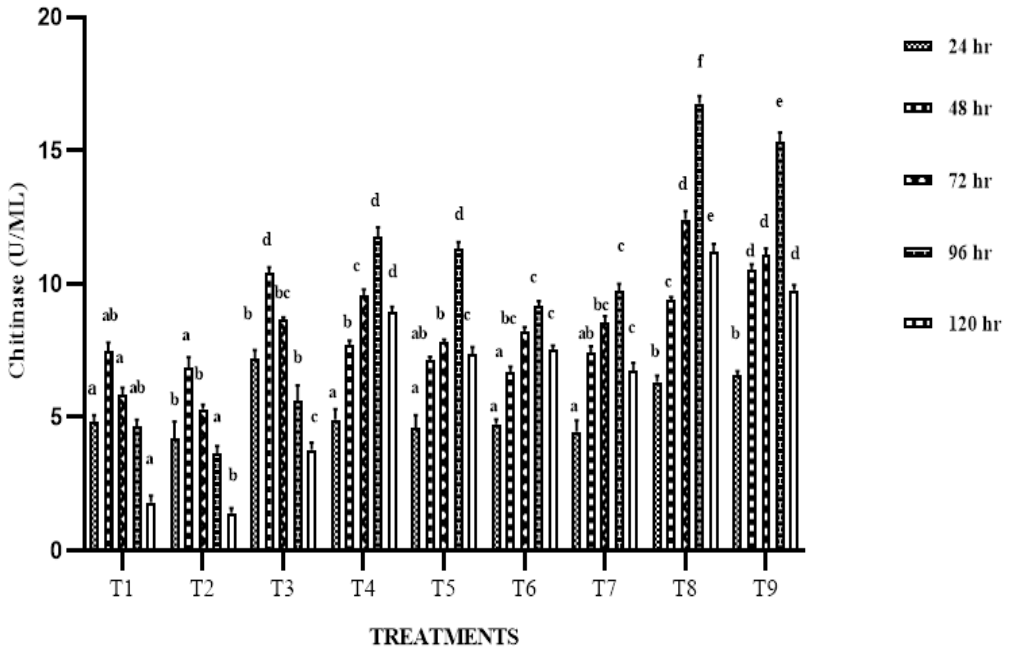
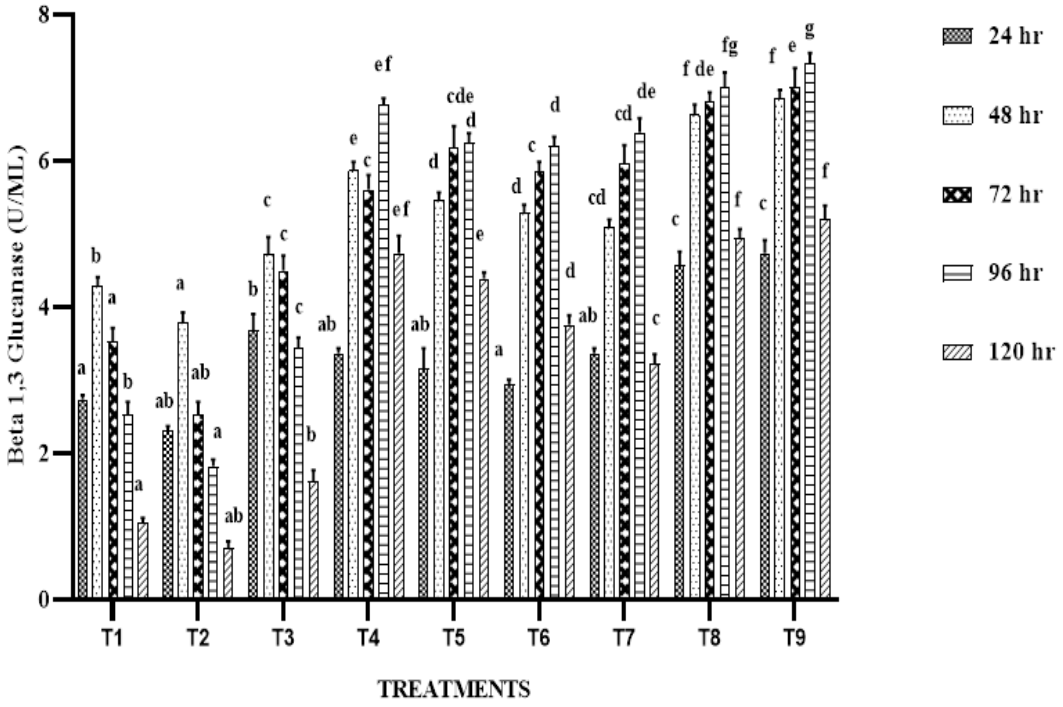


Fig. 2. Production of antagonistic properties of *Bacillus* spp. (P10 & UP11) strains (a) chitinase (b) β-1,3 glucanase (c) protease (d) siderophore

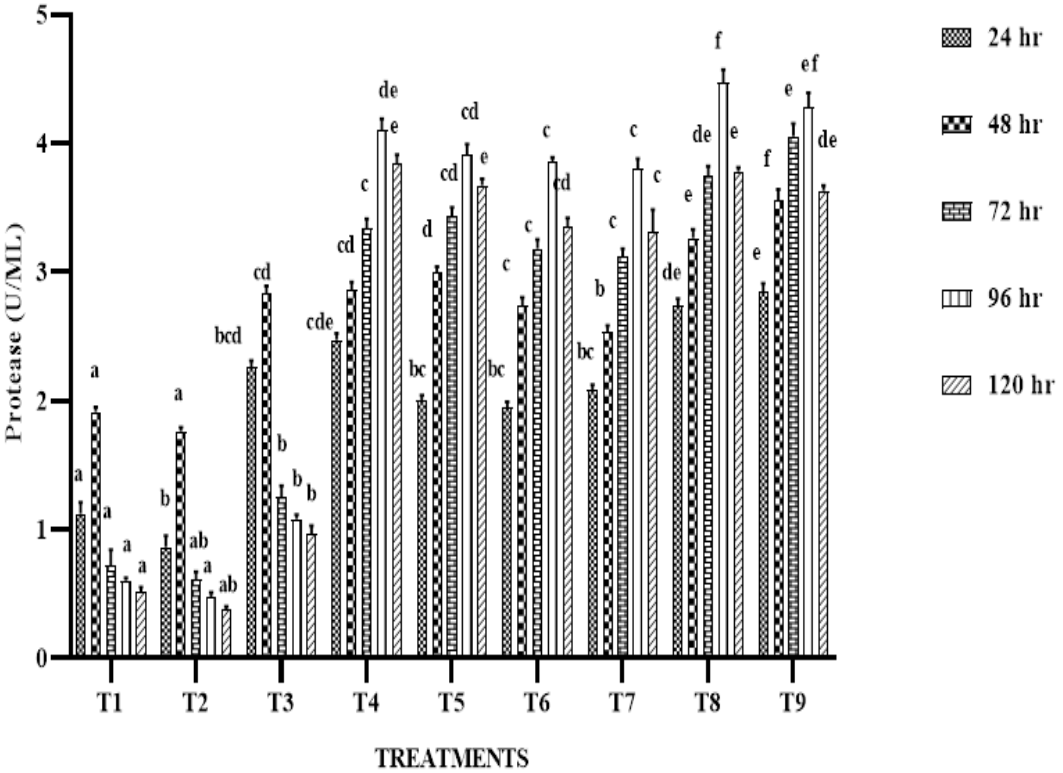
In presence of pathogens I.e., *Alternaria triticina* & *Bipolaris sorokiniana* consortium (CNS) of P10 & UP11shows the maximum chitinase at 96 hrs T8 CNS (4.47), T9 CNS (4.28) followed by the treatments T4(4.10), T5(3.91), T6(3.85), T7(3.80), T3(1.08), T1(0.60) and T2 (0.47). β-1,3-Glucanase activity (U/ML) was also found maximum in consortium T9 CNS (7.32), T8 CNS (7.04) followed by the treatments T4(6.76), T7(6.38), T5(6.24), T6(6.20), T3(3.43), T1(2.52) and T2 (1.82). The protease activity (U/ML) also found maximum in consortium T9 CNS (4.47), T8 CNS (4.28) followed by the treatments T4(4.10), T5(3.91), T6(3.85), T7(3.80), T3(1.08), T1(0.60) and T2 (0.47). the siderophore activity was expressed as in % siderophore unitsT8 CNS (77.03), T9 CNS (76.37) followed by the treatments T5(58.29), T4(55.94), T3(53.55), T6(51.24), T7(48.73), T1(39.25) and T2 (30.42) (Fig. 3).



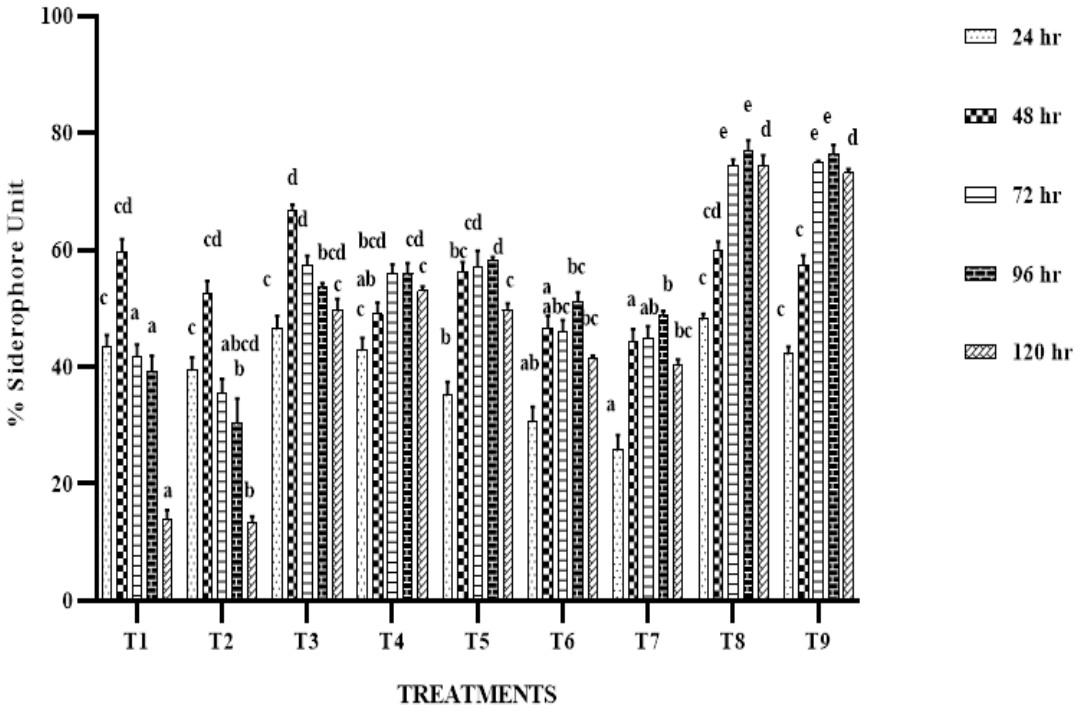
(a)


Beta 1,3 Glucanase (U/ML)
TREATMENTS
T1
T2
T3
T4
T5
T6
T7
T8
T9
24 hr
48 hr
72 hr
96 hr
120 hr

(b)


Protease (U/ML)
TREATMENTS
T1
T2
T3
T4
T5
T6
T7
T8
T9
24 hr
48 hr
72 hr
96 hr
120 hr

(c)



(d)

Fig. 3. Production of cell wall degrading enzymes in absence and presence of fungal pathogens *Alternaria triticina* (F1) &*Bipolaris sorokiniana* (F2) (a) shows effect of different time duration on chitinase activity (b) shows effect of different time duration on β-1,3-glucanase activity (c) shows effect of different time duration on protease activity (d) Siderophore production at different time duration (T1- P10 alone, T2- UP11 alone, T3- P10+UP11 (CNS) T4- P10+F1, T5- P10+F2, T6- UP11+F1, T7- UP11+F2, T8- CNS+F1, T9- CNS+F2. Values are presented as mean ± SE (n = 3)

So, this study revealed that the amount of cell walls degrading enzymes and siderophore of P10 and UP11 alone was highest in early growth phase and when both the antagonist's bacteria and pathogenic fungi are growth both in same media then it was found that the highest during the stationary phase because due to the synergetic action the antagonist's strains secretes more cell wall degrading enzymes and compete for nutrition to suppress the growth of foliar blight pathogens [25-28]. The similar results were also reported in the study by Javeria et al., [29] and Khatri et al., [30]. Additionally, antagonists often release higher amounts of proteases to inhibit the activity of pectinase and chitinase enzymes produced by various plant pathogens [31-33].

4. CONCLUSION

The present research has identified *Bacillus methylotrophicus* and *Bacillus subtilis* as potential biocontrol agents against foliar blight wheat pathogens. These bacteria utilize cell wall-degrading enzymes (CWDEs) such as chitinases, proteases, glucanases and antifungal metabolite like siderophore to disrupt the pathogen cell walls and trigger host defense responses in plants. The secretion of these enzymes and siderophores is linked to direct antagonistic mechanisms that suppress the growth of foliar blight pathogens. The finding suggests that co-culturing these antagonistic strains with pathogens enhances their synergistic effects, directly inhibiting fungal mycelial growth. This study highlights the potential of utilizing *Bacillus* Strains *Bacillus methylotrophicus* and *Bacillus subtilis* are effective biocontrol agents for suppressing foliar blight wheat pathogens.

5. FUTURE SCOPE

Further research is needed to understand the antagonistic mechanisms of P10 and UP11

against Foliar blight pathogens, along with pot and field trials to evaluate their effectiveness and compatibility with agricultural practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, for accomplishing the present research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aboukhaddour R, Fetch T, McCallum BD, Harding MW, Beres BL, Graf RJ. Wheat diseases on the prairies: A Canadian story. Plant Pathology. 2020;69(3):418-432.
2. Al-Sadi AM. Variation in resistance to spot blotch and the aggressiveness of Bipolaris sorokiniana on barley and wheat cultivars. Journal of Plant Pathology. 2016; 97-103.
3. Etesami H, Jeong BR, Glick BR. Biocontrol of plant diseases by Bacillusspp. Physiological and Molecular Plant Pathology. 2023;102048.
4. Miljaković D, Marinković J, Balešević-Tubić S. The significance of Bacillus spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms. 2020;8(7): 1037.
5. Sahgal M. Fungal enzymes in biocontrol of phytopathogens. In Progress in mycology: biology and biotechnological applications Singapore: Springer Nature Singapore. 2022;327-356.
6. Dahiya N, Tewari R, Hoondal GS. Biotechnological aspects of chitinolytic enzymes: a review. Applied microbiology and biotechnology. 2006;71:773-782.
7. Gajera HP, Vakharia DN. Production of lytic enzymes by Trichoderma isolates during in vitro antagonism with Aspergillus niger, the causal agent of collar rot of peanut. Brazilian Journal of Microbiology. 2012;43:43-52.
8. Saravanakumar K, Yu C, Dou K, Wang M, Li Y, Chen J. Synergistic effect of Trichoderma-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of Fusarium oxysporum f. sp. cucumerinum. Biological control. 2016;94:37-46.
9. Ku Y, Yang N, Pu P, Mei, Cao L, Yang X, Cao C. Biocontrol mechanism of Bacillus subtilis C3 against bulb rot disease in Fritillariataipaiensis PY Li. Front Microbiol. 2021;12:756329.
10. Guo M, Li M, Zhang C, Zhang X, Wu Y. Dietary administration of the Bacillus subtilis enhances immune responses and disease resistance in chickens. Front microbiol. 2020;11:1768.
11. Chakraborty K, Kizhakkekalam VK, Joy M, Chakraborty RD. Bacillibactin class of siderophore antibiotics from a marine symbiotic Bacillus as promising antibacterial agents. Applied Microbiology and Biotechnology. 2022;106(1):329-340.
12. Mulk S, Wahab A, Yasmin H, Mumtaz S, El-Serehy HA, Khan N, Hassan MN. Prevalence of wheat associated Bacillus spp. and their bio-control efficacy against fusarium root rot. Front microbiol. 2022;12: 798619.
13. Skidmore AM. Dickinson CH. Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Transactions of the British Mycological Society. 1976;66(1):57-64.
14. Hsu SC, Lockwood J. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Applied microbiology. 1975;29(3):422-426.
15. Sadfi N, Cherif M, Fliss I, Boudabbous A, Antoun H. Evaluation of bacterial isolates from salty soils and Bacillus thuringiensis strains for the biocontrol of Fusarium dry rot of potato tubers. Journal of Plant Pathology. 2001;101-117.
16. Aktuganov GE, Galimzyanova NF, Melent'Ev AI, Kuz'mina LY. Extracellular hydrolases of strain Bacillus sp. 739 and their involvement in the lysis of

micromycete cell walls. Microbiol. 2007; 76:413-420.

17. Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Appl Environ Microbiol. 2002;68(7):3328-3338.
18. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chem. 1959;31(3):426-428.
19. Juarez-Jimenez B, Rodelas B, Martinez-Toledo MV, Gonzalez-Lopez J, Crognale S, Gallo AM, Fenice M. Production of chitinolytic enzymes by a strain (BM17) of Paenibacillus pabuli isolated from crab shells samples collected in the east sector of central Tyrrhenian Sea. International journal of biological macromolecules. 2008;43(1):27-31.
20. Masih EI, Paul B. Secretion of β-1, 3-glucanases by the yeast Pichia membranifaciens and its possible role in the biocontrol of Botrytis cinerea causing grey mold disease of the grapevine. Current microbiology. 2002;44:391-395.
21. Takami H, Akiba T, Horikoshi K. Production of extremely thermostable alkaline protease from Bacillus sp. no. AH-101. Applied Microbiology and Biotechnology. 1989;30:120-124.
22. Jaggi V, Joshi S, Dasila H, Pareek N, Sahgal M. Functional and molecular characterization of wheat rhizosphere bacteria and their antagonistic activity against wheat foliar blight pathogens; 2020.
23. Fan B, Wang C, Song X, Ding X, Wu L, Wu H, Borriss R. Bacillus velezensis FZB42 in 2018: the gram-positive model strain for plant growth promotion and biocontrol. Frontiers in microbiology. 2018; 9:2491.
24. Ghasemi S, Safaie N. Shahbazi S, Shams-Bakhsh M, Askari H. The role of cell wall degrading enzymes in antagonistic traits of Trichoderma virens against Rhizoctonia solani. Iranian Journal of Biotechnology. 2020;18(4):e2333.
25. Firdouse M, Fahima A, Sudha I, Johnson R, Anandham N, Saranya A. Selva Amala, and S. Arunprakash. Survey and Isolation of Rice Bacterial Leaf Blight Caused by Xanthomonas Oryzae Pv. Oryzae. International Journal of Plant & Soil Science. 2023;35(19):1755-60. Available:https://doi.org/10.9734/ijpss/2023/v35i193725.
26. Jarial, Kumud, Sujata Kumari RS. Jarial, Savita Jandaik, and Deepa Sharma. "Prevalence of Bacterial Leaf Spot of Bottle Gourd and Pumpkin in Subtropical Zone of Himachal Pradesh. Journal of Scientific Research and Reports. 2020;26 (9):10-19. Available:hhttps://doi.org/10.9734/jsrr/2020/v26i930305.
27. Zhang Y, Wang XJ, Chen SY, Guo LY, Song ML, Feng H, Li C, Bai JG. Bacillus methylotrophicus isolated from the cucumber rhizosphere degrades ferulic acid in soil and affects antioxidant and rhizosphere enzyme activities. Plant and Soil. 2015;392:309-21.
28. Vicente-Hernández A, Salgado-Garciglia R, Valencia-Cantero E, Ramírez-Ordorica A, Hernández-García, A., García-Juárez, P. and *Macías-Rodríguez*, L. Bacillus methylotrophicus M4-96 stimulates the growth of strawberry (Fragaria× ananassa 'Aromas') plants in vitro and slows Botrytis cinerea infection by two different methods of interaction. Journal of Plant Growth Regulation. 2019;38:765-777.
29. Javeria S, Kumar A, Kharkwal AC, Varma A, Srinivasa N, Sharma P. Evaluation of rhizospheric Trichoderma species strains for producing cell wall-degrading and defense related enzymes in response to Fusarium oxysporum f. sp. lentis. Indian Phytopathology. 2020;73:461-467.
30. Khatri DK, Tiwari DN, Bariya HS. Chitinolytic efficacy and secretion of cell wall degrading enzymes from Trichoderma spp. in response to phyto-pathological fungi. Journal of Applied Biology and Biotechnology. 2017;5(6):1-8.
31. Giovannoni M, Gramegna G, Benedetti M, Mattei B. Industrial use of cell wall degrading enzymes: the fine line between production strategy and economic feasibility. Frontiers in bioengineering and biotechnology. 2020;8:356.
32. Radjacommare R, Venkatesan S. Samiyappan R. Biological control of phytopathogenic fungi of vanilla through lytic action of Trichoderma species and Pseudomonas fluorescens. Archives of Phytopathology and Plant Protection. 2010;43(1):1-17.
33. Reddy EC, Reddy GS, Goudar V, Sriramula A, Swarnalatha GV, Al Tawaha ARM, Sayyed RZ. Hydrolytic enzyme

producing plant growth-promoting rhizobacteria (PGPR) in plant growth promotion and biocontrol. In Secondary metabolites and volatiles of PGPR in plant-growth promotion Cham: Springer International Publishing. 2022;303-312.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/119920