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Research Article

Biocontrol Potential of Chitinase-Producing Soil Bacteria Against *Fusarium proliferatum*

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ABSTRACT

Fusarium proliferatum, a phytopathogenic fungus, causes significant agricultural losses. Chitinase-producing bacteria from post-mining soil have potential as biocontrol agents because they degrade chitin, a major component of fungal cell walls. This study aimed to isolate, characterize, and purify bacterial chitinase and evaluate its antifungal activity against *F. proliferatum*. Bacterial isolates were obtained from post-mining soil in Martabe and screened for chitinase activity using chitin agar medium. The selected isolates were identified through 16S rRNA gene sequencing, and the chitinase enzyme was partially purified using ammonium sulfate precipitation. The antifungal activity against *F. proliferatum* was evaluated using dual-culture and food-poisoning assays. Two isolates, TSU4 and TSU5, exhibited high chitinolytic activity with hydrolytic zone indices of 2.1±0.2 cm and 1.9±0.41 cm, respectively; TSU4 (*Bacillus cereus*) was selected for further study. The 70% ammonium sulfate-precipitated chitinase showed a specific activity of 15.66 U/mg, and the concentrated enzyme inhibited *F. proliferatum* growth by 33.8±0.23%. Chitinases belong to the glycoside hydrolase family 18 (GH18), an evolutionarily widespread group of chitin-degrading enzymes with a conserved catalytic domain that hydrolyzes β-1,4 linkages in chitin, making GH18 chitinases effective for degrading fungal cell walls in biocontrol applications. These findings indicate that the chitinase produced by *B. cereus* TSU4 shows significant antifungal activity and has strong potential as a biocontrol agent against *F. proliferatum*.

1. Introduction

Plant disease caused by phytopathogenic fungi such as *Fusarium proliferatum* remains a major challenge in sustainable agriculture. Although synthetic fungicides are widely used to control these pathogens, their excessive application can lead to pathogen resistance and adverse effects on non-target organisms, soil health, and the environment (Torres-Rodriguez *et al.* 2022), including toxicity to aquatic life and disruption of beneficial soil microbial functions (Xiao *et al.* 2021). Excessive reliance on synthetic fungicides has rapidly driven pathogen resistance, with classes such as Quinone Outside Inhibitors (QoIs) losing efficacy against over 20 species shortly after introduction (Brent and Hollomon 2007). Consequently, despite increasing chemical usage, global crop losses due to fungal diseases remain stagnant at 10–23%, underscoring the urgent need to transition from synthetic dependence toward sustainable alternatives (Savary *et al.* 2019). Therefore, developing sustainable alternatives is essential. Biological control using beneficial microbes, particularly bacteria such as *Bacillus* spp., has emerged as an effective and eco-friendly alternative to synthetic fungicides for managing fungal diseases (Boukaew *et al.* 2022).

In addition to promoting plant growth, beneficial bacteria can enhance plant systemic resistance to pathogen attack. These microbes secrete a range of hydrolytic enzymes, including catalase, cellulase, protease, amylase, xylanase, glucanase, and chitinase, which degrade complex organic polymers in the soil and contribute to pathogen

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suppression (Elshafie and Camele 2022). Microbial chitinases catalyze the hydrolysis of β-1,4-glycosidic linkages in chitin, a key structural polymer of fungal cell walls, leading to cell wall degradation and inhibition of fungal growth (Zhang *et al.* 2020). Numerous bacterial genera, such as *Bacillus, Streptomyces*, and *Pseudomonas*, are known to produce chitinase with potent antifungal activity (Marianah *et al.* 2024; Riseh *et al.* 2024).

Hydrolytic chitinase enzymes can break down the glycosidic linkages in chitin by hydrolyzing chitin polymers into chitin oligosaccharides or N-acetylglucosamine monomers through random hydrolysis of chitin (Akeed *et al.* 2020). Based on the amino acid sequence of glycosyl hydrolases, chitinase and N-acetylhexosaminidase are grouped into families 18, 19, and 20. Families 18 and 19 are part of endochitinases derived from various sources, including plants, viruses, insects, fungi, and bacteria (Veliz *et al.* 2017). Chitinase has been widely used in industry and agriculture. It can facilitate the bioconversion of chitin into valuable products for pharmaceuticals, single-cell proteins, waste management, biomedical applications, biotechnology, and the enzyme industry. Additionally, chitinase has other important applications, such as its efficacy as a biocontrol agent against many pathogenic fungi (Kumar *et al.* 2018).

Chitinolytic bacteria are widespread in diverse environments, such as soil, shrimp waste, water sediments, and animal digestive tracts, where they play a role in the degradation of organic matter. Chitinolytic microorganisms can degrade chitin and have been exploited as biological control against fungal pathogens. Extreme environments such as former mining sites, characterized by low pH, elevated heavy metal concentrations, and limited nutrient availability, select for stress-tolerant bacteria that may also possess antifungal activity. However, chitinase-producing bacteria such as *Bacillus, Pseudomonas,* and other taxa have been reported to exhibit significant antifungal activity against *Fusarium* spp., including *F. oxysporum* and *F. proliferatum* (Morales-Ruiz *et al.* 2021; Khairah *et al.* 2023; Putri *et al.* 2023). However, research specifically targeting chitinolytic bacteria from post-mining soils remains limited. Accordingly, this study aims to isolate and characterize chitinolytic bacteria from soils in former mining areas and evaluate their potential as antifungal agents against the fungus *F. proliferatum*.

2. Materials and Methods

2.1. Selection of Chitinolytic Bacteria

Soil samples were collected from the former Martabe Gold Mine site in South Tapanuli Regency, North Sumatra, Indonesia. Soil samples were air-dried and homogenized before isolation. 10 g of soil were suspended in 90 mL of sterile distilled water and diluted serially to 10^{-6}. Alliquots of each dilution were spread onto chitin agar plates (500 mL) containing 0.35 g K_2HPO_4, 0.15 g KH_2PO_4, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.05 g $FeSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.5 g yeast extract, 1% colloidal chitin, and 2% agar-agar. After incubation at 30°C for 48-60 h, chitinolytic activity was indicated by the formation of clear zones surrounding bacterial colonies, resulting from chitin degradation (Shahbaz and Yu 2020).

2.2. Chitinolytic Bacteria Antagonism against *F. proliferatum*

Dual-culture and food-poisoning methods were used. In the dual culture, *F. proliferatum* and the bacterial isolate were placed 3 cm apart on Potato Dextrose Agar (PDA) plates and incubated at 30°C for 7 days. For the food poisoning assay, 1 mL of bacterial supernatant was mixed with 20 mL of molten PDB (approximately 45-50°C) and then poured into petri dishes. After the medium solidified, a 6 mm plug of *F. proliferatum* was placed in the center of the plate and incubated at 30°C for 7 days (Wang *et al.* 2019).

2.3. Molecular Identification

Bacterial isolates were cultured in NB at 30°C and 120 rpm for 16 hours. Genomic DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA). DNA purity and concentration were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific 2009). The 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR) using primers 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTAC AAGG C3') (Marchesi *et al.* 1998). PCR conditions included a pre-denaturation (94°C, 4 min), 30 cycles of denaturation (94°C, 30 sec), annealing (55°C, 45 sec), elongation (72°C, 1 min, 30 sec), post-elongation (72°C, 7 min), then cooling to 4 °C. Products visualized on a 1% agarose gel (1x TAE buffer, 85 volts, 40 min) under UV transilluminator. Sequenced amplicons were aligned with GenBank information using NCBI's BLAST-N (Basic Alignment Search Tool-Nucleotide), and a phylogenetic tree was constructed using MEGA 12.0 with the Neighbor Joining and 1000 bootstrap.

2.4. Chitinase Activity and Protein Concentration Assay

Chitinase activity was measured per Spindler (1997) by mixing 225 µL of crude extract, 450 µL of 0.3% colloidal chitin, and 225 µL buffer (pH 7), and stirring at 37°C. After 30 min at 30°C, the reaction was stopped, and the mixture was centrifuged. The filtrate was mixed with 750 µL distilled water and 1,500 µL Schales reagent. After 10 min, absorbance was read at 420 nm. Protein concentration was determined by the Bradford method (1976) using the Bovine Serum Albumin standard.

2.5. Partial Amplification of the Chitinase-Encoding Gene

The chitinase-encoding gene (chi) was amplified using degenerate primers ChiF (5'-CCAAATCTCGCATCAGCAAA-3') and ChiR (5'-TCGATTAGACCAAGTCCAGC-3') targeting conserved *B. cereus* regions (Drewnowska *et al.* 2020). Thermal cycling: initial denaturation at 94°C (5 min), 30 cycles with denaturation at 94°C (30 sec), annealing at 54°C (45 sec), elongation at 72°C (40 sec), and final elongation at 72°C (10 min) (Abdel-Salam *et al.* 2018). Amplicons were separated on 1% agarose gel (w/v) using electrophoresis at 70 volts for 35 min.

2.6. Chitinase Precipitation

Precipitated crude extract with ammonium sulfate at (0-80%), chitinase was partially purified using stepwise ammonium sulfate precipitation at saturation levels of 0-20%, 20-30%, 30-40%, 40-50%, 60-70%, and 40-80%, stirred (4°C, 1 h), then stored overnight at 10°C. After centrifugation (15,000 rpm, 10 minutes, 4°C), the pellet was redissolved in 2 mL of 0.1 M phosphate buffer, pH 7 (Liang *et al.* 2014).

2.7. Chitinase Antagonism against *F. proliferatum*

The concentrated chitinase (100 µL) was added to 15 mL of PDA in sterilized petri dishes, then a plug of *F. proliferatum* mycelium was placed centrally. After 7 days of incubation, hyphal damage was examined using a scanning electron microscope (SEM). Samples were fixed with 2.5% glutaraldehyde, washed with phosphate buffer, and dehydrated through a graded ethanol series (30-100%). The dehydrated samples were dried, sputter-coated with gold, and observed using a scanning electron microscope (Wang *et al.* 2019).

3. Results

3.1. Selection of Chitinolytic Bacteria

The selection results from fifteen bacterial isolates on chitin agar demonstrated that several isolates exhibited chitinolytic activity, producing clear zones around the colonies after 48 to 60 hours of incubation (Figure 1). The highest chitinolytic index was observed in isolate TSU4 (2.1±0.2 cm), while the lowest was observed in isolate TSU8 (1.0±0.25 cm).

Seven isolates exhibiting high chitinolytic index values were selected during the screening process and are presented in Table 1. These isolates were considered the most promising candidates due to their superior chitinolytic activity, while the remaining isolates showed comparatively lower activity and were not included in further analysis. Hemolysis and hypersensitivity (HR) assay results against tested pathogens are summarized (hemolysis on blood agar indicating β, α, or γ reactions based on erythrocyte lysis patterns, and HR indicating necrosis or chlorosis response in plant tissue).

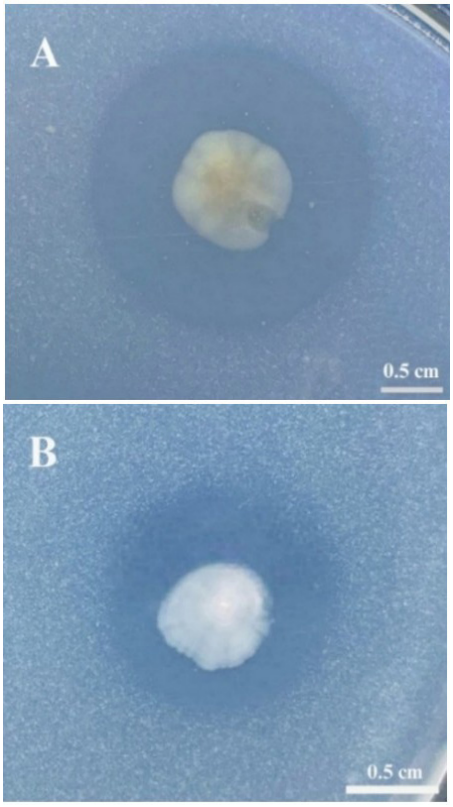

Figure 1. A clear zone developed around the colonies of isolates TSU4 (A) and TSU5 (B) on chitin agar medium after 48–60 hours of incubation at 30°C

Table 1. Results of chitinolytic index calculations for selected isolates, along with their ability to cause red blood cell hemolysis and leaf tissue necrosis

Isolate code	Chitinolytic index (cm)	Hemolysin activity	HR assay
TSU3	1.1 ± 0.05	β-hemolysis	x
TSU4	2.1 ± 0.2	γ-hemolysis	-
TSU5	1.9 ± 0.41	γ-hemolysis	-
TSU6	1.03 ± 0.15	β-hemolysis	x
TSU8	1.0 ± 0.25	β-hemolysis	x
SSB1	2.0 ± 0.1	β-hemolysis	x
SSB2	1.9 ± 0.2	γ-hemolysis	+

(-) no necrosis reaction, (+): necrosis reaction present, (x): not tested; HR: hypersensitivity response

3.2. Bacterial Cells and Culture Filtrate's Antagonistic Effects on the Expansion of *F. proliferatum* Mycelium

The dual culture assay method involves direct bacterial cell antagonism against the growth of *F. proliferatum* mycelium, demonstrating mycelium growth inhibition activities of 22.4±0.14% by isolate TSU4 and 16.1±0.07% by isolate TSU5 on day 7 after treatment. Meanwhile, inhibition using the poisoned-food technique with bacterial culture filtrates yielded mycelial growth inhibition of 28.1±0.39% by isolate

TSU4 and 23.9±0.22% by isolate TSU5. Inhibition of fungal growth from direct bacterial cells and culture filtrates was characterized by a narrowing in the mycelium area of *F. proliferatum* and empty areas on the side facing the bacterial cell culture (Figure 2).

3.3. Identification of Specific Bacterial Strains Through 16S rRNA Gene Sequences

BLAST analysis of the 16S rRNA sequences from isolates TSU4 and TSU5 showed high homology to Bacillus sp. based on query coverage and identity. The highest similarity was observed with *Bacillus cereus*, showing 99.61% sequence identity. A phylogenetic tree constructed with reference sequence from GenBank further supported this classification (Figure 3).

3.4. Partial Amplification of the Chitinase-Encoding Gene

B. cereus TSU4 was selected for chitinase (chi) gene amplification because it exhibited the highest chitinolytic activity. Chi gene amplification was conducted to assess the chitinolytic potential of the isolates. PCR and gel electrophoresis confirmed successful amplification of the chi gene in *B. cereus* TSU4 with an expected product size of ~444 bp (Figure 4).

The chi gene nucleotide sequence was determined, and its deduced open reading frame encodes a 122-amino-acid polypeptide. Alignment with reference chitinase sequences identified 20 conserved residues, including key catalytic residues aspartic acid (Asp107), aspartic acid (Asp109), and glutamic acid (Glu119), characteristic of GH18 chitinases (Figure 5). A phenetic tree based on amino acid sequences showed that the *B. cereus* isolates clustered within the GH18 chitinase clade, indicating high sequence similarity among the GH18 chitinase proteins (Figure 6).

3.5. Concentration of Chitinase Enzyme using Ammonium Sulfate

Chitinase precipitated with 70% ammonium sulfate exhibited a specific activity increase of 15.66 U/mg relative to the crude extract without precipitation (Figure 7). The purity of the precipitated enzyme increased 4.82-fold compared to the crude enzyme extract, with a yield of 14.52% (Table 2).

3.6. Characterization of Optimum Activity of Chitinase Enzyme

Chitinase from both the crude concentrated extracts of *B. cereus* TSU4 exhibited an optimum pH of 6, with activities of 0.59 U/mL and 0.74 U/mL, respectively (Figure 8A). Both extracts also showed maximal activity at 30°C, with activities of 0.64 U/mL and 0.75 U/mL (Figure 8B).

3.7. Inhibition of Concentrated Chitinase on *Fusarium proliferatum*

The concentrated chitinase enzyme from *B. cereus* TSU4 exhibited 33.8±0.23 % inhibition of *F. proliferatum* growth, exceeding the inhibitory effect of the TSU4 culture filtrate. The addition of 5% (v/v) purified enzyme to PDA markedly reduced mycelial growth compared to the control without enzyme (Figure 9).

3.8. Morphological Changes in *Fusarium proliferatum* Hyphae

SEM observations (Figure 10) revealed that *F. proliferatum* hyphae treated with antagonists and concentrated chitinase exhibited structural damage, including swelling and wrinkling, indicative of cell wall degradation, whereas untreated hyphae displayed smooth surfaces without deformities, demonstrating clear morphological differences between normal and damaged hyphae.

4. Discussion

Biological control using beneficial microbes represents a sustainable alternative to synthetic fungicides for managing phytopathogenic fungi. In this study, the isolate exhibiting the highest chitinolytic activity was identified as *Bacillus cereus*. Based on the dual culture assay, TSU4 and TSU5 significantly inhibited the mycelial growth of *F. proliferatum*, as evidenced by the clear inhibition zone observed during incubation. In addition, crude enzyme extracts from both isolates also reduced fungal growth, indicating that extracellular metabolites contribute to the antifungal activity. The strong inhibition observed in the chitinase assay suggests that hydrolytic enzymes, particularly chitinase, play a key role in degrading the fungal cell wall. This is consistent with the screening results,

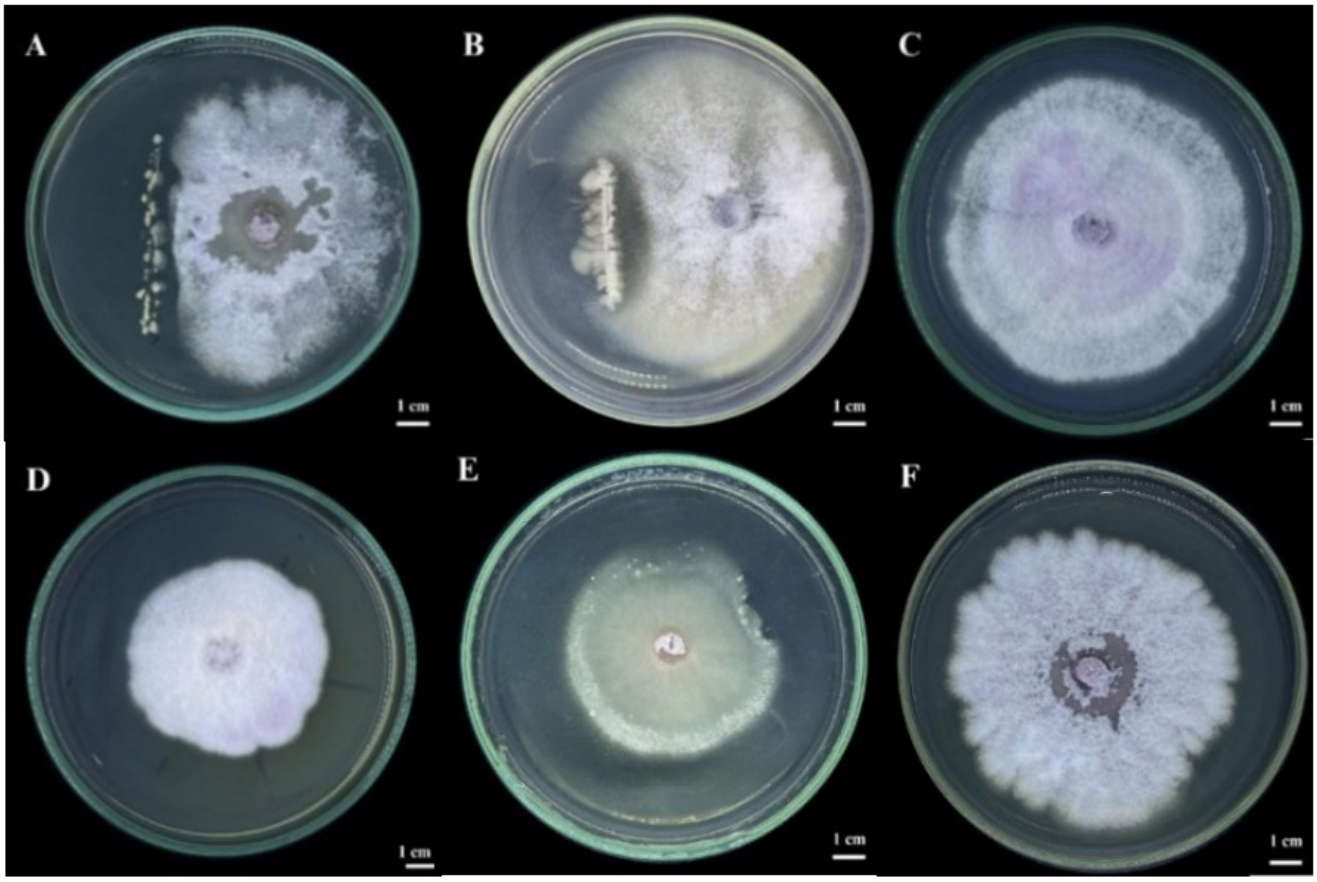

Figure 2. Growth inhibition of *F. proliferatum* by bacterial cells and culture filtrates of isolates TSU4 (A, D), and TSU5 (B, E), compared to controls (C, F) on PDA after 7 days at room temperature

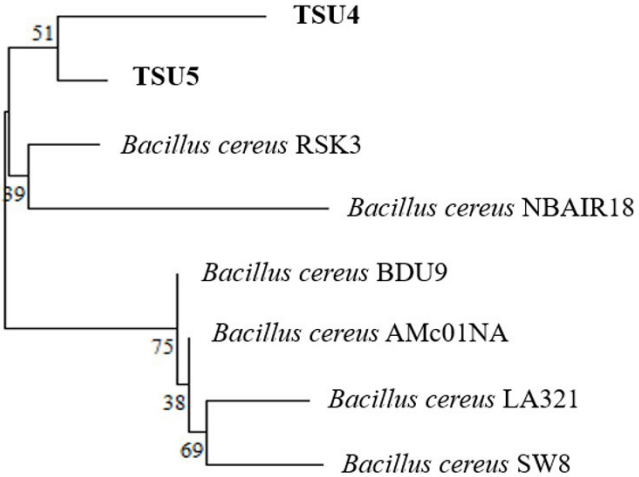

Figure 3. Construction of a phylogenetic tree from 16S rRNA gene sequences of chosen bacterial isolates using the Neighbour Joining method with 1000 times bootstrapping

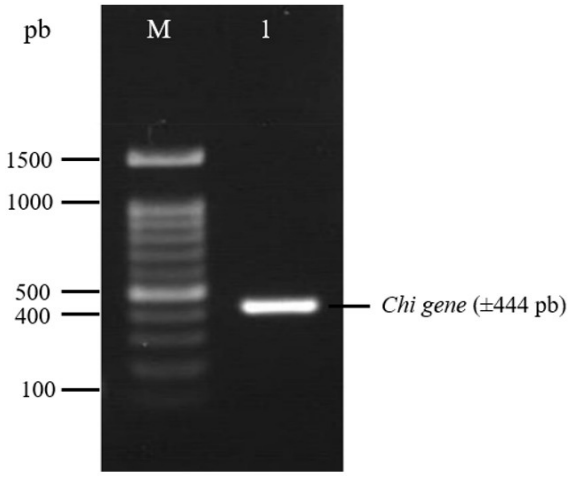

Figure 4. Visualization of the chi gene amplicon on a 1% agarose gel. (M) 1 kb DNA marker, (1) *B. cereus* TSU4

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B.cereus WP_242136560.1          -------------------------------------------FPSWGIYGRNYQVADI   16
B.cereus TSU4                    ----------------------------ISKFTKAKSKNCWVLSSWGVYGRNYQVADI   30
Bacillus sp. WP_149237685.1      ---------------------------------KQGQKIVGYFPSWGIYGRNYQVADI   25
B.cereus WP_336967155.1          ---------------------------------KQSQKIVGYFPSWGVYGRNYQVADI   25
B.cereus SPT86213.1              ---------------------------------KQSQKIVGYFPSWGVYGRNYQVADI   25
B.toyonensis MDT3497571.1        -------------------------------------------FPSWGIYGRNYQVADI   16
                                                                             : ***:**********

B.cereus WP_242136560.1          DASKLTHLNYAFADICWNGRHGNPSTHPDNPNKQTWPCKESSVPLQNKDVPDGTLVLGEP   76
B.cereus TSU4                    DASKLTHLNYAFADICWNGKHGNPSTHPDNPNKQTWNCKESGVPLQNKEVPNGTLVLGEP   90
Bacillus sp. WP_149237685.1      DASKLTHLNYAFADICWNGKHGNPSTHPDNPNKQTWNCKESGVPLQNKEVPNGTLVLGEP   85
B.cereus WP_336967155.1          DASKLTHLNYAFADICWNGKHGNPSTHPDNPNKQTWNCKESGVPLQNKEVPNGTLVLGEP   85
B.cereus SPT86213.1              DASKLTHLNYAFADICWNGKHGNPSTHPDNPNKQTWNCKESGVPLQNKEVPNGTLVLGEP   85
B.toyonensis MDT3497571.1        DASKLTHLNYAFADICWNGKHGNPSTHPDNPNKQTWNCKESGVPLQNKEVPNGTLVLGEP   76
                                 ******************:**************** ****:******:**:********

B.cereus WP_242136560.1          WADVNKSFPGSGTTWEDCDKYARILNFGELKR    108
B.cereus TSU4                    WADVTKSYPGSGTTWEDCDKYAHCGNFGELKR    122
Bacillus sp.WP_149237685.1       WADVTKSYPGLGTTWEDCDKYARCGNFGELKR    117
B.cereus WP_336967155.1          WADVTKSYPGSGTTWEDCDKYARCGNFGELKR    117
B.cereus SPT86213.1              WADVTKSYPGSGTTWEDCDKYARCGNFGELKR    117
B.toyonensis MDT3497571.1        WADVTKSYPGSGTTWEDCDKYARCGNFGELKR    108
                                 ****:**:** **********   *******
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Figure 5. Partial amino acid alignment of chi with reference sequences. Red denotes essential amino acids within the catalytic domain, and yellow denotes conserved residues among *B. cereus, Bacillus* sp., and *B. toyonensis*

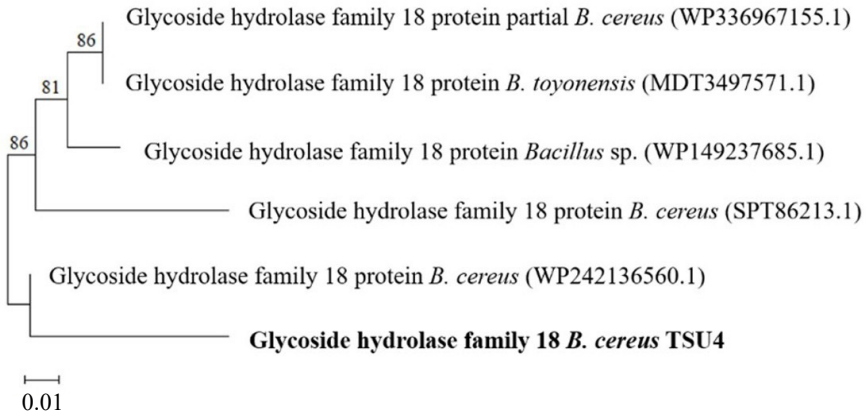

Figure 6. Phenetic tree based on chi gene sequences

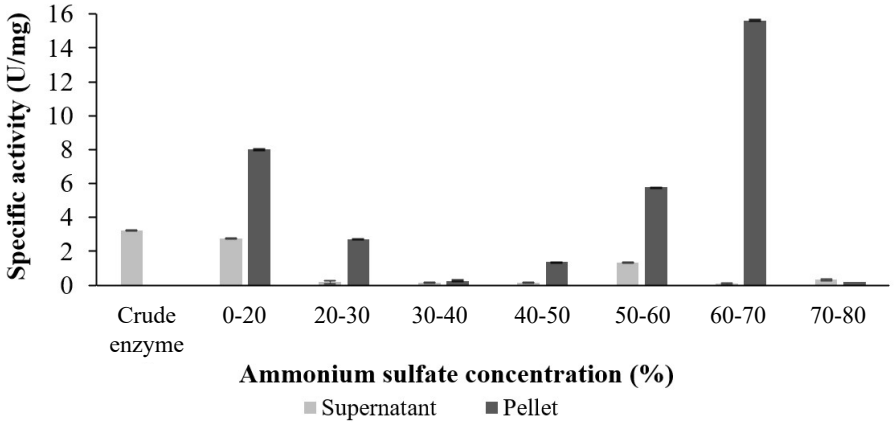

Figure 7. Chitinase activity from the *B. cereus* TSU4 isolate was assessed across a 0-80% ammonium sulfate saturation range

Table 2. Summary of the enzyme activity following ammonium sulfate precipitation from the *B. cereus* TSU4 isolate

Purification step	Volume (mL)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Total activity (U)	Total protein (mg)
Crude enzyme	100	40	12.3	3.252	1	100
Ammonium sulfat 70% (w/v)	7	5.81	0.371	15.66	4.82	14.525

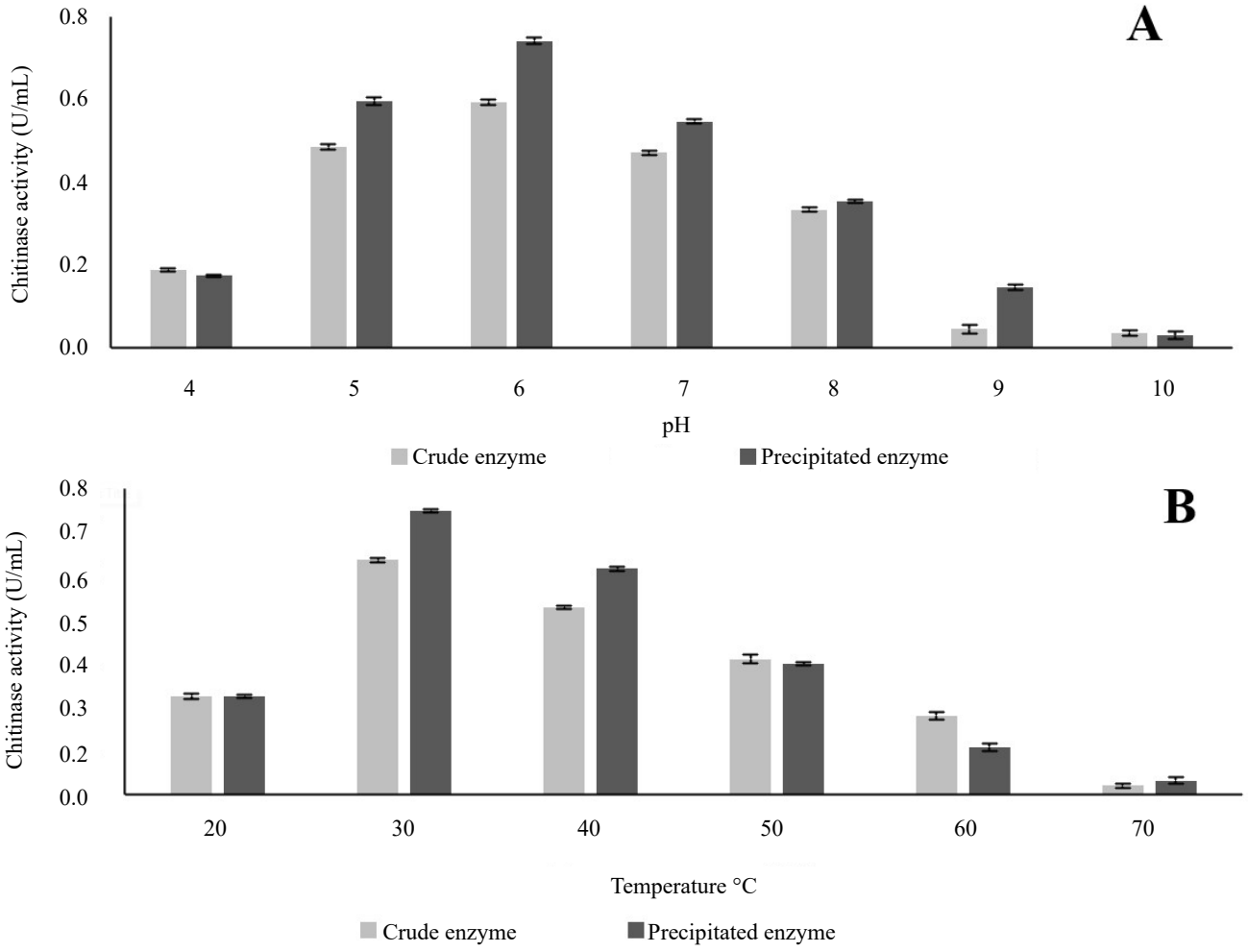

Figure 8. Chitinase activity from the *B. cereus* TSU4 isolate was evaluated over a pH range of 4-10 (A) and a temperature range of 20-70°C (B)

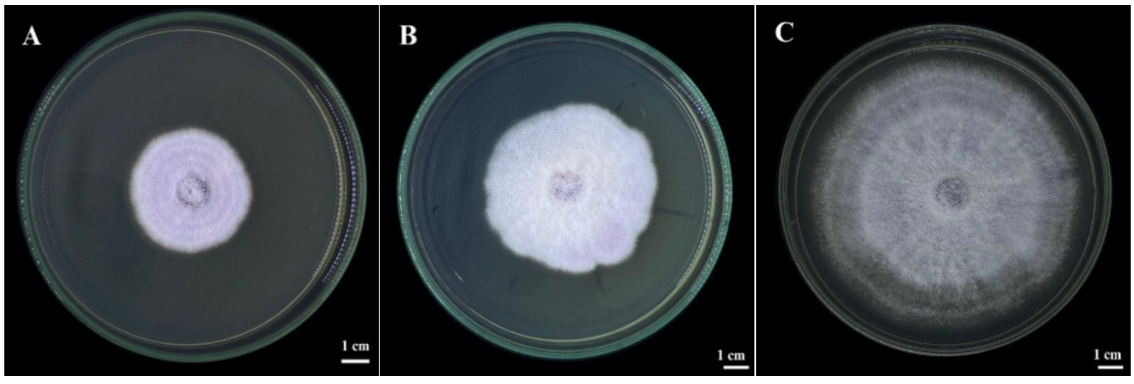

Figure 9. Inhibition of *F. proliferatum* growth on PDA after 7 days at room temperature by concentrated chitinase (A), non-concentrated chitinase filtrate (B), and untreated control (C)

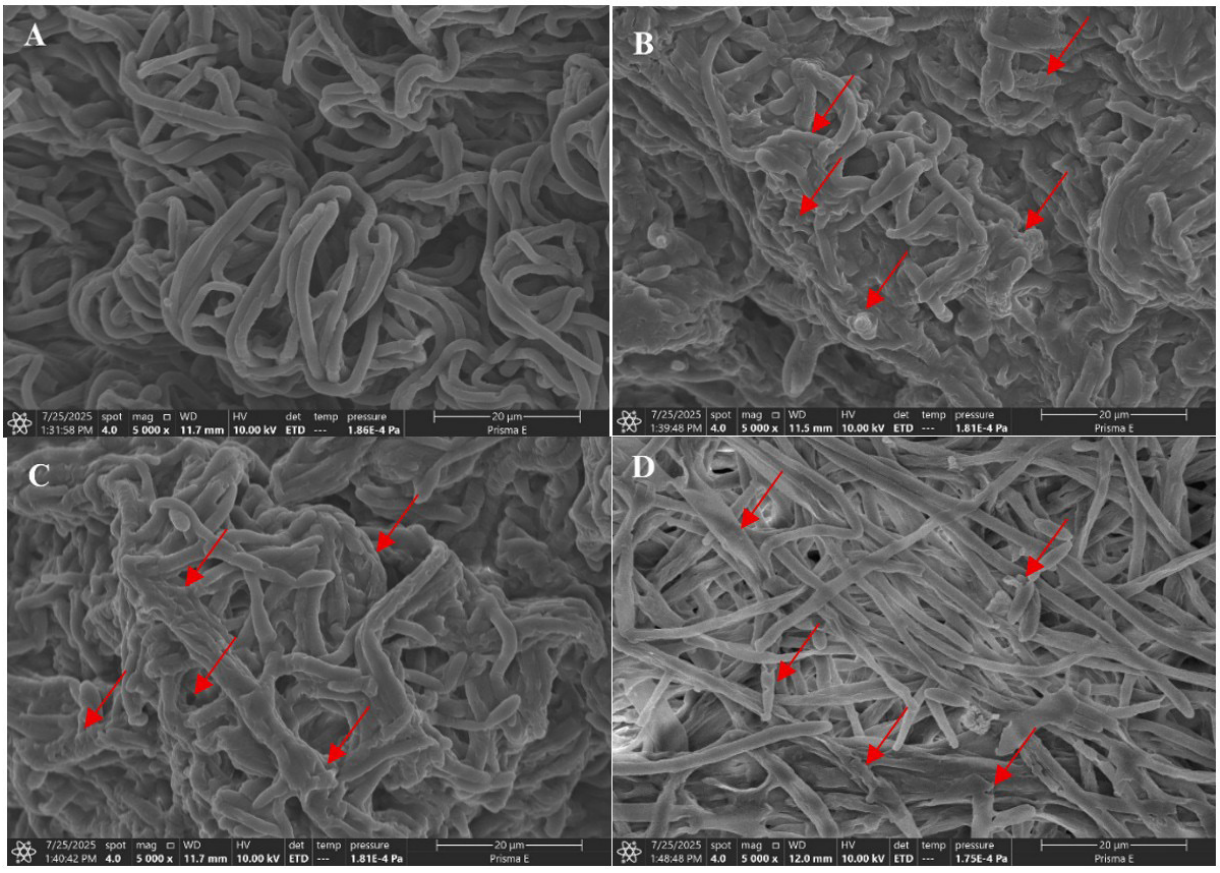

Figure 10. Microscopic observation of *F. proliferatum* hyphal morphology on PDA after 7 days at room temperature: untreated chitinase (A), direct cells *B. cereus* TSU4 (B), concentrated chitinase (C), and positive control with synthetic antifungal ketoconazole (D)

which showed that TSU4 had the highest chitinolytic index, supporting its superior antifungal potential. Similar inhibitory effects of *Bacillus* spp. against *Fusarium* spp. have been reported under *in vitro* conditions (Iqbal *et al.* 2024). Furthermore, the observed antifungal activity may also involve additional metabolites, such as lipopeptides. For example, fengycin produced by *Bacillus* species has been shown to disrupt fungal membranes and cells (Mei *et al.* 2024), which may explain the enhanced inhibition observed in the crude extract treatment.

TSU4 was selected for further study due to its superior overall performance relative to TSU5, as evidenced by its higher activity and greater consistency across experimental replicates. Chitinase enzyme activity in isolate *B. cereus* TSU4 is supported by the detection of the chi gene in the genomes of these bacterial isolates. This activity is supported by the presence of the chitinase (chi) gene in isolate *B. cereus* TSU4. The chi gene is included in the protein group glycoside hydrolase family 18 (GH18). The catalytic domain of GH18 exhibits a $(\beta/\alpha)_8$ fold that places the conserved catalytic motif DxDxE at the base of the substrate groove. The glutamate residue in this motif acts as a proton donor in the β-1,4 glycosidic bond cleavage step (Chen *et al.* 2018). GH18 chitinases exhibit several advantages, including high catalytic efficiency, a deep substrate-binding cleft, and the ability to degrade crystalline chitin, which enhances their effectiveness against fungal pathogens. In addition, GH18 enzymes play a crucial role in biocontrol through efficient chitin degradation and antifungal activity, making them more advantageous than other chitinase families in biocontrol applications (Ezzine *et al.* 2024).

Ammonium sulfate precipitation is a widely used method for purifying bacterial enzymes. The precipitated enzyme from the TSU4 isolate exhibited higher specific activity than the bacterial supernatant, indicating that precipitation at 70% (w/v) effectively removed interfering proteins and other substances. This result is consistent with previous reports that the optimal ammonium sulfate concentration for chitinase purification generally falls within the 60-70% range (Meruvu and Meruvu 2019). Another study reported that chitinase from *B. cereus* BSH-4 showed peak activity of 143.68 U/mL at 80% ammonium sulfate saturation (Hungund *et al.* 2022).

Ammonium sulfate precipitation at 60–80% is commonly used to obtain chitinase fractions with optimal purity and activity (Akeed *et al.* 2020).

Chitinase from the *B. cereus* TSU4 isolate exhibited optimal activity at pH 6 and 30 °C, indicating its preference for slightly acidic conditions and moderate temperature. This optimal condition suggests that the enzyme produced by TSU4 is well adapted to environments commonly associated with fungal growth. The observed optimum at pH 6 is comparable to that reported for chitinase from *B. cereus* C-13 (Suganthi *et al.* 2020),whereas that from NK91 shows optimum activity at pH 7 (Thakur 2022). Both temperature and pH significantly influence enzymatic activity, as suboptimal conditions can alter enzyme conformation, disrupt active site structure, and reduce catalytic efficiency by affecting substrate binding and protein stability (Egilmez and Haspolat 2024). These findings are consistent with previous reports, such as those for chitinase from *B. licheniformis* B307, which also shows optimal activity at pH 6 and 30°C (Akeed *et al.* 2020). Other studies have reported that chitinase enzymes typically exhibit optimal activity at temperatures between 30–60 °C and pH levels of 6-8 (Pamungkas *et al.* 2023; Govindaraj *et al.* 2025). Both temperature and pH significantly influence enzymatic activity, as inappropriate conditions can alter enzyme structure and reduce activity by affecting substrate binding and protein stability (Robinson 2015). These findings demonstrate that the enzymatic activity of TSU4 is strongly influenced by environmental conditions, particularly pH and temperature, which directly affect enzyme stability and substrate interaction.

Ammonium sulfate precipitation significantly enhanced the specificity and potency of the purified enzyme, enabling lower effective dosages and improved biocontrol efficacy of chitinase against fungal growth. Purification of chitinase from *B. subtilis* TV-125A resulted in substantially greater antifungal activity against *F. culmorum* than the crude homogenate, with 35% inhibition (Senol *et al.* 2014). Mechanistically, this inhibition is mediated by the hydrolysis of β-1,4-N-acetylglucosamine linkages in fungal cell wall chitin, leading to weakening of the hyphal cell wall, subsequent hyphal swelling, and eventual hyphal lysis (Ekundayo *et al.* 2022).

Damage to the hyphal cell wall of *F. proliferatum* observed in this study is attributed to chitinase produced by *B. cereus* TSU4, as chitin is a key structural component of the fungal cell wall. Microscopic observations revealed hyphal deformation, swelling, and disruption following treatment with concentrated chitinase, indicating the strong antifungal activity of TSU4. These findings are consistent with previous reports showing that chitinase from *Bacillus* spp. caused structural damage to fungal hyphae, leading to cell wall lysis and growth inhibition (Dukare *et al.* 2020; Morales-Ruiz *et al.* 2021). Furthermore, *F. proliferatum* hyphae treated with TSU4-derived chitinase exhibited morphological changes comparable to those induced by ketoconazole, suggesting a similar inhibitory effect on fungal growth. This indicates that the chitinase produced by TSU4 plays a significant role in disrupting fungal cell wall integrity, thereby inhibiting hyphal development.

Although *B. cereus* is recognized as an opportunistic pathogen, its safety as a biocontrol agent depends on the specific strain. Several studies have demonstrated that certain *B. cereus* strains function as beneficial plant-associated bacteria and can be safely utilized in agricultural applications when lacking virulence factors and toxin-producing genes (Zhao *et al.* 2024). In addition, *Bacillus* spp. are widely regarded as safe and effective microbial inoculants due to their stability, adaptability, and ability to promote plant growth (Gharsallah *et al.* 2025). Therefore, appropriate strain selection and biosafety evaluation are essential to ensure safe application.

In conclusion, *B. cereus* TSU4 chitinase, particularly belonging to the GH18 family, showed enhanced inhibitory activity against *F. proliferatum* after partial purification. The biocontrol effects involved hyphal cell wall damage, supported by SEM observations. These findings indicate the potential of GH18 chitinase from *B. cereus* TSU4 as a biocontrol agent against *F. proliferatum* fungi. Despite the promising findings, this study has several limitations. The experiments were conducted under controlled laboratory conditions, which may not fully reflect field performance. In addition, only selected isolates with high chitinolytic activity were further analyzed.

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