The Potency of UB Forest Bacteria as Chitinolytic Bacteria to Inhibit Anthracnose Disease on Cayenne Pepper

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Abstract  Anthracnose disease is a major disease in cayenne pepper triggered by fungal pathogen Colletotrichum capsici which can cause yield losses of up to 90%. Control of disease generally utilizes synthetic fungicides, but it damages the environment and human health. Hence, alternative controls that are ecofriendly are required. One of the attempts is to make use of chitinolytic bacteria's potential. This study aimed to screen UB Forest chitinolytic bacteria and to determine their potential in suppressing anthracnose in cayenne pepper. Stages of research include a screening of 78 UB Forest bacteria as chitinolytic bacteria, selection of chitinolytic bacteria that are antagonistic against C. capsici, inhibition test of chitinase crude extracts against C. capsici growth, controlling anthracnose disease in cayenne using chitinolytic bacteria, and molecular identification. Based on the results of the selection, there were 76 isolates of UB Forest bacteria classified as chitinolytic bacteria, 74 bacteria antagonistic against C. capsici. Chitinase crude extracts significantly affected C. capsici growth inhibition compared to fungicide treatment. The application of Pseudomonas aeruginosa (UB 11) and Bacillus cereus (UB 12) can control anthracnose disease in cayenne pepper with the intensity of 41.67% and 38.89% respectively with the efficacy level of 58.33% and 61.11%.

Introduction  Cayenne pepper is one of the important horticultural commodities in Indonesia and accounts for the largest inflation in agricultural commodities at 0.08% out of 3.2% of national inflation (Indonesian Bank, 2018). Inflation happens because of increasing prices of cayenne peppers owing to suboptimal production. In the rainy season, the price of red chili is highly increasing due to suboptimal production. One of the factors inhibiting red chili production is the attack of plant-disturbing organisms, especially anthracnose disease.

The most common trigger of anthracnose disease attacking cayenne pepper plants in Indonesia is caused by the attack of the pathogenic fungus C. capsici and C. gloeosporioide (Sudirga, 2016) pathogens. Anthracnose attacks cayenne pepper so that it directly affects and reduces production up to 80% (Najah et al., 2016). Symptoms caused by anthracnose disease include necrotic concave spots and rot on the surface of the fruit (Syahfitri, 2018). Anthracnose disease can be worsened if supported by environmental conditions with high rainfall and humidity intensity (Saxena et al., 2016). According to those reasons, the pathogenic fungus that causes anthracnose disease needs to be controlled. Most farmers control anthracnose disease by using synthetic fungicides with 80% mancozeb, 70% propineb, and dimetomorph. Using synthetic fungicides continuously with excessive doses can pollute the environment
and trigger non-target organisms, resistance, awakening, and death, as well as interfere with human health (Budzinski and Couderchet, 2018). Therefore, it is necessary to develop anthracnose disease control that is eco-friendly based on integrated pest control (IPM).

One of the integrated pest control tactics is the use of natural enemies as biological control agents. Control of plant pathogenic fungi can be done using antagonistic microbes such as chitinolytic bacteria that can produce chitinase enzymes to degrade pathogenic fungal cell walls containing 22-40% chitin (Kim et al., 2017). Chitinolytic bacteria can produce chitinase enzyme which can degrade chitin to N-acetylglucosamine. According to Ajayi et al. (2016), the role of chitinase in plant defense against pathogenic attack occurs by directly inhibiting fungal pathogens through hydrolyzing the mycelial wall and releasing endogenous elicitors by chitinase activity that can trigger host systemic resistance reactions. *Bacillus subtilis* are reported to be effective in controlling *C. gloeosporioides* (Ashwini and Srividya, 2013) and fungal pathogenic *Rhizoctonia solani* (Islam et al., 2012).

Chitinolytic bacteria can be obtained from sites or substrates that contain chitin such as rhizosphere, phylosphere, soil, aquatic environment, shrimp waste, compost, exoskeleton, and thermophilic environment (Wibowo et al., 2017). Forests have high biomass and litter, making them a place of decay on a huge scale (Rubiano-Cardona et al., 2013). The decomposition process involves detritivore insects and decomposing fungi whose bodies are composed of chitin so that when fungi and insects die, it becomes a substrate for chitinolytic microbes (Wieczorek et al., 2014). Therefore, forests are rich in chitinolytic microbes especially bacteria (Kellner and Vandenbol, 2010). University of Brawijaya Forest (UB Forest) is a tropical forest with pine its main vegetation and is known to have a complex microbial community (Hutamy and Yulia, 2019). Microbial biodiversity in forests is influenced by the type of vegetation so that each forest has unique microbial biodiversity characteristics (Lladó et al., 2017). To the best of my knowledge, there has been no study of diversity, abundance, and the ability of chitinolytic bacteria in UB Forest that make this study imperial. Chitinolytic bacteria that have potential as biological control agents (antagonists) against *C. capsici* in UB Forest can be found through the selection process.

Previous studies of UB Forest bacteria exploration were conducted and 78 bacteria were discovered and become bacterial isolates collection from Pest and Disease Departments which are genus *Bacillus* sp., *Pseudomonas* sp., *Xanthomonas* sp., *Erwinia* sp., *Pantoea* sp., and *Clostridium* sp. derived from coffee litter and pine leaves, Solanaceae phylosphere, Cyperaceae plants and Cyperaceae rhizosphere, Solanaceae, and *Leucaena leucocephala* plant rhizosphere. However, the role of those bacteria as chitinolytic bacteria were unknown. Therefore, research on the selection of indigenous bacteria from UB Forest as a chitinolytic bacteria and its potential to inhibit *C. capsici* which causes anthracnose in cayenne pepper is imperative. This research aimed to develop the utilization of UB Forest chitinolytic bacteria which could produce chitinase enzymes to degrade *C. capsici* fungal cell walls. Developing chitinolytic bacteria was carried out through screening of bacterial chitinase activity that had been determined by analyzing the ability of bacteria to inhibit the growth of *C. capsici* and suppressed anthracnose disease on cayenne pepper.

**Materials and Methods**

**Time and Place of Research**

The research was conducted from April to July 2019 at the Plant Disease Laboratory, University of Brawijaya and molecular identification at PT Genetica Science.
Screening of UB Forest Chitinolytic Bacteria

For 72 hours, as many as 78 UB Forest bacteria were grown on chitin media, then stained with congo red. Chitinolytic Index (CI) values are calculated from the diameter of the Clear Zone (CZ) formed divided by the diameter of the bacterial colony (CS) (Faramarzi et al., 2009).

Chitinolytic Bacterial Antagonist Test on C. capsici

The tests used multiple culture methods (Herliyana et al., 2013) with a slight modification. The filter paper was immersed in a chitinolytic bacterial suspension and placed on PDA media at 4 points at a distance of 3 cm from the center of the Petri dish. After 24 hours, the C. capsici was inoculated at the center Petri dish and incubated for 10 days.

Inhibition Testing of Chitinase Crude Extract toward C. capsici

Bacteria were grown on 150 mL of NB medium and incubated in the orbital shaker at 120 rpm for 72 hours, then 10 mL of the culture was centrifuged at 4000 rpm for 20 minutes. One mL supernatant was filtered using a 0.45 µm bacterial filter and inserted in the Eppendorf tube. A total of 30 µL of chitinase crude extract was put into the wells in PDA media within 3 cm from the 3 days old. C. capsici colony based on agar well diffusion method (Wibowo et al., 2017). Percentage of inhibition of fungal growth was measured for 7 days using the Elfina et al. (2015) formula:

\[
\% \text{Inhibition} = \frac{r_1 - r_2}{r_1} \times 100\% \quad (1)
\]

Note: \(r_1\) = r mycelium to the edge of the Petri dish. \(r_2\) = r mycelium to the well.

The experimental design used a Completely Randomized Design (CRD) with 7 treatments consisting of 1 positive control (80% mancozeb fungicide), 1 negative control (sterile distilled water), and 5 selected chitinase crude extracts with 3 replications.

Chitinolytic Bacterial Inhibition Test Against Anthracnose Disease

Experiments were carried out using the fruit bioassay method (Ramanujam et al., 2012). The surface of the chili was sterilized with 70% alcohol and NaOCl and rinsed with sterile distilled water and injured at 3 points and dropped with 0.01 ml suspension of UB Forest chitinolytic bacteria with a density of 10^9 cfu/ml. After 24 hours, the chilies were dropped with 0.01 ml suspension of C. capsici with a density of 10^6 conidia/ml and incubated in a sterile and moist container. The intensity of the symptoms of anthracnose attacks were calculated using the formula from Marlina et al. (2010):

\[
X = \frac{\sum n \times v}{Z \times N} \times 100\% \quad (2)
\]

Note: \(X\) = severity of disease; \(n\) = number of infected fruits; \(v\) = value of each score; \(N\) = total observed fruit; \(Z\) = highest score.

The effectiveness of inhibition was calculated using efficacy level formula (Supriati and Djaya, 2015). The treatment is effective if the efficacy level (EL) is more than or equal to 30%.

\[
\text{EL} = \frac{\text{IPk} - \text{IPP}}{\text{IPk}} \times 100\% \quad (3)
\]

Note: \(E\) = level of fungicide efficacy, \(\text{IPk}\) = intensity of disease attacks in controls (distilled water) \(\text{IPP}\) = intensity of disease attacks in the treatment.

Molecular Identification

Selected chitinolytic bacterial isolates were sent to PT Genetics Science for molecular identification using the 16S rRNA gene marker. DNA was isolated from bacteria then Gen 16S rRNA was amplified with Polymerase Chain Reaction (PCR). Amplicon was sequenced with Bi-directional sequencing using ABI PRISM sequencer, nucleotide base sequence data were analyzed homologously with the Basic Local Alignment Search Tool (BLAST) and analyzed phylogenetic kinship with the Molecular Evolutionary Genetics Analysis (MEGA X) program.
Result and Discussions

Screening Results UB Forest Chitinolytic Bacteria

There were 76 chitinolytic bacteria from 78 UB Forest indigenous bacteria that are able to produce clear zones around the bacterial colony. A total of 39 bacteria have high chitinase activity and 37 bacteria with low chitinase activity based on chitinolytic index values produced (Table 1).

Table 1. UB Forest Bacteria Screening Results as Chitinolytic Bacteria

| Bacterial Code | High Chitinolytic Activity (CI≥2) | Genus of bacteria | Bacterial Code | Low Chitinolytic Activity (CI<2) | Genus of bacteria |
|----------------|----------------------------------|-------------------|----------------|----------------------------------|-------------------|
| UB 10          | 3.3                              | Xanthomonas sp.   | UB 27          | 1.9                              | Erwinia sp.       |
| UB 24          | 2.9                              | Pantoea sp.       | UB 51          | 1.9                              | Pseudomonas sp.   |
| UB 60          | 2.9                              | Pantoea sp.       | UB 30          | 1.8                              | Erwinia sp.       |
| UB 70          | 2.9                              | Xanthomonas sp.   | UB 15          | 1.7                              | Pantoeea sp.      |
| UB 71          | 2.9                              | Erwinia sp.       | UB 43          | 1.7                              | Clostridium sp.   |
| UB 79          | 2.7                              | Erwinia sp.       | UB 48          | 1.7                              | Xanthomonas sp.   |
| UB 8           | 2.7                              | Bacillus sp.      | UB 72          | 1.7                              | Clostridium sp.   |
| UB 1           | 2.5                              | Pantoeea sp.      | UB 74          | 1.5                              | Clostridium sp.   |
| UB 12          | 2.5                              | Pseudomonas sp.   | UB 73          | 1.7                              | Pantoeea sp.      |
| UB 28          | 2.5                              | Pseudomonas sp.   | UB 75          | 1.6                              | Pantoeea sp.      |
| UB 38          | 2.5                              | Pseudomonas sp.   | UB 77          | 1.6                              | Erwinia sp.       |
| UB 4           | 2.5                              | Pseudomonas sp.   | UB 33          | 1.5                              | Erwinia sp.       |
| UB 21          | 2.6                              | Clostridium sp.   | UB 43          | 1.5                              | Pantoea sp.       |
| UB 22          | 2.6                              | Pseudomonas sp.   | UB 40          | 1.4                              | Clostridium sp.   |
| UB 23          | 2.6                              | Pseudomonas sp.   | UB 49          | 1.4                              | Xanthomonas sp.   |
| UB 24          | 2.6                              | Pseudomonas sp.   | UB 65          | 1.4                              | Pantoea sp.       |
| UB 3           | 2.5                              | Pseudomonas sp.   | UB 66          | 1.4                              | Pantoea sp.       |
| UB 12          | 2.4                              | Bacillus sp.      | UB 16          | 1.3                              | Xanthomonas sp.   |
| UB 13          | 2.3                              | Erwinia sp.       | UB 37          | 1.3                              | Pseudomonas sp.   |
| UB 14          | 2.5                              | Pantoea sp.       | UB 41          | 1.3                              | Clostridium sp.   |
| UB 7           | 2.3                              | Erwinia sp.       | UB 62          | 1.3                              | Bacillus sp.      |
| UB 15          | 2.3                              | Pseudomonas sp.   | UB 64          | 1.3                              | Clostridium sp.   |
| UB 16          | 2.3                              | Pantoea sp.       | UB 67          | 1.3                              | Bacillus sp.      |
| UB 17          | 2.4                              | Bacillus sp.      | UB 68          | 1.3                              | Bacillus sp.      |
| UB 18          | 2.2                              | Pantoea sp.       | UB 42          | 1.2                              | Pseudomonas sp.   |
| UB 19          | 2.2                              | Pantoea sp.       | UB 44          | 1.2                              | Pseudomonas sp.   |
| UB 20          | 2.2                              | Pantoea sp.       | UB 45          | 1.2                              | Erwinia sp.       |
| UB 21          | 2.2                              | Pantoea sp.       | UB 47          | 1.2                              | Pseudomonas sp.   |
| UB 22          | 2.1                              | Clostridium sp.   | UB 50          | 1.2                              | Erwinia sp.       |
| UB 23          | 2.1                              | Pantoea sp.       | UB 53          | 1.2                              | Pseudomonas sp.   |
| UB 24          | 2.1                              | Pantoea sp.       | UB 54          | 1.2                              | Bacillus sp.      |
| UB 25          | 2.1                              | Pantoea sp.       | UB 55          | 1.2                              | Pseudomonas sp.   |
| UB 26          | 2.1                              | Pantoea sp.       | UB 57          | 1.2                              | Pantoea sp.       |
| UB 27          | 2.1                              | Pantoea sp.       | UB 58          | 1.2                              | Pantoea sp.       |
| UB 28          | 2.1                              | Pantoea sp.       | UB 61          | 1.2                              | Clostridium sp.   |
| UB 29          | 2.1                              | Xanthomonas sp.   | UB 69          | 1.2                              | Clostridium sp.   |
| UB 30          | 2.1                              | Xanthomonas sp.   | UB 72          | 1.2                              | Pantoea sp.       |
| UB 31          | 2.1                              | Xanthomonas sp.   | UB 76          | 1.2                              | Pantoea sp.       |
| UB 32          | 2.1                              | Xanthomonas sp.   | UB 46          | 1.1                              | Xanthomonas sp.   |
According to Suryadi et al. (2013), chitinase which is secreted by bacteria on chitin media will be bound by chitin particles in the form of colloidal chitin so that chitin becomes deviated and the chitin component in the media will be reduced. It is also supported by Suryanto et al. (2011) that the clear zone formed in chitin media was caused by chitin contained in hydrolyzed media into dissolved monomers or their derivatives in the form of N-acetylglucosamine as bacterial extracellular chitinase.

The high number of indigenous bacteria classified as chitinolytic bacteria in UB Forest is believed to be caused by the source of bacterial isolates having high biomass and decomposition activity by both chitin-composed decomposers and detritivores. This is following Pratiwi et al. (2015), who opined that chitinase-producing bacteria with various characteristics and specific substrate are likely to be found in a biosphere environment that contains a lot of chitinase. The various abilities of bacteria in producing chitinase are predicted as an effort to adapt to various forms, types, and structures of chitin that are available in nature.

Wibowo et al. (2017) stated tropical rain forests are ecosystems that are rich in species diversity of endemic flora, fauna, and microbes. Prokaryotic bacteria are the most abundant and diverse group of organisms in the soil. This organism is important for the transformation of nutrients in the soil and is a major driving force for the biogeochemical cycle, in the top soil layer bacteria play an important role in the process of decomposition of organic matter in the soil. The ability of bacteria to produce chitinase varies greatly because of small differences in the coding gene.

Selection Result of Antagonistic Chitinolytic Bacteria against C. capsici

72 out of 76 UB Forest chitinolytic bacteria were antagonistic towards C. capsici with a range of C. capsici growth inhibition of 6.67-100% and based on the selection results, 5 chitinolytic bacteria were selected for further testing (Table 2).

The antagonism ability of the UB Forest chitinolytic bacteria to suppress the pathogenic fungus C. capsici evidenced by the results of research by de Azevedo and Quecine (2017), Erwinia sp. bacteria which is isolated from anthracnose symptomatic plants, has a high inhibition against Colletotrichum sp. The application of Pseudomonas chitinolytic bacteria is able to give the highest antagonism or inhibitory results against the pathogen Colletotrichum sp. by 37%. The application of chitinolytic bacteria in the form of P. aeruginosa can suppress anthracnose diseases caused by C. gloeosporioides by 48.63% and can cause malformations (abnormalities) in fungal hyphae (Bakthavatchalu et al., 2013). Parhi and Mohapatra (2017) stated that Xanthomonas as a chitinolytic bacteria has been reported to be an important biological control agent in suppressing plant disease. Based on Suryanto et al. (2014), the application of chitinolytic bacteria with the genus Bacillus sp. proven antagonistic against C. gloeosporioides and were able to control anthracnose disease on cocoa leaves.

Table 2. Five Selected Bacteria Tested for Chitinase Crude Extract and Their Inhibition on Cayenne Pepper

| No | Bacterial Code       | Chitinolytic Index | Inhibition Percentage against C. capsici |
|----|----------------------|--------------------|----------------------------------------|
| 1  | UB 9 (Erwinia sp.)   | 2.3                | 100.00%                                |
| 2  | UB 10 (Xanthomonas sp.) | 3.3                | 100.00%                                |
| 3  | UB 11 (Pseudomonas sp.) | 2.5                | 100.00%                                |
| 4  | UB 12 (Bacillus sp.) | 2.4                | 100.00%                                |
| 5  | UB 34 (Bacillus sp.) | 2.6                | 66.33%                                 |

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From five UB Forest chitinolytic bacteria, namely genus *Pseudomonas* sp., *Xanthomonas* sp., *Erwinia* sp., and *Bacillus* sp. the amount of the chitinase enzyme produced was proven by the different chitinolytic index values between the genus of bacteria. The chitinase enzyme generated by each chitinolytic bacteria has distinct types of chitinase, so further research is required to discover specific information about chitinase produced by each bacterial genus. According to Suryadi et al. (2013) a high chitinolytic index indicates that bacteria generate large amounts of chitinase. This is supposed to UB Forest chitinolytic bacteria with a high CI are believed to inhibit fungal growth and can degrade *C. capsici* fungal cell walls with greater ability when compared to bacteria with low CI. Maggadani et al. (2017) chitinase activity can be tested qualitatively through the analysis of clear zones that are located around the growth of bacterial colonies on Agar media containing chitin. The potential of bacteria in producing chitinase enzymes is determined from the value of the chitinolytic index which proves that bacteria can produce chitinase enzymes. That bacteria with the highest chitinolytic index are selected at the chitinase production stage to obtain the potential isolates of chitinolytic bacteria.

**Inhibition of Chitinase Crude Extract against *C. capsici***

Five chitinase crude extracts of UB Forest chitinolytic bacteria were selected to have an inhibition of 8.83-87.77% against *C. capsici*. The inhibitory ability of the bacterial chitinase crude extract of UB 9, UB 11, UB 12, and UB 34 was greater than that of the 80% mancozeb fungicide treatment (Figure 1). Crude extract of chitinolytic bacteria UB 11 (*Pseudomonas* sp.) and UB 12 (*Bacillus* sp.) had the highest inhibitory properties of 74.33% and 87.77%. The effectiveness of chitinase crude extracts from UB 11 (*Pseudomonas* sp.) and UB 12 (*Bacillus* sp.) chitinolytic bacteria had 33 and 43 times more effective inhibition than the use of 80% mancozeb fungicide.

Chitinase crude extract of bacterial can lyse the structure of chitin as the main compound making up the fungal cell wall (Suryadi et al., 2013). The potential of chitinase crude extract in suppressing anthracnose disease had been proven by Nurdin et al. (2015) who reported that the application of bacterial and supernatant formulations containing chitinase crude extract was able to reduce the severity of anthracnose disease. This is supported by Chandrasekaran et al. (2012) who stated that chitinase from bacterial activity plays an important role in controlling fungal pathogens and increasing disease tolerance in plants with the production of chitoooligosaccharides which are further degraded to N-acetylglucosamine by chitobiase.

**Inhibition of UB Forest Chitinolytic Bacteria against Anthracnose Disease**

The intensity of anthracnose disease in the treatment of chitinolytic bacteria UB 11 and UB 12 were lower than that of fungicides, namely with the intensity of the disease 41.67% and 38.89% respectively (Table 3). Thus, it was concluded that UB 11 and UB 12 chitinolytic bacteria were more effective in controlling anthracnose than fungicides. While the treatment of other chitinolytic bacteria were not significantly different according to Jisha et al. (2018).

Application *Pseudomonas aeruginosa* was a greater reduction of anthracnose infection caused by *C. capsici*. The strain possessed chitinolytic and proteolytic activities, produced HCN, siderophores, able to produce salicylic acid at a moderate level, and produced amylase. The isolates induced systemic resistance in chilli corroborated with increased levels of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase.

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The level of efficacy of the application of bacteria UB 11 and UB 12 were 58.33% and 61.11% (efficacy values more than 30%) so that the chitinolytic bacteria were effective in suppressing anthracnose disease in chili. While other treatments have an efficacy value of less than 30%, so it is said to be ineffective.

UB 11 chitinolytic bacteria (*Pseudomonas* sp.) and UB 12 (*Bacillus* sp.) are three times more effective in inhibiting *C. capsici* compared to other biological agents (*Trichoderma harzianum*) from previous studies (Rahman et al., 2015) because of *T. harzianum* have a resistance of only 28%. Thus, UB Forest chitinolytic bacteria have high potential as biological control agents for anthracnose in cayenne pepper. *B. cereus* as chitinolytic bacteria is reported to have the highest ability to produce chitinase and glucanase so that it can inhibit the growth of pathogenic fungi *Pyricularia oryzae* and *Ganoderma boninens* (Suryadi et al., 2013). A study by Sasirekha and Srividya (2016) also asserted that *P. aeruginosa* bacteria isolated from soil rhizosphere can suppress *C. gloeosporioides* that causes anthracnose in chili.

Prasetya et al. (2018) stated that when fungal colonies encountered bacterial colonies, chitin in fungi would induce chitinase formation in bacteria. Bacteria will utilize chitin from fungal hyphae to become a source of carbon, causing hyphae and fungal cell walls of lysis. There are two stages in the mechanism of chitinolytic bacteria in inhibiting fungal growth. At the first stage, the bacteria will produce the enzyme chitinase and damage the structural component of the fungus, namely the cell wall. Damage to the cell wall will affect the permeability of the fungal cell membrane, so that the transport system of fungal intercellular substances becomes disrupted and will inhibit the growth of fungal pathogens. Gohel et al. (2006) stated that the use of microorganisms into biocontrol has two mechanisms at once, namely directly and indirectly on plant pathogens. Biocontrol agents can directly compete with nutrients, growth space, antibiotic production, and produce enzymes that can cause lysis. While indirectly, biocontrol agents can increase plant systemic resistance to pathogens. Chitinase plays a major role not only
in the mechanism of resistance but also in the process of fungal mycoparasites.

Mycoparasites have been studied and developed for biocontrol alternatives as chemical fungicides that dominate agricultural practices. According to Alshehri et al. (2016), bacterial biopesticides are a widely used/appplied and cost-effective solution to microbial-based pesticides so that chitinolytic bacteria have the potential to be environmentally friendly biocontrol alternatives for anthracnose disease.

**Molecular Identification**

The results of BLAST (nucleotide blast) analysis are used to determine the suitability of the nucleotide base sequences from the 16S rRNA sequence with the DNA database (Genbank) from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). UB 11 bacteria (*Pseudomonas* sp.) has a 99.55% similarity with *Pseudomonas aeruginosa* strain S16 (access number MK883097.1). UB 12 bacteria (*Bacillus* sp.) has a 100% similarity with *Bacillus cereus* strain S533-6 (access number MH231418.1) which can be seen in Table 4.

The query coverage value, which is a percentage of the nucleotide length corresponding to the database, is 100%, which indicates that all nucleotide bases correspond to the homologous bacteria. The maximum score shows the similarity between bacterial sequences based on statistical differences from the RNA sequence alignment. The maximum score of the 2412 UB 12 (*Bacillus* sp.) is higher than the 1185 UB 11 (*Pseudomonas* sp.) score. Expectation value (E-value) shows the similarity between the sequences being compared, so the value of 0 on the E-value represents a high similarity with the homologous sequence results. Bacterial sequences from the 16S rRNA gene were analyzed for their kinship with a phylogenetic approach using the MEGA-X program.

The results of the phylogenetic tree construction showed that UB 11 (*Pseudomonas* sp.) and UB 12 (*Bacillus* sp.) had the highest bootstrap value of 100. The bootstrap value represents the level of kinship from a branch point, so the bootstrap value between 95-100 shows that the branching has a kinship level of 100 highs. Bootstrap values between UB 11 *Pseudomonas* sp. with *P. aeruginosa* worth 100, in line with the value between UB 12 *Bacillus* sp. with *B. cereus* which is 99 belonging to one high group. The test bacteria form a monophyletic group which is a taxonic with species originating from the same single ancestor. Whereas *Erwinia pyrifoliae* as a comparative bacterium showed a degree of kinship that was incompatible with the two test bacteria (Figure 2).

**Table 4. Sequence Analysis Results with BLAST**

| Bacterial Isolate | Query length | BLAST Result | Max score | Query coverage | E. Value | Max. Identify |
|-------------------|--------------|--------------|-----------|----------------|----------|--------------|
| UB 11             | 1492         | *Pseudomonas aeruginosa* strain S16 (MK883097.1) | 1185      | 100%           | 0        | 99.55%       |
| UB 12             | 1337         | *Bacillus cereus* strain S533-6 (MH231418.1) | 2412      | 100%           | 0        | 100%         |

**Figure 2.** The bacterial phylogeny of UB 11 and UB 12 bacteria
Conclusions and Suggestion
76 UB Forest chitinolytic bacteria were found and crude extract of chitinolytic bacteria UB 11 and UB 12 were 33 and 43 times more effective in suppressing the growth of C. capsici.

P. aeruginosa (UB 11) and B. cereus (UB 12) were effective in suppressing anthracnose disease with efficacy rates of 58.33% and 61.11%.

Further evaluation of the potential chitinolytic bacteria as Plant Growth Promoting Rhizobacteria (PGPR) and feasibility analysis of biological control products should be carried out.

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