Identification of antagonistic bacteria against peanut stem rot disease (*Sclerotium rolfsii* Sacc.) on the peatland of Kuala Pesisir-Nagan Raya, Indonesia

I Subandar\(^1\), L Hakim\(^2\)*, I Suliansyah\(^3\) and S Syakur\(^2\)

\(^1\)Student of Doctoral Program of Agricultural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia
\(^2\)Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, Indonesia
\(^3\)Faculty of Agriculture, Universitas Andalas, Padang, Indonesia
\(^4\)Faculty of Agriculture, Universitas Teuku Umar, West Aceh, Indonesia

Corresponding author: lkm_hakiem@unsyiah.ac.id

Abstract. Peanut cultivation in peatlands has major obstacles in terms of controlling plant diseases. This research aimed to identify and characterize bacteria which have the potential as biological control agents against *S. rolfsii* in the peatlands of Kuala Pesisir Nagan Raya. This research was conducted in the Kuala Pesisir sub-district, Nagan Raya District, Aceh, Indonesia. The research activities include isolation, morphological identification, pathogenicity testing, antagonistic testing, and molecular identification. After the first screening, 25 bacterial isolates were to be observed further. At the root of the peanut plants the colonies were mostly bacillus, some of them were coccus. In gram staining, there were many gram-negative isolates compared to gram positive. Non-pathogenic bacterial isolates have good growth potential (85\% - 100\%), except for B3 bacterial isolates (74\%). The peanut seeds in non-pathogenic bacteria that have good germination were in the range of 80\% - 96\% of the germination capacity. Peatland bacteria that interact with type D have the potential to act as antagonistic agents against *S. rolfsii*. There are bacteria which were promising to act as an antagonistic agent against *Sclerotium rolfsii* in the research area. Based on morphological and DNA characteristics, the bacteria were identified as *Bacillus* sp.

1. Introduction

The cultivation of peanuts (*Arachis hypogaea* L.) on peatlands have often encounter problems. Aside from problem such as drainage, acidic soil, nutrient and pest, disease aspect is also an important issue to address in planting crops on peat soil. Peatlands are rich in organic matter and have high acidity; hence, it is prone to develop plant diseases, especially those caused by fungi. One of the most commonly found fungi is peanut stem rot disease caused by *Sclerotium rolfsii* Sacc. The abundance of organic matter in peatlands provide sufficient nutrients for these pathogens to survive without any host; coupled with the highly acidic soil properties which are suitable for these pathogens to live, especially fungi.

Disease control with the use of pesticides will create new dilemmas for the environment [1]. The use of pesticides will only serve a temporary effect on pathogens. Moreover, the use of pesticides
without strictly following the recommended rules can adversely affect non-target organisms; which, in turn, it will actually benefit the pathogens in the future.

Soil pathogen fungi such as *S. rolfsii* can form sclerotia when environmental conditions are unfavorable [2]. The sclerotia can remain in the soil for a long time [3]. Hence, although the use of pesticides is effective against the active body of fungi (mycelium), it is not necessarily effective against sclerotia. The use of fungicides such as Benomyl, Oxyquinoline, and Hymexazol only affects about 50% of the viability of this fungus’ sclerotia [4]. While the rest can germinate whenever environmental conditions are favorable for the pathogen.

Biological control is one of the efforts potential to overcome peanut stem rot, namely by utilizing agents which are antagonistic and have the properties to inhibit pathogens. The use of antagonistic microorganisms as biological control agents is more environmentally friendly and sustainable. However, the success of biological control with the introduction of antagonistic agents from outside the region is susceptible to constraints regarding the adaptation ability of the new agents. Utilization of indigenous bacteria in the peatlands of Kuala Pesisir Nagan Raya can be a solution to the problem of peanut diseases, especially stem rot caused by *S. rolfsii*. Several known bacterial genus which are known to be antagonistic to *S. rolfsii* such as *Bacillus, Pseudomonas, Streptomyces, and Actinomycetes* [5,6,7].

Based on the description above, it is necessary to identify and characterize bacteria which have the potential as biological control agents against *S. rolfsii* in the peatlands of Kuala Pesisir Nagan Raya. This research is important considering that no information has been found related to antagonistic bacteria that has the potential to suppress *S. rolfsii*, the cause of peanut stem rot in the peatlands of Kuala Pesisir Nagan Raya.

2. Materials and methods
The research was conducted from July 2018 to March 2019 in Peatlands, Kuala Pesisir District, Nagan Raya Regency, Aceh Province, Indonesia. Isolation, identification, pathogenicity testing, and inhibition test were carried out at the Plant Disease Laboratory, Faculty of Agriculture, Syiah Kuala University. DNA sequencing was carried out by PT Genetika Science Indonesia. The materials used in this study were peat soil samples from weeds around the land area of research, peanut rhizosphere, and groundnut roots, *S. rolfsii* culture obtained from diseased plants, nutrient agar (NA), potato dextrose agar (PDA), aquadest, and alcohol. The tools used are tweezers, ose, 6 mm diameter mold, alcohol burner, measuring glass, beaker glass, Erlenmeyer flask, hotplate magnetic stirrer, Petri dish, autoclave, incubator, and shaker.

2.1. Peatland bacteria isolation
Bacteria from peatlands were isolated from peanut farm in Kuala Pesisir District, Nagan Raya District, Aceh Province. The sources of the inoculum came from three sources, namely the rhizosphere of weeds outside the area of cultivation of groundnuts (coded B), peanut rhizosphere (coded KT), and the roots of peanut (coded AK). Isolation using serial dilution method. Each isolate measured 1 ml was poured into a Petri dish containing NA (Nutrient Agar) medium. They were then incubated for three days at 28°C. The 10\(^{-4}\) dilutions were a series of dilutions taken to observe the growing bacteria. The growing bacteria were observed, then separated based on morphological differences in NA media. Each bacterial isolate was purified for further characterization testing.

2.2. Morphological identification
Bacteria were identified morphologically, including sizes (such as pinpoint, small, medium, large); color; colony forms (round, irregular, rooted); colony margins (entire, undulate lobate, serrate, and filamentous); elevations (include flat, raised, convex, and umbonate). The microscopic characteristics such as the shape (bacillus, coccus) and gram (+, -) were observed using the gram stain method [8].

---

---
2.3. Pathogenicity test
Peanut seeds were sterilized using CaClO$_3$ and hot water immersion. Then it is put into a Petri dish containing pure cultures of peatland bacteria. Nine seeds were placed in each isolate. Germination observations were observed at 7 DAI (day after inoculation) and 14 DAI. Healthy seeds showed that the bacteria are non-pathogenic. Pathogenicity testing was also carried out by injecting the bacteria into tobacco leaves, the areas showing necrosis indicated that the bacteria are pathogenic. To support this pathogenicity test, growth potential and germinability tests were also carried out [9]. The maximum growth potential (MGP) was observed at 14 days after planting using the formula:

$$\text{MGP} (\%) = \frac{\sum \text{seeds showing sprouting symptoms}}{\sum \text{seedling sown}} \times 100\%$$

The germinability (G) was observed based on the percentage of normal germination at the first (7 days) and the second (14 days) counts. Germinability was calculated using a formula:

$$\text{G} (\%) = \frac{\sum \text{calculate normal seedling I} + \text{calculate normal seedling II}}{\sum \text{seedling sown}} \times 100\%$$

2.4. Evaluation of the inhibition of $S. \text{rolfsii}$ on dual culture test
Pathogenic fungi molds were grown together with bacterial isolates to be tested for their antagonistic ability; both grown in a 10 mm petri dish. Pathogenic fungi isolates used a mold with a diameter of 5 mm, while the bacterial isolates to be tested for antagonism were scratched using ose with a location opposite the pathogenic fungi with a distance of 3 cm. The parameters observed were:

2.4.1. Pathogen mycelium radius (mm). Observation of the mycelium radius was carried out to determine whether the pathogen mycelium grown with the biological control agent candidates had increased or decreased growth.

2.4.2. The inhibition percentage (%). The percentage of inhibition of $S. \text{rolfsii}$ mycelium by potential antagonistic bacteria was calculated using the formula $H = 100 x (r1-r2)/r2$, where $H =$ percentage of $S. \text{rolfsii}$ fungus inhibition by antagonistic bacteria, $r1 =$ radius of $S. \text{rolfsii}$ fungal colonies which grow in the opposite direction to the antagonistic bacteria, and $r2 =$ the radius of the $S. \text{rolfsii}$ fungal colony that grow in the direction of the antagonist bacteria [10].

2.4.3. Interaction Type. Visually observing the type of interaction between pathogenic fungi and antagonistic bacteria was carried out at 4 DAI until one of the pathogens radii reached the edge of the petri dish. The types of interaction were observed namely (A) without interaction, the growth of both fungi and bacteria mingled with each other; (Bi) intermingling growth with overlapping blends; (Bii) intermingling growth where the growth of one of the isolates stops; (C) there is a little inhibition with an inhibition zone distance of 1 - 2 mm; and (D) inhibition occurs with an inhibition zone distance of > 2 mm [11].

2.4.4. Zone of inhibition distance (mm). Inhibition zone distance was measured if there was an interaction in category C or D in the types of interactions above.

3. Results and discussion
There were 67 bacterial colonies observed as the results of the isolation of the diversity of microorganisms on peatlands in the Kuala Pesisir Nagan Raya District. The isolates were obtained from three sources, namely the rhizosphere of weeds around peanut farm, peanut rhizosphere and peanut roots. Isolates that have morphological similarities with the other isolates were eliminated; except those which have similar characteristics to the biological control agents. It was hoped that different types of isolates will be found even though their morphological appearances are the same. Pathogenic isolates ($S. \text{rolfsii}$) were also not included in the observation. After the isolates that have similarities and pathogenic isolates were eliminated, 25 bacterial isolates were to be observed further.
### 3.1. Morphological characteristics

In general, the bacterial colonies found were of yellowish white, whitish yellow, white, and yellow colors. The size of these bacteria varied from pinpoint size, small, moderate and large. There are only two forms of colony observed, namely circular and irregular. Colony surfaces were flat, raised, convex, and umbonate. The edges of the colony were entire, lobate, serrate, filaments, undulate. In the observation under the microscope, the observed bacterial cell forms consisted of bacillus, coccus, diplobacillus, diplococcus, streptobacillus and streptococcus (Table 1).

**Table 1. Morphological characteristics of bacteria isolated from peatlands**

| Isolate | Color        | Size         | Shape       | Elevation | Margin  | Cell form       | Gram |
|---------|--------------|--------------|-------------|-----------|---------|-----------------|------|
| B1      | yellowish white | moderate     | irregular   | flat      | lobate  | bacillus        | -    |
| B2      | yellowish white | pinpoint     | circular    | convex    | entire  | coccus          | -    |
| B3      | yellowish white | pinpoint     | circular    | flat      | entire  | bacillus        | +    |
| B4      | yellowish white | pinpoint     | circular    | convex    | entire  | bacillus        | +    |
| B5      | yellowish white | pinpoint     | circular    | flat      | entire  | coccus          | +    |
| B6      | yellowish white | small        | irregular   | convex    | filament | bacillus        | -    |
| B7      | yellowish white | moderate     | circular    | flat      | serrate | bacillus        | -    |
| B8      | yellowish white | moderate     | circular    | umbonate  | serrate | coccus          | -    |
| KT1     | yellowish white | small        | circular    | raised    | undulate | streptobacillus | -    |
| KT2     | yellowish white | moderate     | circular    | flat      | undulate | bacillus        | +    |
| KT3     | yellowish white | small        | circular    | convex    | lobate  | coccus          | +    |
| KT4     | yellowish white | small        | circular    | flat      | undulate | diplobacillus   | -    |
| KT5     | yellowish white | moderate     | circular    | flat      | entire  | streptobacillus | -    |
| KT6     | yellowish white | moderate     | circular    | flat      | undulate | streptococcus   | -    |
| KT7     | yellowish white | pinpoint     | circular    | convex    | entire  | diplococcus     | +    |
| KT8     | yellow        | moderate     | irregular   | flat      | lobate  | streptococcus   | -    |
| KT9     | yellowish white | moderate     | circular    | convex    | lobate  | bacillus        | +    |
| KT10    | yellowish white | pinpoint     | circular    | convex    | serrate | bacillus        | +    |
| AK1     | whitish yellow | small        | circular    | convex    | entire  | coccus          | +    |
| AK2     | whitish yellow | pinpoint     | circular    | raised    | entire  | bacillus        | -    |
| AK3     | white         | large        | irregular   | flat      | lobate  | bacillus        | -    |
| AK4     | white         | moderate     | irregular   | convex    | lobate  | coccus          | +    |
| AK5     | whitish yellow | pinpoint     | circular    | convex    | entire  | bacillus        | -    |
| AK6     | yellowish white | pinpoint     | circular    | convex    | entire  | bacillus        | -    |
| AK7     | yellowish white | small        | circular    | convex    | entire  | bacillus        | +    |

Bacterial colonies obtained from the root areas of weeds around the plants showed that all colonies were yellowish white. Colonies of bacteria are mostly in dots, some were moderate, and some were small. Colonies were mostly round shaped and some were irregular. Most of the colony surfaces were flat, some were convex and some were umbonate. Colony margins were mostly flat, some colonies had serrate margins and some were in the form of filaments and lobate. In the observation under the microscope, the bacteria were dominated by the form of bacillus and coccus, most of them were gram-negative bacteria.

In the root zone of peanuts, colonies were mostly yellowish white and some were yellow. Most of the colonies were moderate in size, some were small, and some were dots. Some of the colonies were round in shape and some were irregular in shape. Most of the surface of the colony elevated flat, some were convex and some were raised. The edges of the colony were mostly undulate and lobate, a small
part of the entire and some were serrate shaped. The cell shape under the microscope was more diverse than the root zone of weeds and the roots of peanut plants. Cell forms were streptobacillus, bacillus, streptococcus, coccus, diplobacillus, and diplococcus. The bacterial colonies based on gram staining had almost the same number of isolates between gram positive and gram negative.

At the roots of the peanut plant, the color of the colonies were more diverse, namely white, whitish yellow, yellowish white and white. Most of the colonies were small and pinpoint and a few were moderate and large. The forms of the colonies were mostly round and partly irregular. Most colonies had a convex surface and some were flat and raised. The edges of the colony were mostly flat and some were lobate. The colonies were mostly bacillus, some of them coccus. In gram staining, there were many gram-negative isolates compared to gram positive.

3.2. Pathogenicity test

The results of bacterial pathogenicity testing on peanut seeds showed that B8, KT2, KT3, KT4, AK1, AK4, AK5, and AK6 were pathogenic to peanut seeds characterized by rot in germinated peanut seeds and necrosis on tobacco leaves. Non-pathogenic bacterial isolates have good growth potential (85% - 100%), except for B3 bacterial isolates (74%). The peanut seeds in non-pathogenic bacteria that have good germination were in the range of 80% - 96% of the germination capacity, while the lowest germination was found in isolate B3 (69%) (Table 2).

Table 2. Pathogenicity test, growth potential and germination of peanut seeds against bacteria isolate from peatlands

| Isolate | Pathogenicity | growth potential | Germinability |
|---------|---------------|------------------|--------------|
| control | +             | 0.00 a           | 0.00 a       |
| B1      | -             | 81.48 efgh       | 77.78 efg    |
| B2      | -             | 88.89 fgh        | 83.33 fgh    |
| B3      | -             | 74.07 defg       | 68.52 def    |
| B4      | -             | 85.19 efgh       | 77.78 efgh   |
| B5      | -             | 92.59 gh         | 79.63 efgh   |
| B6      | -             | 85.19 efgh       | 79.63 efgh   |
| B7      | -             | 96.30 h          | 87.04 gh     |
| B8      | +             | 66.67 cde        | 59.26 bcd    |
| KT1     | -             | 96.30 h          | 85.19 fgh    |
| KT2     | +             | 70.37 cdef       | 61.11 cd     |
| KT3     | +             | 66.67 cde        | 53.70 bcd    |
| KT4     | +             | 62.96 bcd        | 53.70 bcd    |
| KT5     | -             | 100.00 h         | 96.30 h      |
| KT6     | -             | 92.59 gh         | 94.44 gh     |
| KT7     | +             | 70.37 cdef       | 61.11 cd     |
| KT8     | -             | 85.19 efgh       | 77.78 efgh   |
| KT9     | -             | 88.89 fgh        | 79.63 efgh   |
| KT10    | -             | 92.59 gh         | 85.19 fgh    |
| AK1     | +             | 74.07 defg       | 64.81 cde    |
| AK2     | -             | 92.59 gh         | 83.33 fgh    |
| AK3     | -             | 85.19 efgh       | 77.78 efgh   |
| AK4     | +             | 66.67 cde        | 59.26 bcd    |
| AK5     | +             | 55.56 bc         | 51.85 bc     |
| AK6     | +             | 48.15 b          | 44.44 b      |
| AK7     | -             | 85.19 efgh       | 79.63 efgh   |

The numbers followed by different letters in the same column are significantly different at α=5% (Duncan’s test).
3.3. Antagonists test in dual culture

The antagonistic tests of isolated bacteria from peatlands were carried out in dual cultures in a 100 mm petri dish. Observations included pathogen radius, types of interaction, inhibition percentage and zone distance of inhibition.

In peatland bacterial colonies, the lowest pathogenic mycelium radii were KT9 followed by B3, AK3, and AK7 respectively. The highest mycelium radii were B0, B4, B6, and KT1. Type D interactions were only found in KT9 isolates, while other isolates (B1, B4, B6, KT5, KT6, AK5, and AK6) were without any interactions. The highest percentage of inhibition was in isolates KT9, while the lowest were found in isolates B4, B6, KT1, then B2, KT5, KT6, AK1, AK2, B5, B7, B8, KT2, AK5, AK6. The inhibition zone was only found in KT9 isolates with a wide inhibition zone distance (including type D), while other bacterial isolates did not have any inhibition zone (Table 3).

**Table 3. Dual culture test of S. rolfsii against antagonistic bacteria isolated from peatlands.**

| Isolate | Pathogen mycelium radius (mm) | Interaction type* | Percentage of inhibition (%) | Zone of inhibition distance (mm) |
|---------|-------------------------------|-------------------|-------------------------------|---------------------------------|
| control | 57.39 e | 0 | 0.00 a | - |
| B1 | 51.36 cd | A | 10.21 cd | - |
| B2 | 56.49 e | Bi | 1.56 ab | - |
| B3 | 46.53 b | Bii | 18.81 e | - |
| B4 | 57.12 e | A | 0.44 a | - |
| B5 | 55.51 e | Bi | 3.17 abc | - |
| B6 | 56.77 e | A | 1.03 a | - |
| B7 | 53.77 de | Bii | 6.25 bc | - |
| KT1 | 56.85 e | Bi | 0.90 a | - |
| KT5 | 56.68 e | A | 1.22 ab | - |
| KT6 | 55.85 e | A | 2.65 abc | - |
| KT8 | 47.72 bc | Bii | 16.78 e | - |
| KT9 | 33.95 a | D | 40.80 f | 6.94 |
| KT10 | 47.95 bc | Bii | 16.17 de | - |
| AK2 | 55.77 e | Bi | 2.77 abc | - |
| AK3 | 46.16 b | Bii | 19.58 e | - |
| AK7 | 46.68 b | Bii | 18.68 e | - |

The numbers followed by different letters in the same column are significantly different at $\alpha=5\%$ (Duncan's test). *(A) Without interaction; (B) intermingling growth with overlapping blends; (Bii) intermingling growth where the growth of one of the isolates stops; (D) inhibition occurs with an inhibition zone distance of > 2 mm.

KT9 bacteria obtained from the peat rhizosphere in peat soils have the potential to act as antagonistic bacteria which can act as biological control agents against *S. rolfsii*. KT9 bacteria were able to inhibit *S. rolfsii* in multiple culture tests so that the growth of the pathogen was inhibited on agar media. The mycelium radius to pathogens on KT9 was the lowest compared to other isolates (33.95 mm), as well as the percentage of inhibition (40.80%). Isolate of KT9 have a fairly large inhibition zone distance of 6.94 mm.

The small radius of pathogen mycelium indicates that bacteria from peatlands inhibit the growth of pathogen’s mycelium, so that these bacteria have the potential to act as biological control agents. Type D is the best type of interaction in terms of *S. rolfsii* inhibition by bacteria from peatlands. Peatland bacteria that interact with type D have the potential to act as antagonistic agents against *S. rolfsii*. The percentage of inhibition indicates the ability of these bacteria to inhibit the growth of fungal colonies. The greater the percentage of inhibition, the greater the potential of the bacteria as a biological control agent for *S. rolfsii*.
3.4. Molecular identification

The PCR results using universal primers, namely 16s sRNA (Figure 1), showed that the KT9 bacterial isolate had a DNA fragment length of 1401 bp. Based on the results of DNA sequencing (Table 4), the top 10 hit of Blasts which were obtained from the NCBI database and phylogenetic tree (Figure 2) which showed that the KT9 bacterial isolate was related to Bacillus sp. Bacteria within the KT9 isolate have morphological characteristics and DNA structure similar to those of Bacillus sp. It is yellowish white, moderate in size, circular colony shape, convex elevation, and the colony periphery in the form of a lobate; under the microscope these bacteria are gram-positive bacilli. The results of molecular identification to the phylogenetic stage reinforce the above morphological characteristics that the bacteria are related to Bacillus sp.

![Gel Photo-PCR products from peatland bacteria with potential as antagonistic agent against S. rolfsii](image_url)

**Figure 1.** Gel Photo-PCR products from peatland bacteria with potential as antagonistic agent against S. rolfsii

| Sample | Sequences |
|--------|-----------|
| Assembly of 2 sequence 1401 bp |
| 1 | GGTGATCCCTCA CCGACTTCTCG GTGTTACCAA CGTCTGATGTT GTGACGGGCGA GTGTTACGAC |
| 61 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 121 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 181 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 301 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 361 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 421 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 481 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 541 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 601 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 661 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 721 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 781 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 841 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 901 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 961 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1021 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1081 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1141 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1201 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1261 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| KT9 (bacteria) |
| 1 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 61 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 121 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 181 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 301 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 361 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 421 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 481 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 541 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 601 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 661 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 721 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 781 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 841 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 901 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 961 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1021 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1081 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1141 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1201 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1261 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
| 1321 | GCGCAGGGGAA CTTATTCCTAC GCAGCTGCTAG GTGTTACGAG TCGTCTGCTAC TACCTGTTGCA |
Bacillus sp. bacteria are antagonistic to *S. rolfsii* by forming a zone of inhibition in the media due to the presence of mycolytic enzyme compounds in the form of proteases and glucans produced by these bacteria so that they can degrade the cell walls of the pathogenic fungi [12]. Bacillus sp. as a biological control agent was also able to inhibit the growth of plant pathogenic fungi by producing secondary metabolites as their biocontrol properties [13]. These bacteria can produce antibiotics in the form of antimicrobial compounds such as 2,4 diacetyl-phloroglucinol, phenazine derivatives, extracellular lytic enzymes such as chitinolytic enzymes, or byproducts in the form of hydrogen cyanide (HCN) or siderophores. [14,15,16].

Antagonistic agent bacteria from *Bacillus* sp. dominate peat soils, especially in the rhizosphere and topsoil [13,17], such as in the peat swamp forests of Kalimantan. *Bacillus* sp bacteria isolates were found in various samples at different pH levels [18]. An increase in the pH level of peat soils will eliminate the inhibitory effect of peat on colonization by most soil bacteria [17].

**Figure 2.** Phylogenetic tree of KT9 bacterial isolates using the Neighbor Joining (unrooted Tree) by NCBI Blast Tree Method.

4. Conclusion
It can be concluded that in the Kuala Pesisir - Nagan Raya peatlands, there are bacteria that have the potential to act as an antagonistic agent against *Sclerotium rolfsii*. Based on morphological and DNA characteristics, the bacteria were identified as *Bacillus* sp.

References
[1] Gill H K and Garg H 2014 Pesticide: environmental impacts and management strategies *Pesticides-toxic aspects* 8 (Croatia: In Tech) p 187
[2] Ferreira S A and Boley R A 1992 *Sclerotium rolfsii* (Manoa: University of Hawaii)
[3] Punja Z K 1985 *Annu. Rev. Phytopathol.* 23 97–127
[4] Khattabi N, Ezzahiri B, Louali L and Oihabi A 2001 *Phytopathol. Mediterr.* 40 143–148
[5] Sumartini S 2012 *J. Penelit. dan Pengemb. Pertan.* 31 27–34
[6] Abidin Z, Aini L Q and Abadi A L 2015 *J. Hama dan Penyakit Tumbuh.* 3 1–10
[7] Lê N C 2011 Diversity and biological control of *Sclerotium rolfsii*, causal agent of stem rot of groundnut (Netherlands: Wageningen University)
[8] Cappuccino J G and Sherman N 2014 *Microbiology: A Laboratory Manual* 10th Ed (Boston: Pearson Education)
[9] Sadjad S Murniati E and Ilyas S 1999 *Parameters of Seed Vigor Testing from Comparative to Simulated* (Jakarta: Grasindo) p 185
[10] Skidmore A M 1976 Interaction in relation to biological control of plant pathogen *Microbiology of aerial plant surfaces* (Massachusetts: Academic Press) 507–524
[11] Skidmore A M and Dickinson C H 1976 Trans. Br. mycol. Soc. 66 57–64
[12] Shui W E E 2017 Isolation of mycolytic enzyme producing bacteria from the environment in Sarawak, and evaluation of their potentials as biocontrol agents against Ganoderma boninense (Swinburne: University of Technology)
[13] Szentes S, Radu G L, Laslo É, Lányi S and Mara G 2013 Crop Prot. 52 116–124
[14] Raaijmakers J M, Paulitz T C, Steinberg C, Alabouvette C and Moënne-Loccoz Y 2009 Plant Soil 321 341–361
[15] Suryanto D, Wibowo R H, Siregar E B M and Munir E 2012 African J. Microbiol. Res. 6 2053–2059
[16] Shahraki M, Heydari A and Hasanzadeh N 2009 Iran. J. Biol. 22 71–84
[17] Boehm M J, Madden L V and Hoitink H A J 1993 Appl. Environ. Microbiol. 59 4171–4179
[18] Yuliar, Abidin Z and Mangunwardoyo W 2011 Indones. J. For. Res. 8 144–157