Antagonistic potential of *Streptomyces cellulosae* SM12 against *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4

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**Abstract.** A study was carried out to screen 25 Actinomycetes isolates that have ability to act as biocontrol agents Actinomycetes against *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4. Among 25 Actinomycetes isolates, only Actinomycetes SM12 can inhibit the growth of both *Ganoderma* sp. TB3 (52 %) and *Ganoderma* sp. TB4 (60 %). Antagonistic assay using cross streak method with a longer period of delayed antagonism showed higher inhibition effect to the growth of *Ganoderma* sp. TB3 (76 %) and *Ganoderma* sp. TB4 (85 %). Identification of Actinomycetes SM12 based on 165 rRNA revealed that Actinomycetes SM12 belong to *Streptomyces cellulosae*. The Actinomycetes SM12 isolate produces rectinaculum-apertum spore chain and forms a colony that exhibited white to greyish color. Enzyme activity test showed that the cultures are positive in amylase, protease, lipase and cellulase production respectively.

**Keywords:** Antagonistic, *Ganoderma* sp., *Streptomyces cellulosae*

1. Introduction

Actinomycetes is known to produce many bioactive compounds such as antibiotics, antifungal substances, plant growth factors and enzymes [1]. Research that has been done by Fadhilah et al. [2] successfully isolated 28 isolates of Actinomycetes from Pramuka Island, Kepulauan Seribu, DKI Jakarta. Tan et al. [3] and Shariffah-Muzaimah et al. [4] stated that Actinomycetes are able to control the growth of *Ganoderma* spp.  

As a fungal pathogen, *Ganoderma* spp. was able to infect the stem [5] and root [6] of plants and can infect more than 44 species from 30 plant genera [5]. Oil palm tree or Kelapa sawit (*Elaeis guineensis*) [5] and *Acacia* sp. [7] are the main targets of *Ganoderma* spp. infection. Infection of fungal pathogen may result in serious problems for the agricultural industry, especially oil palm industry.  

Actinomycetes isolates from the mangrove ecosystem at Pramuka Island were assumed to have antagonistic activity towards *Ganoderma* spp. The purpose of the research is to screen the antagonistic potential of the collected Actinomycetes against *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4 also to characterize the morphology, enzyme properties and to identify the selected a Actinomycetes.

2. Materials and method

Twenty five Actinomycetes isolates, *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4 were provided from Microbiology Laboratory, Department of Biology, FMIPA Universitas Indonesia. The Actinomycetes...
have been isolated from mangrove sediment of Pramuka Island, Kepulauan Seribu, DKI Jakarta. Actinomycetes isolates were grown in Cross Streak Medium (CSM) agar containing 0.3 % yeast extract, 0.3 % peptone, 0.3 % casein, 0.8 % soluble starch, 0.05 % K2HPO4, 0.05 % MgSO4.7H2O, 0.2 % NaCl and 1.5 % agar [8]. *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4 were isolated from forest area in Universitas Indonesia. Both isolates were purified and cultured in Potato Dextrose Agar (PDA).

### 2.1. Screening of antagonistic activity

Screening was carried out using the dual plug method [9]. The Actinomycetes isolates were streaked into Cross Streak Medium (CSM) agar plate, and allowed to grow until sporulation (5–7 days). *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4 were also inoculated into Potato Dextrose Agar (PDA) plates and incubated for 5–7 days at 30 °C. The edge of the Actinomycetes colonies was plugged (Ø 5 mm) and placed a side on a fresh PDA plate and allowed to grow for 5 days. Then, after delayed for 5 days, *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4 were also plugged (Ø 5 mm) and placed 2 cm away from Actinomycetes colony in PDA plates. The dual cultures plates were incubated at 30 °C for 5–7 days. The results of screening were indicated by the width of treated *Ganoderma* sp. isolates and calculated using Percent Inhibition of Radial Growth (PIRG) formula as follows [4]:

$$PIRG = \frac{\text{width of mycelium control} - \text{width of mycelium treated}}{\text{width of mycelium control}} \times 100\%$$

### 2.2. Longer delayed antagonistic test of selected Actinomycetes

The Actinomycetes isolate with the highest PIRG was selected and used for longer delayed antagonistic test culture. The selected Actinomycetes was grown in ‘Zhang Starch Soil Extract (ZSSE) agar which contains 0.5 % soluble starch, 0.1 % KNO3, 1 % agar and 1,000 mL soil extract [10]. The Actinomycetes isolate was incubated for 13 days at 30°C until it sporulated heavily. The test was done in similar conditions as the screening method but the delay period of *Ganoderma* sp. isolates inoculation were extended. Selected Actinomycetes was inoculated, 9 days prior to *Ganoderma* sp., into PDA plates. The dual culture was incubated for 7 days at 30 °C.

### 2.3. Characterization of selected Actinomycetes

The selected isolate Actinomycetes from the screening was observed macroscopically on ZSSE agar. Features such as surface colony, reverse colony, and colony texture were collected. Microscopic of Actinomycetes was observed on slide culture under Leica DM 500 microscope and the measurement was done using Leica LAS EZ 3.0 software program. The selected Actinomycetes isolate was also tested for the production of different extracellular enzymes such as amylase, cellulase, protease, lipase and chitinase.

#### 2.3.1. Amylase activity.

The medium used for amylase activity test was starch agar which contains 0.3 % meat extract, 0.5 % peptone, 0.2 % soluble starch and 1.5 % agar [11]. Selected Actinomycetes was streaked on starch agar plate and incubated at 30 °C for 7 days. After the incubation period, 1 % of iodine solution was flooded on the starch agar plates and clear zone of starch hydrolysis was considered as amylase positive [11, 12].

#### 2.3.2. Cellulase activity.

Cellulase activity test was done on Carboxymethylcellulose (CMC) agar which contains 0.05 % CMC, 0.01 % NaNO3, 0.01 % K2HPO4, 0.005 % MgSO4, 0.005 % yeast extract and 1.5 % agar [13]. Selected Actinomycetes was streaked on CMC agar and incubated for 7 days at 30 °C. After the incubation period, the plate was flooded with 0.1 % Congo red and left for 30 minutes. Excess of Congo red was removed and the plate is flooded again with NaCl 1 M and left for 30 minutes. Appearance of clear zone around the isolate indicates cellulolytic activity [14].
2.3.3. Protease activity. The selected Actinomycetes isolate was streaked on Skim Milk Agar (SMA) which contains 0.3 % yeast extract, 0.5 % NaCl, 1.8 % agar and 1 % skim milk [15]. Plates were incubated for 7 days at 30 °C. A clear zone of skim milk hydrolysis gave indication of a protease producing microorganism [12, 15].

2.3.4. Lipase activity. Lipolytic enzyme activity was carried out in Tween-20 agar that was prepared based on modified method of Gopinath et al. [16]. The selected Actinomycetes was streaked on Tween-20 agar which contains 1 % Peptone, 0.5 % NaCl, 0.01 % CaCl₂, 2 % agar and 1 % Tween-20. Selected Actinomycetes was inoculated on tween-20 agar, then was incubated for 7 days at room temperature. Lipolytic enzyme activity was indicated by the presence of calcium salts precipitate around the colony [16].

2.3.5. Chitinase activity. The selected Actinomycetes isolate was streaked on Colloidal Chitin Agar (CCA) which contains 0.07 % KH₂PO₄, 0.03 % K₂HPO₄, 0.05 % MgSO₄, 0.0001 % FeSO₄, 0.0001 % ZnSO₄, 0.0001 % MnCl₂, 2 % agar and 0.5 % colloidal chitin. Preparation of colloidal chitin was done according to Hsu et al. [17]. Selected Actinomycetes on CCA was incubated for 7 days at 30 °C. Positive result of chitinase activity was indicated by appearance of clear zone around the colony [17].

2.4. Molecular identification of selected Actinomycetes

Amplification of 16S ribosomal sequence from DNA template of selected Actinomycetes was carried out by using the primer (27F 5’ (AGAGTTTGATCMTGGCTCAG) 3’) and (1492R 5’ (TACGGYTACCTTGTTACGACTT) 3’) [18] in a thermal cycler (SimpliAmp, Applied Biosystem). The master mix for PCR reaction used Go Taq Green for 25ul reaction (Promega). The cyclic conditions were as follows: initial denaturation at 94 °C for 3 min, denaturation with 35cycles at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 2 min and finally held at 4 °C. The PCR products (1500 bp) of the sample were identified by 1 % agarose gel electrophoresis. Automated sequencing was carried out using the primer (785F 5’ (GGATTAGATACCCTGGTA) ‘3) and (907R 5’ (CCGTCAATTCMTTTR AGTTT) 3’) [19] provided by Macrogen (Korea). Species identification using neighbour joining in Mega X software with selection from BLAST result with minimum >99 % sequences similarity [20].

3. Results and discussion

3.1. Screening of antagonistic activity

The screening results revealed that some of the isolates have antagonistic activity (table 1) but only one isolate, Actinomycetes SM12 that has antagonistic activity against both of Ganoderma sp. TB3 and Ganoderma sp. TB4. Based on the PIRG value (table 1), only Actinomycetes SM12 isolate was selected for further study.

3.2. Longer delayed antagonistic test

Result from delayed antagonistic test exhibited that Actinomycetes SM12 (figure 1) gave higher PIRG value. Table 2 showed that PIRG value of delayed antagonistic test (9 days) was 76 % for Ganoderma sp. TB3 and 85 % for Ganoderma sp. TB4. This result indicates that delayed antagonistic test will increase PIRG value from previous antagonistic test (table 1) which have PIRG of Ganoderma sp. TB3 (52 %) and Ganoderma sp.TB4 (60 %). Actinomycetes belongs to a group of bacteria which usually grow slowly. On the other hand, Ganoderma spp. is a fungi which have hyphae and grow faster than actinomycyes. Inoculation of Actinomycetes SM12, 9 days prior to Ganoderma was assumed to give more time for Actinomycetes SM12 to grow and reach the stationary phase. In the stationary phase, the bacteria will produce many secondary metabolites including the antifungal substances. That may have caused inhibition of the growth of Ganoderma SM12 to increase. Shariffah-
Muzaimah et al. [4] stated that pre-inoculation of Actinomycetes will allow the bacteria to grow first prior to *Ganoderma* sp. The delay will give advantage to Actinomycetes to colonize the substrate and more become more competitive against the pathogen.

3.3. Characterization of selected Actinomycetes

Selected Actinomycetes SM12 that grew on ZSSE agar exhibited white to greyish surface colony with brown ochre reverse colony color. The texture of Actinomycetes SM12 colony is powdery. Actinomycetes SM12 under the microscope (figure 2) showed formation of branching rectinaculum-apertum (RA) spore chain which is straight, wavy or spiral on the tip of its chain [21]. Its spore size ranging from 1–2 μm. The thickness from each of Actinomycetes SM12 hypha ranging from 0.4 until 1.2 μm.

Actinomycetes SM12 isolate was subjected to various kind of enzyme tests such as amylase, protease, cellulase, lipase and chitinase. Results showed that Actinomycetes SM12 gave positive results to all of enzyme tests (figure 3). Various kind of enzymes will give benefit to allow actinomyces SM12 to use many kinds of substrates. The Actinomycetes play important role in decomposition,

### Table 1. Percent Inhibition of Radial Growth (PIRG) of Actinomycetes by plug method.

| Isolates | PIRG (%) | Isolates | PIRG (%) |
|----------|----------|----------|----------|
|          | *Ganoderma* TB3 | *Ganoderma* TB4 | *Ganoderma* TB3 | *Ganoderma* TB4 |
| SM 1     | 0.00      | 0.00      | SM 14     | 22.97     | 0.00      |
| SM 2     | 0.00      | 0.00      | SM 15     | 32.86     | 0.00      |
| SM 3     | 0.00      | 0.00      | SM 16     | 0.00      | 0.00      |
| SM 4     | 10.76     | 0.00      | SM 17     | 0.00      | 0.00      |
| SM 5     | 0.00      | 0.00      | SM 18     | 29.68     | 0.00      |
| SM 6     | 0.00      | 0.00      | SM 19     | 0.00      | 0.00      |
| SM 7     | 49.24     | 0.00      | SM 20     | 31.14     | 0.00      |
| SM 8     | 0.00      | 0.00      | SM 21     | 0.00      | 0.00      |
| SM 9     | 0.00      | 0.00      | SM 22     | 0.00      | 0.00      |
| SM 10    | 0.00      | 0.00      | SM 23     | 0.00      | 0.00      |
| SM 11    | 21.62     | 0.00      | SM 24     | 0.00      | 0.00      |
| SM 12    | 52.86     | 60.91     | SM 25     | 0.00      | 0.00      |
| SM 13    | 0.00      | 0.00      |           |           |           |

**Figure 1.** Longer delayed antagonistic test using dual plug method from Actinomycetes SM12: (a) control *Ganoderma* sp. TB3, (b) Actinomycetes SM12 against *Ganoderma* sp. TB3, (c) control *Ganoderma* sp. TB4, and (d) Actinomycetes SM12 against *Ganoderma* sp. TB4.
Table 2. Longer delayed antagonistic test using dual plug method of 9 days Actinomycetes SM12 towards *Ganoderma* sp. after 7 days incubation period.

| Isolate     | Mean of treated mycelium (mm) | Control mycelium width (mm) | PIRG (%) | Inhibition zone (mm) |
|-------------|-------------------------------|----------------------------|----------|----------------------|
| *Ganoderma* sp. TB3 | 8.89                          | 37.09                      | 76       | 9.81                 |
| *Ganoderma* sp. TB4 | 4.81                          | 32.94                      | 85       | 11.98                |

especially in complex polymer [22]. The ability of Actinomycetes SM12 to produce chitinase will benefit the bacteria as biocontrol because chitinase is a potential antifungal agent which can suppresses pathogenic fungal plant [23].

3.4. Molecular Identification of selected Actinomycetes

Based on analysis of 16s rRNA sequencing using primer 785F 5' (GGA TTA GAT ACC CTG GTA) 3' and 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3' and phylogenetic tree analysis (figure 4), Actinomycetes SM12 isolate was closely related to *Streptomyces cellulosae*. Neighbour joining analyses was conducted comparing 10 sequences with sequence similarity >99 % (NR_043815.1, NR_112346.1, LC034307.1, NR_043835.1, NR_112329.1, NR_041158.1, NR_112379.1, NR_112338.1, NR_112511.1, and NR_112390.1). The Actinomycetes SM12 is grouped into one clade with *Streptomyces cellulosae*.

![Figure 2. Microscopic morphology of 48 hours Actinomycetes SM12 slide culture.](image)

![Figure 3. Enzymes activity test of Actinomycetes SM12 including (a) amylase, (b) cellulase, (c) protease, (d) lipase and (e) chitinase results, (arrow: area of positive enzyme activity).](image)
Figure 4. Phylogenetic analyses of Actinomycetes SM12.

4. Conclusion
Among 25 isolates that were screened for their antagonistic activity against Ganoderma sp. TB3 and Ganoderma sp. TB4, Actinomycetes SM12 was the most potential isolate. Based on enzyme activity tests of Actinomycetes SM12, the isolate has the ability to synthesize extracellular enzymes such as amylase, cellulase, protease, lipase and chitinase. Molecular identification using 16S rRNA reveals that Actinomycetes SM12 was belong to Streptomyces cellulosae.

Acknowledgments
The research was financially supported by Grant PITTA B from Universitas Indonesia to Dr. rer. nat Yasman M.Sc (No. 476/SK/R/UI/2019).

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