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Isolation of actinomycetes from peatland to suppress the growth of *Ganoderma boninense* the causal agent of basal stem rot disease in oil palm

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Abstract. *Budi MBS, Giyanto, Tondok ET. 2022. Isolation of actinomycetes from peatland to suppress the growth of* Ganoderma boninense *the causal agent of basal stem rot disease in oil palm. Biodiversitas 23: 5914-5922.* Basal stem rot is a major constrain in oil palm cultivation. The aim of this study was to obtain actinomycetes isolates that can suppress the growth of *Ganoderma boninense*. The research stages included rejuvenation of actinomycetes isolate stock cultures, observation of morphological characters, analysis of the potency of actinomycetes as biological control agents against *G. boninense* (antibiosis, volatile organic compound, effect of bioactive compounds in actinomycete culture supernatants and their concentration levels in suppressing *G. boninense*), physiological characterization and molecular identification was also done of selected actinomycetes. All actinomycetes colonies were grey and brown in color with rectiflexible and vertilicillate spore chain types. Three of the seven isolates tested, namely AKT19, AKT28, and AKT52, were able to suppress the growth of *G. boninense* up to 100% based on the dual culture test and the percent inhibition of *G. boninense* growth was 12.27; 17.50; and 23.23% by volatile organic compounds. At 10% concentration, the bioactive compounds of three actinomycetes isolates inhibited the growth of *G. boninense*, i.e., 89.17, 88.30 and 86.50%, respectively. Further results revealed that isolate AKT19 was the strongest suppressor (70.93) of *G. boninense* at a concentration of 0.5%, while isolates AKT 28 and 52 inhibited only 15.30 and 4.80%, respectively. All potential actinomycetes isolates produce chitinase, glucanase, cellulase, phosphatase enzymes, fixes nitrogen and secreted indole acetic acid growth hormone. Based on the analysis of 16S rRNA gene, it was noted that all potential isolates were identified as *Streptomyces gelaticus*.

Keywords: Antibiosis, actinomycetes, biological control agents, *Ganoderma boninense*, *Streptomyces gelaticus*, sustainable agriculture

INTRODUCTION

Basal stem rot disease (BSR) is the main disease in oil palm plantations caused by the fungus *Ganoderma boninense*. In Southeast Asian countries that are mostly engaged in the oil palm industry, such as Indonesia and Malaysia, this disease poses a serious threat to the production and export of oil palm (Naher et al. 2013; Afandi et al. 2018). BSR disease is reported to cause 30-50% damage in oil palm plantations (Ibrahim et al. 2020). The total loss caused by basal stem rot disease in oil palm producing countries such as Indonesia and Malaysia even reached 500 million USD/year and became the country with the highest losses due to BSR disease (Rees et al. 2012).

Some controls that have been carried out to suppress the infection of fungus *G. boninense* include controlling technical culture, planting holes in holes, making isolation trenches and using several resistant varieties (Naher et al. 2012). Control with technical and mechanical culture is less efficient and often fails due to the characteristics of *G. boninense* as a soil-borne pathogen that has high saprophytic ability and a very wide host range (Rees et al. 2012). Efforts to control BSR disease are mostly carried out with chemical fungicides, but with consideration of environmental safety, the use of biological agents has been widely developed as alternative control technique. Several antagonistic microorganisms that have been used to control BSR disease include *Bacillus cepacia*, *Enterobacter* sp., and *Pseudomonas aeruginosa* (Bivi et al. 2010; Suryanto et al. 2012). Several endophytic bacteria from the group *Pseudomonas aeruginosa, Serratia marcescens, Burkholderia cepacia* and have also been reported to be used to control BSR disease (Zaiton et al. 2006). Use of *Trichoderma harzianum* and *Trichoderma* sp have also been reported but have not yet yielded maximum results (Alviodinasyari et al. 2015).

Actinomycetes are a group of Gram-positive bacteria and have a structure that resembles a fungus, which has soft filaments such as hyphae and mycelia (Madigan et al. 2006). Actinomycetes have high potential to be developed and used as biological control agents because actinomycetes are known to increase resistance and promote plant growth. Various antimicrobial compounds produced by actinomycetes, especially *Streptomyces* species (Hasani et al. 2014). The antimicrobial compound includes antimycin, tetracycline, streptomycin, erythromycin, chloramphenicol, ivermectin, and rifampin (Todar 2008). The antimicrobial by actinomycetes is also known to act as antifungal compounds that suppress the growth and development of pathogens. One of the antifungal compounds produced is urauchimycins which is a group of antimycin compounds. This compound can act

as antifungal by inhibiting electrons that act as mitochondrial respiration (Sharma et al. 2015).

Actinomycetes from the genus *Streptomyces* produce the most antibiotics compared to other species. It is estimated that 80% of antibiotic products are produced by *Streptomyces* (Singh et al. 2006). Actinomycetes are known to be effective in suppressing several plant pathogens including *Verticillium dahlia, Fusarium oxysporum* and groups of oomycetes such as *Phytophthora* sp and *Pythium ultimum*. The antagonistic mechanisms included hyperparasitism, antibiosis, producing cell wall degrading enzymes and induction of plant resistance (Errakhi et al. 2009). Actinomycetes as prokaryotic microbes are able to play an important role in the nutrient cycle, nitrogen fixation, secondary metabolite production and promote plant growth (Olanrewaju and Babalola 2019). As plant growth promoting rhizobacteria (PGPR), actinomycetes are reported to be able to produce phytohormones, namely auxins, gibberellins, and cytokinins (El-Tarabily et al. 2020).

The expansion of oil palm plantations in Indonesia continues and even on peat soil. *Ganoderma boninense*, the causal agent of BSR disease, has not been widely explored in oil palm in peat soil as biological agents of actinomycetes. The aim of this study was to obtain actinomycete isolates from peat soil capable to suppress the growth of *G. boninense* to be developed as biological agents for controlling BSR disease in of oil palm.

MATERIALS AND METHODS

Rejuvenation of actinomycetes isolate culture stocks and confirmation based on general morphological characteristics

Actinomycetes isolates were acquired from the Plant Bacteriology Laboratory, Department of Plant Protection, Bogor Agricultural University and isolated from peatlands in Musi Banyuasin Regency, South Sumatra Province. The isolates used were named AKTb, AKT19, AKT28, AKT41, AKT52, AKT56, and AKT57. The conformation of actinomycetes included colony color, substrate mycelium color, pigment produce in medium, and the shape of spore chain. All the isolates were grown on two medium, namely YSA medium (yeast extract 2.0 g soluble starch 10.0 g agar 15.0 g and 1 L distilled water) and ISP2 medium (yeast extract 4.0 g, malt extract 10.0 g, dextrose, 4.0 g, bacto agar 20.0 g and 1 L distilled water) (Akbar et al. 2017). *Ganoderma boninense* isolates were obtained from fruiting bodies appeared on oil palm plants infected with *G. boninense*. The inoculum was grown on Himedia *potato dextrose agar* (PDA) media and molecularly identified using primers ITS1 and ITS4.

Potency test of actinomycete isolates as biological agents of *Ganoderma boninense*

Growth inhibition of Ganoderma boninense *by actinomycetes using dual culture technique*

All the isolates of actinomycetes were tested for their ability to inhibit the growth of *G. boninense* isolate SMU as described by Kunova et al. (2016). Actinomycetes isolates were scratched from the sides of petridish and incubated for 3 days. After incubation, *G. boninense* isolate was placed on the other side of the petri dish for further incubation for 7 days. Observations were made on the presence or absence of a clear zone and also the relative inhibition of pathogen using the formula.

$$H = \frac{R1-R2}{R1} \times 100\%$$

Where:

H : Relative inhibition of *G. boninense* growth by actinomycetes

R1: Radius *G. boninense* colony close to actinomycetes culture

R2: Control

Effect of volatile organic compound (VOC) produced by actinomycetes on the growth of Ganoderma boninense

Ganoderma boninense was cultured on PDA and actinomycete was grown on ISP2 media. The two cultures in petri dishes were cupped together and sealed with parafilm, then incubated for 7 days. In control treatment, the same method was used, actinomycetes were not culture on ISP2 medium. Observations were made on 7th day by measuring the diameter of the fungal colonies and compared with the control treatment (Yuan et al. 2012).

Test the effect of bioactive compounds produced by actinomycetes on the growth of Ganoderma boninense

Preparation of bioactive compounds was carried out by growing actinomycetes on liquid ISP2 medium and incubating on a shaker at 114 rpm for 7 days. The supernatant of actinomycetes cultures was separated and sterilized by 0.22 □m millipore filter to obtain sterile crude bioactive compounds (Susilowati et al. 2007). ISP medium was added with actinomycetes bioactive compounds. Furthermore, *G. boninense* was grown on PDA medium containing 10% (v/v) of bioactive compounds. As a control, *G. boninense* was grown on PDA medium without the addition of actinomycetes bioactive compounds. Observation of inhibition was carried out after 7 days of incubation. The percentage of inhibition (I) was determined using the formula.

$$I\,(\%) = \frac{DK-DP}{DK} \times 100\%$$

Where:

DK: Diameter of *G. boninense* colonies on control (cm)

DP: Diameter of *G. boninense* colonies in treatment (cm)

Three selected isolates with the highest percentage inhibition to *G. boninense* were further tested using several concentrations of bioactive compounds at 3, 2.5, 2, 1.5, 1, 0.5 and 0% according to Achmad (2015). *Ganoderma boninense* was grown in liquid PDB medium containing actinomycetes bioactive compounds with various concentrations as mentioned above. On the seventh day mycelia of *G. boninense* were separated from liquid media by filtering the mycelia using Whatman filter paper no 1

and then placed in an oven for 24 hours at 60°C. The weight of *G. boninense* biomass was measured based on the formula by Pratomo (2006).

Physiological characterization of selected actinomycetes isolates

Chitinase production

Chitinase production by actinomycetes was detected using chitin agar medium containing 0.1 g $MgSO_4.7H_2O$, 1 g KH_2PO_4, 1 g NaCl, 7 g $(NH4)_2SO_4$, 2 g yeast extract, 1 g tryptone, 15 mL colloidal chitin, 20 g bacto agar and 1 L of distilled water. 3 mm diameter of actinomycetes culture pieces were inoculated in the center of media and then incubated for 3 days. The overgrown medium of actinomycetes was flooded with 1 mL of 0.1% congo red dye solution and rinsed with 1M NaCl solution. The presence of a clear zone around the bacterial colonies indicated chitinolytic activity and chitinolytic index was calculated based on Hariprasad et al. (2015).

Glucanase production

The ability of actinomycetes to degrade glucan was carried out using glucan agar media containing 1.5 g KH_2PO_4, 0.65 g Na_2PO_4, 2.5 g NaCl, 0.05 g $CaCl_2$, 1.25 g peptone, 0.5 g yeast extract, 0.5 g $(NH4)_2SO_4$, 10 mL glucan substrate, 20 g of bacto agar and 1 L of distilled water. Actinomycetes culture pieces with a diameter of 3 mm diameter were inoculated in the center of media and then incubated at 30°C for four days. Furthermore, the medium overgrown by actinomycetes was flooded with 1 mL of 0.1% congo red dye solution and rinsed with 1M NaCl solution. The clear zone formed was observed and the glucanase activity index was calculated based on Dawood and Muhammed (2015).

Cellulase production

Carboxymethyl cellulose (CMC) agar media containing 1.36 g KH_2PO_4, 1 g $(NH4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$, 2 g NaCl, 1 g yeast extract, 0.01 g $CaCl_2.2H_2O$, 5 g CMC, 15 g of bacto agar and 1 L of distilled water. Actinomycetes culture pieces with a diameter of 0.3 mm were inoculated in the center of media and then incubated at 30°C for four days. The medium that had been overgrown with actinomycetes was flooded with 1 mL of 0.1% congo red dye solution and rinsed with 1M NaCl solution. The clear zone formed was then calculated as cellulase index based on Hastuti et al. (2014)

Phosphatase production

The production of phosphatase by actinomycetes was tested using pikovskaya agar medium containing 10 g dextrose, 0.5 g yeast extract, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 0.0001g $MnSO_4 \cdot H_2O$, 0.0001 g $FeSO_4$, 5 g $Ca_3(PO_4)_2$, 0.2 g KCL, 15 g bacto agar and 1 L distilled water. A clear zone was formed as a result of phosphate solubilizing activity around actinomycetes colonies (Sarker et al. 2014). The clear zone was measured by calculating the phosphate dissolution index based on Ulfiyanti and Zulaikha (2015).

Nitrogen fixation

The test was carried out by culturing actinomycetes on NFB (nitrogen-free bromthymol blue) semi agar containing 0.05 g $FeSO_4.7H_2O$, 4 g KOH, 5 g malic acid, 0.01 g $MgSO_4.7H_2O$, 0.01 g $MnSO_4.H_2O$, 0.5 g K_2HPO_4, 0.002 g NaCl, 0.01 g $CaCl_2$, 0.002 g $Na_2MO_4.2H_2O$, 1.75 g bacto agar, 2 mL of 0.5% bromothymol blue in 1 L distilled water (Döbereiner et al. 1976). The culture was incubated at room temperature for 5-7 days. The formation of pellicle under the surface of media indicated that actinomycetes isolate was able to fix nitrogen from air for their growth.

Production of indole acetic acid (IAA)

The ability of actinomycetes to produce IAA compounds was tested based on Patten and Glick (2002). Actinomycetes were cultured in ISP2 broth media containing L-tryptophan (L-trp) 2 mg mL^{-1}, then incubated at room temperature and shaken at 150 rpm for 3 days. Actinomycetes supernatants were separated using a 0.22 m millipore filter to obtain crude metabolites. A total of 1 mL of crude metabolite was mixed with Salkowsky's reagent (1 mL of 0.5 M FeCl3 and 49 mL of 35% perchloric acid) with a ratio of 1:2 and incubated in a room without light for 30 minutes (Gordon and Weber 1951). If the suspension turn pink color, it indicates that actinomycetes isolate produced IAA.

Identification of actinomycetes isolates by sequencing of 16S rRNA gene

Extraction of total DNA from actinomycetes isolates was carried out using PrestoTM Mini DNA Bacteria Kit Geneaid according to the protocol of product. 16S rRNA gene amplification was carried out by PCR in a volume of 25 µL consisting of 12.5 µL Thermo Scientific PCR master mix (2x), 1 µL 27F forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') (Bruce et al. 1992), 1 µL reverse primer 16Sact1114R (5'-GAGTTGACCCCGGCRGT-3', 1 µL DNA template and 9.5 µL Nuclease free water (NFW). PCR amplification was achieved with one cycle of an initial denaturation at 94°C for 2 minutes, followed by 30 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and elongation/extension at 72°C for 2 minutes and one cycle for a final extension at 72°C for 2 minutes. The PCR products were further analyzed by electrophoresis in 0,1% agarose gel and visualized using UV transilluminator. The PCR products were then sequenced through sequencing service company. Sequencing results were aligned with the data on GenBank using the Blast-N (Basic Local Alignment Search Tool-Nucleotida) program from the National Center for Biotechnology Information (NCBI) website.

Data analysis

All treatments were repeated three times. The data obtained were collected in MS Excel and analyzed using analysis of variance (ANOVA). The significance of the parameters was tested using the least significant difference multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Morphological characteristics of actinomycetes

All actinomycetes isolates had aerial mycelium with different types of spore chains. Isolates AKT19, AKT28 and AKT52 had the same spore chain shape, namely rectiflexibles and isolates coded AKTb, AKT41, AKT56 and AKT57 showed verticilliate spore chains (Table 1). This is in accordance with Liu et al. (2016), who reported that the genus *Streptomyces* has classic polyspores that form long chains and have more than 50 spores or commonly referred to as spore chains. Several types of spore chains that show characteristics of the genus *Streptomyces* include rectiflexibiles, retinaculiaperti, spira and verticillate. In addition, *Streptomyces* colonies were usually covered with aerial hyphae. The color of actinomycetes colonies of isolates AKT19, AKT28 and AKT52 were grey and produced yellow pigment on ISP 2 and YSA media. AKTb and AKT41 isolates showed brown colony color with brown pigment on medium. Colony colors on AKT56 and AKT57 isolates were grey, brown and both produced brown pigment in ISP2 and YSA growing medium (Table 1).

Streptomyces belongs to the Streptomycetaceae family, and suborder Streptomycineae. *Streptomyces* grow as filamentous mycelia in the soil. The adult actinomycetes colony consists of two different types of mycelia, namely substrate mycelium (vegetative) and air mycelium (aerial mycelium). Substrate mycelium and aerial mycelium have different roles. Substrate mycelium plays a role in absorbing nutrients needed for growth (Hastuti 2014). Substrate mycelium can penetrate the substrate or grow on the surface, while aerial mycelium grows vertically. Aerial mycelium covers the surface of colony so that the colony looks hairy and powdery (Islam et al. 2014). Aerial mycelium acts as a reproductive organ, when environmental nutrients are reduced, aerial mycelium develops to form a spore chain like the adult phase in its life cycle. The spores germinate and develop into a new mycelium (Koepff et al. 2018)

Growth inhibition of *Ganoderma boninense* by actinomycetes

Seven actinomycetes isolates tested by dual culture technique showed inhibition of *G. boninense* from 33.3 to 100%. AKT19, AKT28 and AKT52 isolates showed the highest inhibition up to 100% (Table 2). All the three isolates showed widest zone of inhibition compared to other isolates (Figure 1). AKT41 and AKT57 isolates showed 45% and 50.13% percentage of inhibition, respectively, while the lowest percentage of inhibition was shown by AKT56 isolates with a value of 33.33% (Table 2). One of the characteristics of actinomycete group of bacteria as biological control agents against several soil-borne pathogens is that they are able to produce bioactive compounds that can effectively suppress the growth of pathogens (Adegboye and Babalola 2012).

Table 2. Growth inhibition of *Ganoderma boninense* by actinomycetes

Actinomycetes isolates	Growth inhibitions of *G. boninense* (%)
AKT b	45.0 ± 5.00 bc
AKT19	100.00 ± 0.00 a
AKT28	100.00 ± 0.00a
AKT41	56.43 ± 12.38b
AKT52	100.00 ± 0.00a
AKT56	33.33 ± 9.11c
AKT57	50.13 ± 9.48bc

Note: Data are the means ± standard deviation of three replicates. The numbers in each treatment in the same column followed by the same letter show that they are not significantly different according to the Tukey test at the 5% level.

Tabel 1. Morphological characteristics of actinomycetes isolates

Parameters	Actinomycetes isolates						
	AKTb	AKT19	AKT 28	AKT41	AKT52	AKT56	AKT 57
Aerial mycelium	+	+	+	+	+	+	+
Spore chain shape							
Rectiflexibiles	-	+	+	-	+	-	-
Spirales	-	-	-	-	-	-	-
Vertilicilate	+	-	-	+	-	+	+
Rectinaculiaperti	-	-	-	-	-	-	-
Colony color, mycelium and pigment on YSA medium							
Colony color	White	Brown	Grey	Brown	Grey	Brown	Brown
Pigment production to media -		-	-	-	-	-	-
Under colony	White	Brown	Grey	Brown	Grey	Brown	Brown
Colony color, mycelium and pigment on ISP2 medium							
Colony color	Brown	Grey	Grey	Brown	Grey	Grey	Brown
Pigment production to media	Brown	Yellow	Yellow	-	Yellow	-	Brown
Under colony	Brown	Yellow	Yellow	Brown	Yellow	Brown	Brown

Note: +: Presence of spore chain; -: absence of spore chain.

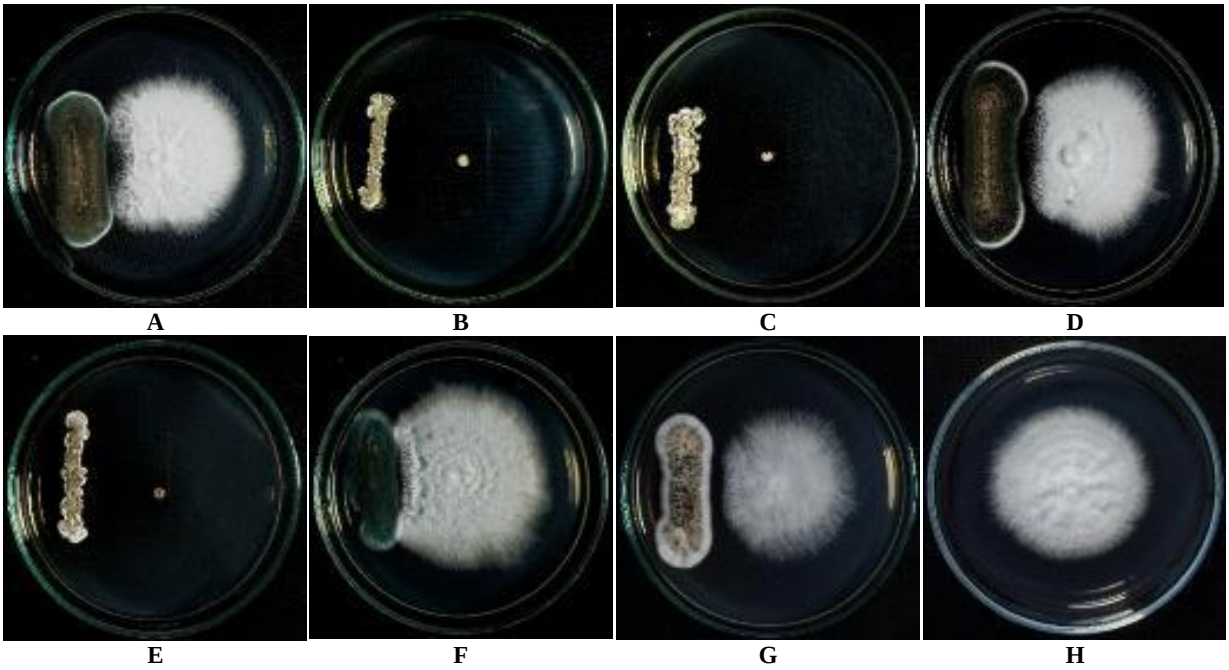


Figure 1. Dual culture test between actinomycetes isolates and *Ganoderma boninense* A) Actinomycetes isolate AKTb vis *G. boninense;* B) AKT19 vis *G. boninense;* C) AKT28 vis *G. boninense;* D) AKT41 vis *G. boninense;* E) AKT52 vis *G. boninense;* F) AKT56 vis *G. boninense;* G*)* AKT57 vis *G. boninense* 7; H) Control

Three of the seven actinomycetes isolates from peat soil, namely AKT19, AKT28 and AKT52 isolates were able to inhibit the growth of *G. boninense* up to 100% (Figure 1). This is in line with previous studies which reported that actinomycetes could suppress fungal growth ranging from 80% to 100% (Muzaimah et al. 2015). *Streptomyces* spp. showed strong antagonistic activity against the dikaryotic mycelium of *G. boninense* with the percentage of inhibition reaching more than 70% (Yusoff et al. 2021). Degradation of the cell wall of *G. boninense* resulted in abnormal hyphae morphology and changes in hyphae morphology resulted in abnormal growth of *G. boninense* (Suryanto et al. 2012). In the dual culture test conducted by Lim et al. (2018), bioactive compounds produced by *Streptomyces* spp. played an important role in inhibiting the growth and density of *G. boninense*. Actinomycetes are also known to suppress the growth of other soil-borne pathogens such as *V. dahliae, F. oxysporum, B. cinerea, R. solani,* and *A. flavus* (Aouar et al. 2020).

Effect of VOCs and bioactive compounds on the growth of *Ganoderma boninense*

The effect of VOC produced by actinomycetes isolates on the growth of *G. boninense* was less effective. The relative inhibition ranged from 2.87% (AKT41) to 20.23% (AKT52). Several actinomycetes isolates indicated no real difference in suppressing *G. boninense* growth (Table 3). Previous research indicated that volatile organic compounds produced by actinomycetes showed an inhibition on the growth of plant pathogens (Wang et al. 2013). Several VOC compounds produced by the genus *Streptomyces* which are known to suppress the growth of soil-borne pathogens are trans-1,10-dimethyl-trans-9-decalol (geosmin) and 2-methylisoborneol (MIB). This compound is also responsible for the odor produced by actinomycetes (Jiang et al. 2007). VOC compounds produced by the *Streptomyces* genus can cause abnormal morphology of pathogen and suppress the growth of pathogenic hyphae (Cordovez et al. 2015).

Streptomyces platensis F-1 produces VOC compounds which are antifungal against *Rhizoctonia solani*, *S. sclerotiorum*, and *Botrytis cinerea* (Wan et al. 2008). Similarly, VOCs produced from *Streptomyces globisporus* JK1 were able to suppress *Penicillium italicum* (Li et al. 2010). VOC compounds produced by *Streptomyces albulus* NJZJSA2 is known to inhibit mycelia growth of pathogenic fungi. The VOC compound produced by *Streptomyces albulus* NJZJSA2 was able to inhibit the growth of *Fusarium oxysporum* by 56.3% and inhibit *Sclerotium sclerotiorum* up to 100% after 60 days after incubation. The resulting VOC cannot kill fungal mycelia, however only inhibits the growth of fungus (Wu et al. 2015).

Results of present investigation showed that the bioactive compounds of actinomycetes supernatant at a concentration of 10% varied in inhibiting the growth of *G. boninense* (Table 3). The three highest relative inhibition percentages i.e., 89.17%, 88.83%, and 86.50% were shown by AKT19, AKT 52 and AKT52. Meanwhile, AKT41 isolate did not show any inhibitory activity on the growth of *G. boninense*. Actinomycetes are known to be producer of bioactive compounds that can act as antifungals such as ribostamycin, benxylmalic, landomycin and Salinomycin (Lim et al. 2018). Several strains of bacteria belonging to the genus *Streptomyces* can also produce antibiotics such as

vancomycin, erythromycin, tetracycline, streptomycin and chloramphenicol (Hasani et al. 2014). These compounds are capable of damaging cell walls, disrupting cell membrane function, and interfering with protein and nucleic acid synthesis of fungal pathogens (Purnomo et al. 2017).

Based on the ability to inhibit *G. boninense* growth, three potential isolates, namely AKT19, AKT28 and AKT52 were further investigated at different level of concentration. Result revealed that AKT19 isolates showed the strongest inhibition of *G. boninense* at a concentration of 0.5% which suppressed growth by 70.93%, while isolates AKT 28 and AKT52 inhibit only 15.30% and 4.80% growth, respectively. Bioactive compounds at low concentrations can able to suppress the mycelial biomass of *G. boninense*. The concentration of metabolites added to the growth media showed differences in the biomass of *G. boninense* (Table 4).

The data indicated that actinomycetes AKT19 isolate had a very strong activity to inhibit the growth of *G. boninense*. Previous result reported that application of bioactive compounds with a concentration of 4% suppressed the growth of several pathogenic fungi such as *G. boninense*, *Alternaria* sp., *Fusarium* sp., *Rhizopus stolonifer, A. niger., A. flavus, Alternaria* sp., *Fusarium* sp., *Rhizopus stolonifer, Pythium ultimum* and *Helminthosporium* (Sharma and Parihar 2010). Reduced biomass and decreased growth are due to fungal malformations such as thickened, shortened hyphae structures, hyphae fragments that are destroyed and undergo lysis (Azura et al. 2016).

Characteristic of selected actinomycetes in production of chitinase, glucanase and cellulase

All the selected actinomycetes isolates were able to degrade chitin, glucan and cellulose substrates as indicated by the presence of a clear zone close to the colony. The highest glucanase, chitinase and cellulase indexes were shown by isolate AKT28 (Table 5).

These characteristics can support the development of actinomycetes isolates as biological agents. For example, the ability to produce chitinase enzymes is thought to increase the ability to suppress the development of *Ganoderma boninense* through the degradation of chitin as a constituent of fungal cell walls. Actinomycetes can produce chitin and glucan-degrading enzymes which are the main components of the cell wall of Ganoderma (Ma et al. 2019). According to Prapagdee et al. (2008), enzymes that are generally produced by bacteria belonging to the genus *Streptomyces* are chitinase enzymes and 1.3 β-glucanase which can lyse chitin and glucan compounds in fungal cell walls, respectively. Actinomycetes are a group of microorganisms that have an significant role in producing cellulase enzymes to decompose organic material (Das et al. 2014).

Physiological characterization of selected actinomycetes as bio-fertilizers and growth promotor

Further characterization of selected isolates indicated that none of the isolates produced phosphatase, but only AKT28 isolate has the ability to fix nitrogen. All the isolates produced indole acetic acid, promote plant growth (Table 6).

Table 3. Growth inhibition of *Ganoderma boninense* by volatile organic compound and bioactive compound actinomycetes isolates

Actinomycetes isolates	Growth inhibition of *G. boninense*	
	Relative inhibition by VOC (%)	Relative inhibition by bioactive compounds
AKTb	7.97 ± 6.91a	37.50 ± 33.07ab
AKT19	12.27 ± 11.38a	89.17 ± 3.82a
AKT28	17.50 ± 9.62a	88.33 ± 3.82a
AKT41	2.87 ± 4.96a	0.00 ± 0.00b
AKT52	20.23 ± 18.12a	86.50 ± 6.206a
AKT56	4.47 ± 4.30a	27.50 ± 23.84b
AKT57	16.53 ± 18.87a	18.77 ± 32.50b

Note: Data are the means ± standard deviation of three replicates. The numbers in each treatment in the same column followed by the same letter show that they are not significantly different according to the Tukey test at the 5% level.

Table 4. Growth inhibition of *Ganoderma boninense* at different concentration of bioactive compound produced by selected actinomycetes isolates

Conc. (%)	Growth inhibition of *G. boninense* (%)		
	AKT19	AKT28	AKT52
0	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
0.5	70.93 ± 7.01b	15.30 ± 9.05b	4.80 ± 2.65b
1.0	74.23 ± 6.98ab	13.97 ± 16.52b	4.70 ± 5.28b
1.5	75.93 ± 1.62ab	27.90 ± 15.32ab	8.97 ± 5.87b
2.0	73.40 ± 3.34ab	27.77 ± 14.94ab	7.40 ± 6.39b
2.5	74.37 ± 3.61ab	17.87 ± 14.49b	12.00 ± 18.04b
3.0	83.67 ± 2.22b	61.80 ± 10.36a	49.53 ± 21.38a

Note: Data are the means ± standard deviation of three replicates. The numbers in each treatment in the same column followed by the same letter show that they are not significantly different according to the Tukey test at the 5% level.

Table 5. Ability of three actinomycetes isolates to degrade chitin, glucan and cellulose substrates

Isolates	Glucanase index	Chitinase index	Cellulose index
AKT19	0.31 ± 0.07b	018 ± 0.04a	1.09 ± 0.51a
AKT28	0.39 ± 0.03b	0.31 ± 0.09a	1.27 ± 0.37a
AKT52	0.27 ± 0.11b	0.25 ± 0.04a	1.10 ± 0.10a

Note: Data are the means ± standard deviation of three replicates. The numbers in each treatment in the same column followed by the same letter show that they are not significantly different according to the Tukey test at the 5% level.

Table 6. Production of phosphatase, nitrogen fixation, and IAA by actinomycetes isolates

Isolates	Phosphatase index	Nitrogen fixation	Producer IAA
AKT19	-	-	+
AKT28	-	+	+
AKT52	-	-	+

Gupta et al. (2014) reported that bacterial isolates with ability to produce phosphatase, nitrogen fixation and secreting IAA are able to increase plant growth. Phosphate and nitrogen are essential in the synthesis of chlorophyll, proteins, enzymes, DNA and RNA (Santi et al. 2013). One of the characteristics of bacteria known to be able to dissolve nitrogen is to form a pellicle or ring and cause a change in the color of NfB medium from yellow to bluish yellow (Islam et al. 2019). Furthermore, IAA is significant hormone in stimulating plant root development, increasing resistance to pathogens and promoting plant growth (Joshi and Bath 2011).

Molecular identification of selected actinomycetes

Amplification of 16S rRNA gene successfully resulted DNA fragment (amplicon) with a size of about 1200 base pair as shown in Figure 3. Alignment of all sequences of the amplicon to the GenBank database indicated that all actinomycetes isolates have similarities with *Streptomyces gelaticus* strain NRRL B-2928 originating from the United States (Table 7). Based on research conducted by Osman et al. (2020), *Streptomyces gelaticus* is known to act as a biocontrol agent in suppressing the growth of *Alternaria solani* and also *Botrytis fabae* in tomato plants.

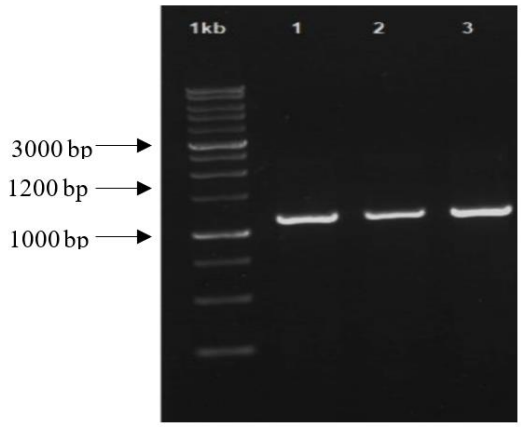
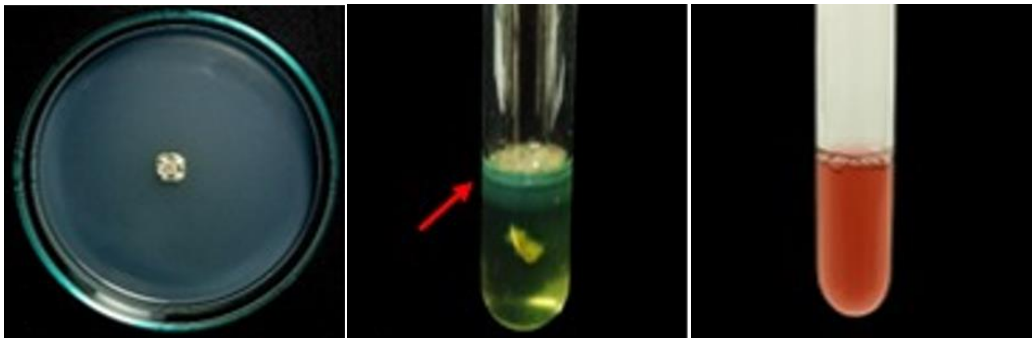


Figure 3. Visualization of amplified actinomycetes potential DNA bands using specific primers actinomycetes 27F/16Scatt111R on 0.1% agarose gel with 1 kb DNA marker. (1) AKT19, (2) AKT28, (3) AKT52



A **B** **C**

Figure 2. Characterization potential actinomycetes as biofertilizers and plant growth promoters A) phosphate solubilizing activity, B) nitrogen fixation activity, C) IAA hormone

Table 7. Homology of nucleotide sequences of AKT19, AKT28 and AKT52 isolates

Isolates	Isolates in GenBank	Query cover	Identity (%)	Accession number
AKT19	*Streptomyces gelaticus* strain NRRL B-2928	100%	99.73%	NR_043488.1
	S. gelaticus strain NBRC 12866	100%	99.73%	NR_112308.1
	S. atratus strain NRRL B-16927	100%	99.55%	NR_043490.1
	S. atratus strain NBRC 3897	100%	99.55%	NR_112503.1
	S. sanglieri strain NBRC 100784	100%	99.46%	NR_041417.1
AKT28	*Streptomyces gelaticus* strain NRRL B-2928	100%	99.54%	NR_043488.1
	S. gelaticus strain NBRC 12866	100%	99.54%	NR_112308.1
	S. sanglieri strain NBRC 100784	100%	99.45%	NR_041417.1
	S. atratus strain NRRL B-16927	100%	99.36%	NR_043490.1
	S. atratus strain NBRC 3897	100%	99.36%	NR_112503.1
AKT52	*Streptomyces gelaticus* strain NRRL B-2928	100%	99.63%	NR_043488.1
	S. gelaticus strain NBRC 12866	100%	99.63%	NR_112308.1
	S. sanglieri strain NBRC 100784	100%	99.54%	NR_041417.1
	S. atratus strain NRRL B-16927	100%	99.45%	NR_043490.1
	S. atratus strain NBRC 3897	100%	99.45%	NR_112503.1

Conclusion

The selection results obtained three potential isolates with inhibitory abilities *G. boninense* growth of 33.33 to 100% was shown by isolate AKT19, AKT28, and AKT52. The effect of VOC compounds and bioactive compounds on potential isolates suppressed the growth of *G. boninense* with sequential inhibition ranging from 12.27 to 20.23% and 86.50 to 89.17%. Isolates AKT19, AKT28, and AKT 52 were able to degrade glucan, chitin, and cellulose, produce IAA hormone, and cannot dissolve phosphate but isolate AKT28 was able to bind nitrogen. Molecular identification shows that isolates AKT19, AKT28, and AKT52 had the highest homology with *Streptomyces gelaticus* strain NRRL B-2928 originating from the USA.

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