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Bacterial selection and characterization of chitinase enzyme from bacteria controlling *Fusarium proliferatum*

MIFTAHUL KHAIRAH[1], NISA RACHMANIA MUBARIK[2,♥], LISDAR A. MANAF[3]

[1]Program of Microbiology, Graduate School, Institut Pertanian Bogor. Jl. Raya Dramaga, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel./Fax.: +62-251-8622833, ♥email: nrachmania@apps.ipb.ac.id
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

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Abstract. *Khairah M, Mubarik NR, Manaf LA. 2023. Bacterial selection and characterization of chitinase enzyme from bacteria controlling* Fusarium proliferatum. *Biodiversitas 24: 1926-1933.* The phytopathogenic fungus *Fusarium proliferatum* causes wilt rot disease of onion bulbs and many agricultural losses. The biocontrol of *F. proliferatum* can be approached with chitinase from microorganisms, such as endophytic bacteria. The chitinase enzyme can inhibit and control fungus growth by degrading chitin, the main component of the fungal cell wall. This study aimed to select, characterize, and semi-purifying the chitinase produced by endophytic bacteria and to evaluate its antifungal activity against *F. proliferatum*. The results showed ABS 4.1.2 isolate produced chitinase and had antagonistic activity against *F. proliferatum*. The prospective ABS 4.1.2 isolate was identified based on 16S rRNA gene as *Pseudomonas aeruginosa.* The precipitated chitinase using 60% ammonium sulfate (w/v) showed a specific activity of 19.69 U/mg and increased the purity by 3.60 fold. SDS-PAGE analysis showed that chitinase had an estimated molecular weight of 32 kDa and 65 kDa. The chitinase activity of crude extract and precipitated chitinase were optimum at pH 7 and the temperature of 35°C and 45°C, respectively. The precipitated chitinase showed a higher inhibition compared to 12 hrs of cell culture and crude enzymes against *F. proliferatum*. The precipitated chitinase and crude enzymes significantly exhibited destructive activity toward *F. proliferatum* mycelium.

Keywords: Biocontrol, chitinase, *Fusarium proliferatum*, *Pseudomonas aeruginosa* ABS 4.1.2

Abbreviations: BLAST: Basic Local Alignment Search Tool; BSA: Bovine Serum Albumin; DNA: Deoxyribose Nucleic Acid; IPBCC: IPB Culture Collection; LB: Luria-Broth; NB: Nutrient Broth; NCBI: National Center of Biotechnology Center; NFW: Nuclease-Free Water; NJ: Neighbor-Joining; PCR: Polymerase Chain Reaction; PDA: Potato Dextrose Agar; rRNA: ribosomal-Ribose Nucleic Acid; SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

INTRODUCTION

Onion (*Allium cepa* L.) is a vegetable commodity that has important meaning for society in terms of nutritional and economic value, so demand will continue to increase (Wulandari and Hidayat 2016). Although the average consumption of onion each year increases, the production level tends to decrease. Onion production in Indonesia generally increased by from 2013 to 2018 by 1.5 tons, but in 2015 decreased by 0.39% compared to 2014 (BPS 2018). The phytopathogenic fungus attack is still one of the problem to increasing onion production, mainly caused by *Alternaria* and *Fusarium* (Basuki 2016).

Fusarium proliferatum is widely known as a phytopathogenic fungus that attacks more than 25 plants, including onion and garlic, and can produce mycotoxins (Stankovic et al. 2007; Yamazaki et al. 2013; Avasthi et al. 2018). Diseases caused by *F. proliferatum* are rot and wilting of onions and attack both the growth and harvest phases (Proctor et al. 2010; Le et al. 2021). Primary infection of *F. proliferatum* occurs when the fungus penetrates directly into roots and becomes necrotic. Roots became semi-transparent, shrunken, and rot. When the disease symptoms become severe, the entire bulb could rot, and shrunken tissue was observed on the bulb (Stankovic et al. 2007).

Management of this pathogen becomes substantial because of the large number of losses caused by *F. proliferatum.* To control this disease, synthetic fungicides such as carbendazim, bavistin, benomyl, and thiram are commonly used to control *Fusarium* and other soil-pathogenic fungi (Le et al. 2021). However, several studies show that excessive fungicide usage influences harmful residues in the ecosystem affects non-target microorganisms, and induces resistance in phytopathogenic strains (Villarreal-Delgado et al. 2018; Dukare et al. 2019). Thus, environmentally friendly technologies in reducing the use of synthetic fungicides in agriculture are highly desired.

Microbial antagonists are widely used as biocontrol of many plant diseases caused by phytopathogens. Among them, endophytic bacteria are widely known as one of the plant biocontrols by producing siderophores, antifungal compounds, and various hydrolytic enzymes, such as

chitinase (Yu et al. 2011; Saber et al. 2015). Chitinase is an enzyme capable of hydrolyzing chitin into N-acetylglucosamine (Herdyastuti et al. 2010). Chitinase can be used as a biocontrol agent for plant diseases caused by phytopathogenic fungus by lysing chitin, the main component of the fungal cell wall. Previous studies have demonstrated the utilization of endophytic bacterial chitinase as a biocontrol of plant diseases caused by pathogenic fungi (Seo et al. 2012; Singh and Gaur 2016; Hamaoka et al. 2021). A study by Kumar (2022) showed that *Pseudomonas stutzeri* isolated from *Withania somnifera* produced chitinase enzyme and suppressed the growth of *Fusarium oxysporum* and *Rhizoctonia solani*. *Serratia marcescens* was isolated from the rubber tree and also produce chitinase and was involved in the antifungal mechanism against *F. oxysporum* f.sp. *cubense* (Tan et al. 2015). However, studies on the utilization of onion endophytic bacterial chitinase in inhibiting the growth and control of *F. proliferatum* have never been reported. Therefore, research on fungal cell wall-degrading enzymes as a biocontrol agent has become an essential issue.

This paper describes the biocontrol properties of onion endophytic bacteria against the phytopathogenic fungus, *F. proliferatum in vitro*. Its chitinolytic activity was evaluated through semi-purification and characterization of chitinase produced by the onion endophytic bacterium for enzyme specification and bacterial biocontrol properties.

MATERIALS AND METHODS

Procedures

Microbial isolate

Bacterial isolates ABS 4.1,2, ABP 5.2.2, ABP 5.1, and BBP 5.21 isolated from onion endophytic bacteria collection of IPB Culture Collection (IPBCC) were grown on Nutrient Agar (NB) media and incubated at room temperature. *F. proliferatum* was isolated from rot and wilting of onions, maintained on Potato Dextrose Agar (PDA), and used in an antagonistic assay (Murtado et al. 2020).

Screening of F. proliferatum *growth inhibiting bacteria*

Screening of bacteria inhibiting the growth of *F. proliferatum* was performed using the dual culture method (Haidar et al. 2016). One plug of *F. proliferatum* with a diameter of 10 mm was transferred to the PDA media at a distance of 3 cm from the edge of the petri dish. Bacterial isolates were streaked on PDA media at 3 cm from the fungus and incubated for 10 days at ± 28°C. The percentage of inhibition of bacteria on the growth of *F. proliferatum* was calculated using the formulation of Fokkema et al. (1973):

$$\text{Inhibition of Radial Growth (\%)} = \frac{R1 - R2}{R1} x100$$

Where:
R1 : the mycelium radius control (mm)
R2 : the mycelium radius toward the bacteria (mm)

Bacterial isolates showing inhibitory activity against *F. proliferatum* were measured for their chitinase activity by growing the isolates in a liquid chitin medium and incubating them at 37°C at 120 rpm for 72 hours. The cell culture was then centrifuged for 15 minutes at 13,000 xg. The supernatant obtained was an enzyme crude extract which was measured for its chitinase activity. The isolate with the best chitinase activity was designated as the selected isolate (Asril et al. 2014).

Identification of selected bacterial isolates based on the 16S rRNA gene

Bacterial isolates were cultured on Luria-Broth (LB) medium for 16 hours in a shaking incubator at 120 rpm at 30°C. The genomic DNA was extracted regarding the PrestoTM Mini gDNA Bacteria Kit and amplified by the 16S rRNA gene using a Polymerase Chain Reaction (PCR) machine with primers 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3') with a target fragment of ± 1300 bp (Marchesi et al. 1998).

The PCR conditions used were as: pre-denaturation (94°C for 1 minute), annealing (55°C for 55 seconds), elongation (72°C for 1 minute 30 seconds), and extension (72°C for 10 minutes) for 35 cycles. The PCR product was visualized using an electrophoresis machine on a 1% (w/v) agarose gel added with 3 µL peqGreen DNA dye (PeqLab, Germany) by electrophoresis at 70 volts for 40 minutes. The nucleotide base sequences were aligned with the GeneBank data using the BLAST-N (Basic alignment Search Tool-Nucleotide) program on the NCBI (National Center for Biotechnology Information) online site. Phylogenetic tree construction was carried out using the Mega 10.0 program with the Neighbor-Joining (NJ) method with bootstrap 1000.

Determination of the optimum production of bacterial chitinase

Two loopful of selected bacterial isolates were grown on nutrient broth media containing 0.3% colloidal chitin and then incubated for 15 hours at 120 rpm, 37°C. Approximately 1% inoculum culture (10^8 cells/mL) was inoculated into 100 mL nutrient broth which was added with 0.3% colloidal chitin as production medium and incubated in an incubator shaker at 37°C at 120 rpm. Cultures were collected every 6 h until 54 h and optical density were measured spectrophotometrically at 600 nm. The same cell culture was centrifuged for 15 minutes at 13,000 xg at 4°C. The supernatant obtained was a crude enzyme measured for its chitinase activity.

Determination of chitinase enzyme activity and protein content

Chitinase activity was measured using the method as given by Spindler (1997). The crude extract of the extracellular enzyme was added to 450 µL of 0.3% colloidal chitin and 225 µL of 0.1 M phosphate buffer at 37°C, pH 7.0, and agitated at 120 rpm. The mixture was incubated at 30°C for 30 minutes. The mixture was centrifuged at 10,000 rpm for 15 minutes, and 750 µL of

distilled water and 1500 µL of Schales reagent (K-ferricyanide and 0.5 M Na_2CO_3) were added to the filtrate, and the mixture was heated at 100°C for 10 minutes. Chitinase activity was determined by measuring the absorbance at 420 nm and using GlcNAc as standard. One unit of enzyme activity is defined as the amount of enzyme that produces 1 µmol N-acetylglucosamine per minute. The Bradford method (1976) measured protein concentration using Bovine Serum Albumin (BSA) as a standard.

Ammonium sulfate precipitation

Precipitation of the chitinase enzyme was carried out with ammonium sulfate (Scopes 1994). The free cell supernatant was precipited with ammonium sulfate in the range of 0-80%. The addition was accompanied by stirring for 1 hour at 4°C, then stored in a centrifuge tube overnight at 10°C and centrifuged at 15,000 g for 15 minutes at 4°C. The precipitated was dissolved in 0.1 M phosphate buffer (pH 7.0), and the chitinase activity and protein content were measured.

Electrophoresis

Electrophoresis was performed using Sodium Dodecyl Sulfate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) by the method of Laemmli (1970) with 4% acrylamide concentration in the stacking gel and 10% acrylamide in the separation gel. After electrophoresis, gels were stained with Coomassie brilliant blue G-250.

Characterization of chitinase activity

Determination of the optimum pH and temperature of chitinase enzyme activity using the Asril et al. (2014). Measurement of the optimum pH of crude enzyme and precipitated chitinase in the pH range (4.0-10.0) at the highest production time using 0.3% colloidal chitin as a substrate in a buffer. The buffers used were 0.1 M citrate buffer (pH 4.0-6.0), 0.1 M phosphate buffer (pH 7.0-8.0), and 0.1 M glycine-NaOH buffer (pH 9.0-10.0). Determination of the optimum temperature of chitinase activity by testing the crude enzyme and precipitated chitinase at the optimum pH with a temperature range of 25°C-60°C (5°C interval).

Inhibition of chitinase against Fusarium proliferatum

Inhibition of chitinase against *F. proliferatum* using cell culture, crude enzymes, and precipitated chitinase was observed by agar well diffusion method (Melent'ev et al. 2001). Approximately 100 µL of cell culture, crude extract enzymes, and precipitated chitinase were placed in wells 3 cm from the edge of the petri dish containing PDA media. The mycelium of the pathogenic fungus *F. proliferatum* was placed 3 cm from the well and then incubated at ± 28°C for 10 days (Asril et al. 2014). The inhibition growth (%) was calculated using the Fokkema method (1973).

Effect of chitinase enzyme on the mycelial morphology of F. proliferatum

Microscopic observation was carried out by observing the tip of the mycelium in the inhibition zone of *F. proliferatum* using a light microscope (Leica EC4 camera microscope). The tip of the mycelium of *F. proliferatum* was taken and placed on a glass slide. Abnormal mycelial growth and morphology of *F. proliferatum*, such as lysis, swelling, and distortion of mycelium, were observed (Lorito et al. 1992).

Data analysis

Data of enzyme activity was obtained from three replicates and analyzed using Microsoft Excel 2019 for standard deviation and standard error.

RESULTS AND DISCUSSION

Screening of *F. proliferatum* growth inhibiting bacteria

The screening results showed that there were 3 isolates, viz. ABS 4.1.2, ABP 5.2.2, and ABP 5.1, which found to inhibit the growth of *F. proliferatum* (Table 1). Isolates ABS 4.1.2 had inhibition percentage and specific chitinase activity higher than other isolates, so ABS 4.1.2 isolate were chosen for further test.

Identification of selected bacterial isolates based on the 16S rRNA gene

The selected bacteria capable of inhibiting the growth of *F. proliferatum* were identified molecularly based on the 16S rRNA gene. The 16S rRNA gene sequence alignment showed that ABS 4.1.2 isolate had a 99.68% similarity with *Pseudomonas aeruginosa* DSM 50071 (Figure 1).

Determination of the optimum production of bacterial chitinase

Pseudomonas aeruginosa ABS 4.1.2 grew on nutrient broth media and was added with 0.3% colloidal chitin at 37°C and pH 7.0. The growth of bacterial isolates increased from 0 to 30 h and tended to be stable up to 54 h. Chitinase produced increased with optimum activity at 12 h of incubation with the specific activity of chitinase of 6.89 U/mg and decreased after 24 h of incubation (Figure 2).

Table 1. Screening of *F. proliferatum* Growth Inhibiting Bacteria

Isolates code	Inhibition zone (%) ± SD	Specific chitinase activity(U/mg) ± SD
ABS 4.1.2	44.44 ± 0.0	5.76 ± 0.05
ABP 5.2.2	41.11 ± 1.57	5,63 ± 0.07
ABP 5.1	40.74 ± 1.28	5.40 ± 0.0
BBP 5.21	0	0

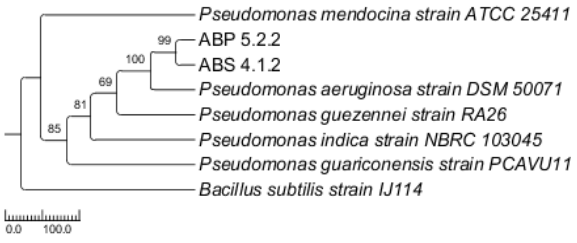

Figure 1. Phylogeny tree analysis of 16S rRNA gene sequences of potential isolate ABS 4.1.2. *Bacillus subtilis* strain IJ114 was used as an outgroup for this analysis

Ammonium sulfate precipitation

The results of the precipitation of the chitinase enzyme with ammonium sulfate showed that the crude enzyme *P. aeruginosa* ABS 4.1.2 was able to be precipitated at a concentration of ammonium sulfate 60% (w/v) with a total specific activity of 19.69 U/mg and increased its purity to 3.60 fold (Table 2).

Electrophoresis

Molecular weight chitinase from crude enzyme and ammonium sulfate precipitation *P. aeruginosa* ABS. 4.1.2 was analyzed by SDS-PAGE. The results of SDS-PAGE showed that were two bands in the crude enzyme, and one band precipitated chitinase with an estimated molecular weight of 65 kDa and 32 kDa (Figure 3).

Characterization of chitinase activity

The chitinase activity of the crude extract and the precipitated chitinase of *P. aeruginosa* ABS 4.1.2 was in the pH range of 4-10 with optimum activity at pH 7. The chitinase activity of the crude enzyme and precipitated chitinase were 0.759 U/mL and 0.767 U/mL, respectively (Figure 4). Determination of optimum temperature showed that the chitinase activity of the crude enzyme had optimum activity at 35°C and precipitated chitinase at 45°C with activity 0.767 U/mL and 0.768 U/mL respectively (Figure 5).

In vitro inhibition test of *F. proliferatum* using chitinase

Inhibition of the phytopathogenic fungus *F. proliferatum* using cell culture, crude enzymes, and precipitated chitinase of *P. aeruginosa* ABS 4.1.2 was observed in present study (Figure 7). The precipitated chitinase by *P. aeruginosa* ABS 4.1.2 can inhibit the growth of *F. proliferatum* was 33,82 % after 7 days of incubation, greater than 12 h cell culture and crude enzymes (Figure 6).

Effect of chitinase enzyme on the mycelial morphology of *F. proliferatum*

A change of mycelium morphology of *F. proliferatum* in the presence of crude enzyme and precipitate chitinase was observed in 7 days of incubation. Crude enzyme lysis mycelium of *F. proliferatum*. The precipitated chitinase caused vacuolization, swelling, and distortion of the *F. proliferatum* mycelium (Figure 8).

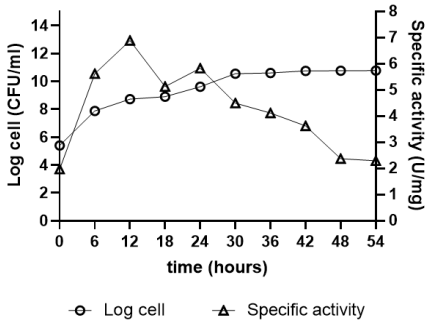

Figure 2. Bacterial cell growth and chitinase activity of *P. aeruginosa* ABS 4.1.2 on production media enriched with colloidal chitin

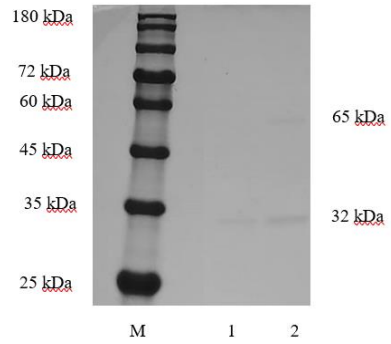

Figure 3. SDS-PAGE of chitinase *P. aeruginosa* ABS 4.1.2 from different purification steps. M: molecular weight markers; 1: ammonium sulfate precipitation; 2: crude enzymes

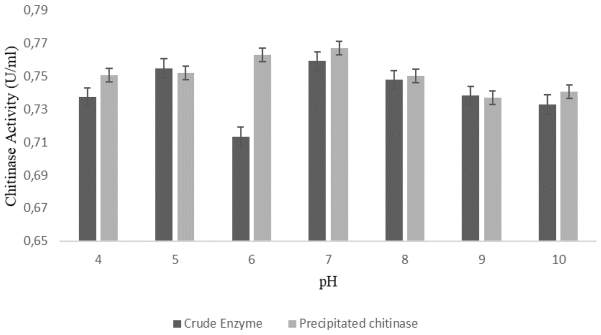

Figure 4. Effect of PH on chitinase activity of crude enzyme and precipitated chitinase of *P. aeruginosa* ABS 4.1.2

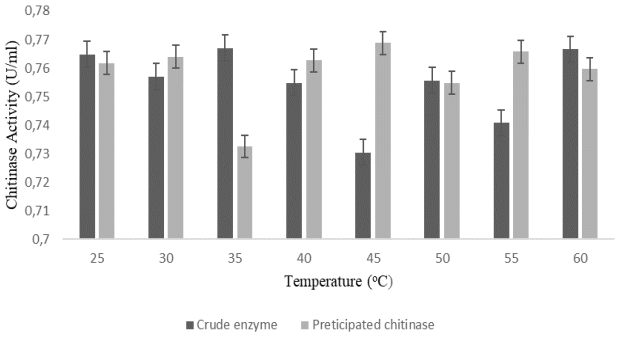

Figure 5. Effect of temperature on chitinase activity of the crude enzyme and precipitated chitinase of *P. aeruginosa* ABS 4.1.2

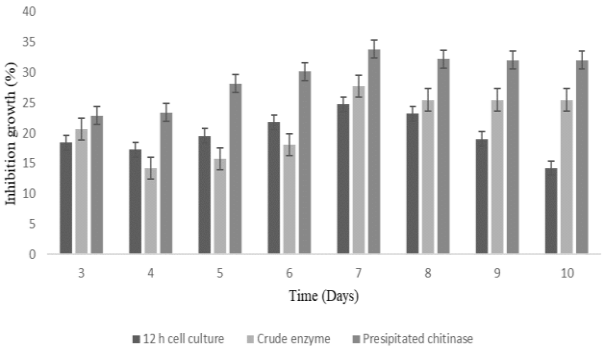

Figure 6. In vitro inhibition test of *F. proliferatum* using chitinase

Table 2. Purification steps of chitinase enzyme produced by *P. aeruginosa* ABS 4.1.2

Purification step	Volume (mL)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude enzyme	100	13.429	73.428	5.46	1	100
Ammonium sulfate 60% (w/v)	1.5	0.058	1.154	19.69	3.60	1.572

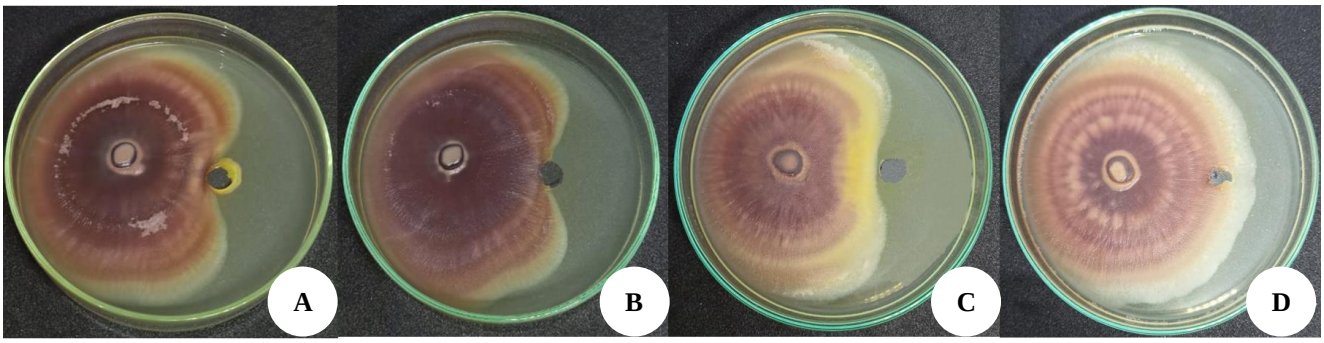

Figure 7. Inhibition of *Fusarium proliferatum* using chitinase after 7 days incubation. 12 h cell culture (A); crude enzyme (B); precipitated chitinase (C); control (D)

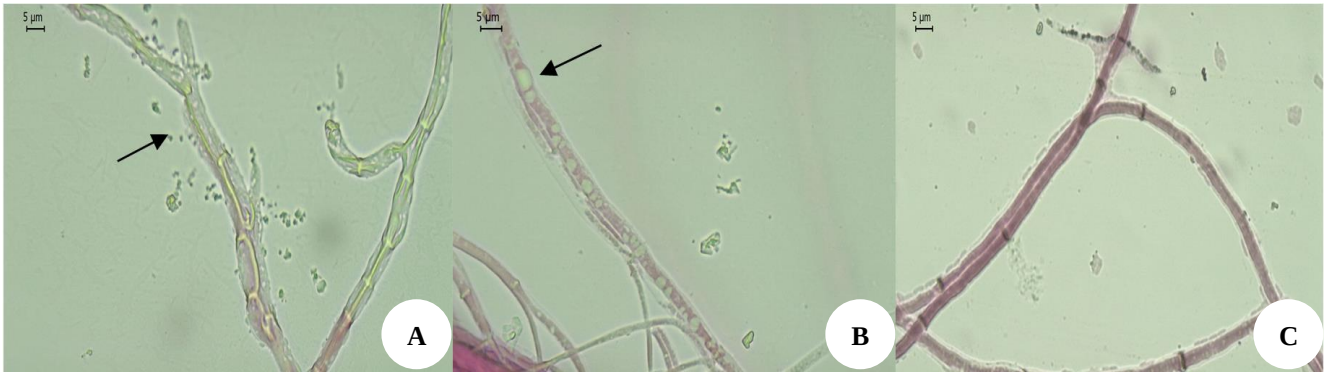

Figure 8. Effect of chitinase enzyme from *P. aeruginosa* ABS 4.1.2 on hyphae morphology of *F. proliferatum* in 7 days' incubation. Lysis of *F. proliferatum* mycelium in the presence of the crude enzyme (A); swelling, distortion and vacuolization of *F. proliferatum* mycelium grown in the presence precipitated chitinase (B); normal mycelium of *F. proliferatum* (C)

Discussion

Endophytic bacteria ABS 4.1.2 can inhibited the growth of *F. proliferatum* mycelium with high inhibition percentage compared to the other isolates. The antagonistic activity was indicated by an inhibition zone between bacteria and fungi. The percentage inhibition compliance with the inhibition zone among bacteria against *F. proliferatum* that indicated that antifungal compounds, including the hydrolytic chitinase enzyme, play a role in inhibiting the growth of the mycelium of *F. proliferatum*. Chitinases with other hydrolytic enzymes such as β-1-3 glucanase, cellulases, and proteases, play a role in inhibiting the growth of fungi by modifying, lysing, and damaging the structure of the fungal cell wall (Mota et al. 2017). The study by Khan et al. (2018) also reported that the hydrolytic enzymes chitinase, pectinase, and protease produced by *Bacillus simplex* play a role in suppressing the growth of *Fusarium* spp.

Isolate ABS 4.1.2 was identified as *Pseudomonas aeruginosa*. Genus *Pseudomonas* is widely known as a producer of various secondary metabolites, siderophores, and hydrolytic enzymes such as chitinase, which play a role in the biocontrol of plant diseases, promote plant growth and induce systemic resistance in plants (Sharma et al. 2018; Ghadamgahi et al. 2022). Bacteria from this genus include *P. fluorescens*, *P. aureofaciens*, *P. chlororaphis*, *P. putida*, *P. aeruginosa*, and *P. syringae* are known to have biocontrol abilities (Panpatte et al. 2016). A study by Banerjee et al. (2020) revealed that *Pseudomonas azotoformans* could be used as biocontrol by producing chitinase, HCN, and siderophores and suppressing the growth of *Fusarium solani*.

Pseudomonas aeruginosa ABS 4.1.2 produced extracellular chitinase, which could hydrolyze substrates such as colloidal chitin. The optimum chitinase activity occurred at 12 h of incubation. The specific activity of

chitinase was seen in the logarithmic phase when cells were dividing. Bacteria produce extracellular chitinase enzymes to degrade colloidal chitin in the media and absorb nutrients for bacterial growth. The degradation of chitin requires the work of endochitinase and exochitinase. Endochitinase will hydrolyze chitin randomly and produce oligomers, while exochitinase will hydrolyze chitin from the non-reducing end (Veliz et al. 2017). The N-acetylglucosamine oligomers metabolized into energy, carbon, and nitrogen sources, which can be used to stimulate bacterial growth (Wang et al. 2010).

Crude enzymes obtained from the highest production of *P. aeruginosa* ABS 4.1.2 could be precipitated and increased its activity using ammonium sulfate at 60 % (w/v). Precipitation of enzymes using ammonium sulfate to separate proteins from non-proteins. The interaction between the hydrophobic sides form aggregates when the solubility of the protein decreases and causes the protein molecules to precipitate to the maximum concentration (Scopes 1994). The percentage of ammonium sulfate saturation for each isolate was different. The saturation level of chitinase deposition ranges from 30%-85% (Jankiewicz et al. 2012; Asril et al. 2014). Akocak et al. (2015) study reported that the antifungal chitinase activity of *P. fluorescence* PB27 increased after precipitating it with a concentration of 80% ammonium sulfate. The SDS-PAGE of the crude extract enzyme and precipitated chitinase *P. aeruginosa* ABS 4.1.2 was 32 - 62 kDa. The molecular weight of chitinase *Pseudomonas* sp. ranges from 30 kDa - 68 kDa (Wang et al. 2008; Wang et al. 2010; Suganthi et al. 2015).

The highest activity of both enzymes of *P. aeruginosa* ABS 4.1.2 was at pH 7.0 and had a broad pH range between 4-10. Chitinase derived from bacteria has activity over a wide range of pH. Most chitinase from various bacteria has optimum activity in the pH range of 4-9 (Stoykov et al. 2015). Other studies have also shown that the chitinase activity of *P. fluorescence* is optimum at pH 7 (Suganthi et al. 2015). The crude enzyme and precipitated chitinase activity were optimum at temperatures 35°C and 45°C, respectively. Most of the chitinase produced by bacteria also has a wide temperature range. Various reports showed that the optimum activity of bacterial chitinase at a temperature range of 30-75°C (Jankiewicz et al. 2012; Senol et al. 2014; Chalidah et al. 2018). Temperature and pH are essential factors that affect the activity of enzymes in catalyzing substrates. Chitinases are produced by microbes with a wide range of temperatures, and pH is excellently applied as biocontrol. Wang et al. (2014) also reported that antifungal proteins with a wide range of pH and temperature could be used as biocontrol.

The precipitated chitinase showed a higher percentage of inhibition compared to 12 hours of cell culture and crude extract enzyme after 7 days of incubation. The precipitated chitinase has higher activity than the crude extract enzyme, resulting in a wider range of inhibition. The chitinase produced will lyse chitin, a component of the fungal cell wall that inhibits its growth (Nagpure et al. 2014; Afzal et al. 2017). Other reports showed that purified chitinase can inhibit the growth of *Bacillus subtilis* TV15 and the growth of *Fusarium culmorum* (Senol et al. 2014). Chitinase antifungal activity produced by *Pseudomonas* spp. NS-1 and *Bacillus* spp. NS-22 resulted in damage to the hyphae of the fungus *Fusarium udum* (Dukare et al. 2020).

A change in the morphology of *F. proliferatum* mycelium in the presence of crude enzymes and precipitated chitinase showed the role of chitinase enzymes in changing shape and lysing hyphae. The crude enzyme lysis mycelium of *F. proliferatum*, whereas vacuolization, swelling, and distortion of *F. proliferatum* occur in the presence of precipitated chitinase. Hydrolytic enzymes such as chitinase, β-1,3-glucanase, and proteases play a role in lysing the cell wall of the fungus (Jadhav and Sayyed 2016). Previous reports have also shown that the enzymes chitinase and β-1,3-glucanase produced by *B. licheniformis* MH48 exhibit antifungal activity by lysis, degradation, and deformation of *F. oxysporum* mycelium (Won et al. 2018). Chitinase produced by *P. fluorescens* causes changes in the shape of *Aspergillus flavus* spores and hyphae (Akocak et al. 2014). The inhibition of the growth of *Pestalotiopsis versicolor* by *Bacillus siamensis* S3 and *Bacillus tequilensis* S5 indicated the damage of hyphal structure and leakage of cell contents as an effect of chitinase, β-1,3-glucanase and other metabolites produced by these bacteria (Ali et al. 2020). Therefore, it can be concluded that *P. aeruginosa* ABS 4.1.2 and its chitinase enzyme are a potential biocontrol against phytopathogen, especially *F. proliferatum*. However, further study on other antifungal compounds and evaluation ability of plant growth-promoting bacteria as biocontrol phytopathogen is needed.

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