Isolation of non-symbiotic Nitrogen-fixing bacteria on andisol land affected by Sinabung eruption

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Abstract. Andisol soils affected by the eruption of Mount Sinabung have different levels of ash thickness, each thickness has a different microbial activity and nutrient status. The aim of this research was to find non-symbiotic nitrogen-fixing bacteria isolates in several volcanic ash thicknesses. This research was conducted at the Soil Biology Laboratory, Faculty of Agriculture, University of Sumatera Utara. Isolation of non-symbiotic nitrogen-fixing bacteria was carried out using a nitrogen-free growth medium, Jensen medium. The results indicated that 7 bacterial isolates were able to grow on nitrogen-free medium, these isolates had different shapes (B1-B7). In the land which is not covered with ash there are 2 isolates, in the soil covered in thin ash (<2 cm) there are 2 isolates, in the soil covered in medium ash (2-5 cm) there are 2 isolates and in the soil covered in thick ash (> 5 cm) there are 1 bacterial isolate.

1. Introduction

Mount Sinabung is a volcano in the Karo highlands, Karo District, Sumatera Utara. Mount Sinabung experienced an eruption in 2013. The Sinabung eruption released grey-black smoke and volcanic ash material. Ash eruption can damage health and crops in the affected area [4].

Volcanic ash covering the soil surface can affect soil conditions. The researches results of [9;13-18;21]. Andisol soil pH affected by Mt. Sinabung eruption ranges from 4.4 to 5.6. The low pH of soils affected by the eruption of Mt. Sinabung can result in disruption of soil organism activity [4;5;20]. [10] soil pH affected by the eruption of Mount Sinabung based on various ash thicknesses that is 4.10 to 5.59, this indicated that the thicker the ash covering the soil, the lower the pH.

Acidic soil reaction (pH) affects the development of soil microorganisms that live in it. Fungi can live and tolerate soil pH ranges from pH 4 - 6.5 while for bacteria prefer soil conditions with a pH ranging from 6-7 [6].

The eruption from Mt. Sinabung also resulted in reduced organic matter in the soil hence the N content in the soil was low. This happens because the soil is exposed to ash causing soil organisms difficult to survive hence the decomposition process is inhibited [20]. The analysis results of [12] the chemical properties of soil exposed to volcanic ash with a depth of 0-5 cm have a very low C-Organic that is 0.91%. The results of [19] N total soil affected by volcanic ash ranged from very low to low at 0.04% - 0.20%. The analysis results of [14] reported that the levels of C-organic and N total of soil affected by the eruption were 5.74% and 0.56%. This happens because the source of N in the soil is reduced and the microorganisms that function in binding N in the air are also reduced. The ratio of
fixated N and soil organic matter content is 5 - 20: 1000, which means that for every 5-20g of N fixed fixation, an overhaul of 1000 g of soil organic matter is required [7].

Nitrogen (N) is one of the most widely distributed elements in nature. Nitrogen fixation can occur symbiotically or non-symbiotically. Microorganisms that play a role in non-symbiotic fixation include Azospirillum, Azotobacter, and Beijerinckia (which are active in acidic soil conditions), Bacillus, Enterobacter, etc. Besides these bacteria, Blue-green algae can also fixate nitrogen [7].

2. Material and method

This research was conducted at the Soil Biology Laboratory, Agrotechnology Study Program, Faculty of Agriculture, Universitas Sumatera Utara, Medan and carried out in April to September 2019. The land used in this research was land that is distinguished by several thicknesses of ash, namely A0: already processed, A1: Thin (<2 cm), A2: Medium (> 2-5 cm), A3: Thick (> 5 cm) [22]. The media used are Jensen's media for the composition per litre of aqua dest: (Sucrose 20 g, K2HPO4 1 g, MgSO4.7H2O 0.5 g, NaCL 0.5 g, FeSO4 0.1 g, CaCO3 2 g, jelly 20 g, aqua dest 1 L). Ingredients for gram staining include Violet Crystal Solution, Iodine Solution, 70% Alcohol Solution, Safranin Solution. As well as chemicals used for analysis in the laboratory. The parameters observed were pH (electrometry), C-Organic (Walkley and Black), and total microbes.

Isolation of soil bacteria was carried out by stratifying dilution and using the Pour Plate method. The first step was to make a suspension of soil samples (composite) from locations A, B, C and D by taking 10 g of soil and put it in sterile distilled water as much as 90 ml, then homogenized using a shaker. Furthermore, a dilution technique was carried out on the ground suspension, a dilution factor of 10<sup>-1</sup> to 10<sup>-5</sup> by taking 1 ml of a sample suspension, then pipetted and put into a test tube containing 9 ml of distilled water (10<sup>-1</sup>). Then the suspension of the dilution factor 10<sup>-1</sup> pipetted 1 ml and put into a test tube containing 9 ml of distilled water (10<sup>-2</sup>), the same treatment was carried out on the dilution factor 10<sup>-3</sup> to the dilution of 10<sup>-5</sup>. Next, 1 ml was taken from each of the dilution factors of 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> to be inoculated into the 20 ml Jensen medium on a petri dish using the pour plate method and incubated for 72 hours at 30°C until found a growing colony.

3. Results and discussion

Based on the research results conducted on several thicknesses of ash obtained data of soil temperature, humidity, C-organic and soil pH which can be seen in Table 1 below.

| Location | Soil Temp. (°C) | Humidity (%) | C-Organic (%) | pH H2O |
|----------|----------------|--------------|---------------|--------|
| I        | 19             | 78           | 7.55          | 5.2    |
| II       | 19             | 73           | 5.22          | 5.14   |
| III      | 20             | 60           | 0.64          | 5.33   |
| IV       | 20             | 40           | 0.75          | 4.38   |

Location I: Ash Thickness (0 cm); Location II: Ash Thickness (<2 cm); Location III: Ash Thickness (2-5 cm); Location IV: Ash Thickness (>5 cm)

Based on Table 1, it can be seen that the highest C-organic is at the location I (land not covered with ash) with C-organic of 7.55% and the lowest C-organic is at location IV (soil covered with ash >5cm thick) with C-organic of 0.75%. This indicated that the thicker the soil is covered with volcanic ash, the lower the C-organic content, and vice versa. This was in accordance with the literature of [12].
which stated that the activity of microorganisms in soils not exposed to volcanic dust is higher than that of soils exposed to volcanic dust, this is due to the pH and C-organic which are also higher when compared to the pH and C-organics in samples volcanic ash. The analysis results of [13] reported that the level of C-organic of andisols soil affected by Sinabung eruption was 4.7%. [10] reported that the levels of C-organic soil affected by Sinabung eruption ranged from 4.41 - 7.34%. The lowest C-organic in soils was with ash thickness >8cm. The analysis of [19] reported that the C-organic soil affected by the Sinabung eruption ranged from 0.91 to 7.19%. [20] stated that volcanic ash that has covered the surface for a long time will settle and harden depending on the thickness level. This will affect soil aeration, respiration, availability of oxygen and organic matter in the soil which affects the life of organisms in the soil.

The highest soil pH is at the location I (land not covered with ash) with a pH of 5.42 and the lowest pH at location IV (soil covered with ash >5cm thick). This is due to the volcanic ash of Mount Sinabung is dominated by high sulfur content which causes a decrease in pH. This is in accordance with the literature of [23] which stated that the high content of Mn, Fe, and S in Sinabung volcanic ash were 67.80 ppm, 2500.65 ppm, and 130.20 ppm respectively. This situation caused the pH of Mt. Sinabung volcanic ash to be classified as very low. In fact, one of the volcanic ash samples is 3.6. This is also in accordance with the analysis results of [14] that the soil pH affected by the eruption of Mount Sinabung is very acidic with a value of 4.29. [20] stated on the A0 ash thickness (without ash) the pH value was 4.50 but the pH decreased at the A3 ash thickness (> 8cm) to 3.74. The analysis results of [5] reported soil pH affected by the eruption of Sinabung is 3.46 - 5.02 where the thicker the ash, the lower the soil pH.

Bacterial isolation in soil samples using a nitrogen-free medium (Jensen) was obtained by 7 bacterial isolates that had different colony characteristics. Based on the isolation of bacteria can be observed parameters such as bacterial populations and have a relationship with soil pH conditions at several thicknesses of ash at 4 different locations. This can be seen in Table 2 below.

| Location | Number of Isolate | Isolate Code | Population \( \times 10^6 \) |
|----------|------------------|--------------|-----------------------------|
| I        | 2                | B1,B5        | 14                          |
| II       | 2                | B2,B6        | 6                           |
| III      | 2                | B3,B7        | 10                          |
| IV       | 1                | B4           | 5                           |

Location I: Ash Thickness (0 cm); Location II: Ash Thickness (<2 cm); Location III: Ash Thickness (2-5 cm); Location IV: Ash Thickness (>5 cm)

Based on Table 2, it can be seen that there are 7 types of bacterial isolates from several thicknesses of ash, namely code B1-B7, where at location I there are 2 isolates with codes (B1 and B5), location II there are 2 isolates with code (B2 and B6), location III there are 2 isolates with code (B3 and B7) and location IV there are 1 isolate with code (B4).

The highest number of isolates was found in soil without ash, covered with thin ash and covered with medium ash while the lowest number of isolates was found in soil with thick ash. This indicated that the thicker the thickness of ash, the fewer types of bacterial isolates and vice versa. This happens because the soil with a thickness of ash >5cm has a very acidic pH of 4.38, hence some types of bacteria are unable to move at a pH that is too acidic. This is in accordance with the literature of [6] which stated that acid soil reaction (pH) affects the development of soil microorganisms that live in it. Fungi can live and tolerate soil pH ranges from pH 4-6.5 while bacteria prefer soil conditions with a pH of 6-7. The analysis results of [5] [20] reported that the pH of Mt. Sinabung volcanic ash ranges
from 3.3 to 3.5 while the pH of the soil ranges from 4.4 to 6.5, this can result in disruption of soil organism activity.

**Table 3. Morphological Characteristics of Non-Symbiotic Nitrogen-Fixing Bacteria**

| No | Isolate Code | Micro | Form | Gram   |
|----|--------------|-------|------|--------|
| 1  | B1           | Round | Negative |
| 2  | B2           | Rod   | Negative |
| 3  | B3           | Rod   | Negative |
| 4  | B4           | Rod   | Negative |
| 5  | B5           | Round | Negative |
The highest non-symbiotic nitrogen-fixing bacterial population in soil without ash with a population of $14 \times 10^5$ and the lowest bacterial population on soil with thick ash thickness ($> 5$cm) with a population of $5 \times 10^5$, it can be concluded that the thicker the volcanic ash, the microbial population is getting smaller. This is in accordance with the literature of [10] which stated land that is exposed to volcanic ash will affect the respiration of soil microorganisms because the thick ash will make the soil solid and will disrupt soil aeration. The availability of oxygen will affect the presence and activity of soil microorganisms due to obstruction of air circulation due to mixing of soil with volcanic ash which makes it difficult for microorganisms to carry out activities in the soil. [2] stated that volcanic dust significantly increases the saturation of H, increases the saturation of bases and increases S-available in the soil which then affects the number of microorganisms in the soil.

This indicated that there is a relationship between microbial populations, the amount of isolates and soil pH, in which the thicker the volcanic ash covered the soil, the lower the soil pH will be, with a pH that is too acidic, bacteria are unable to grow and develop, and under these conditions will affect the type of isolate that grows where only certain isolates can grow.

Each bacterial species has different characteristics from one species to another species both morphological characteristics or others. The difference in character can be used as a guide in identifying the taxonomic position. This can be seen in Table 3 below.

Based on the data in Table 3 above, it can be seen that observations of bacterial isolates have different cell shapes. The data obtained is in accordance with some morphological data of nitrogen-fixing bacteria that have been reported in previous studies by [8]. The variations in character of the bacteria that were successfully observed indicated various types of Nitrogen-fixing bacteria that were successfully isolated at this stage.

Based on Table 3 above, it is identified that in general, Nitrogen-fixing bacteria are characterized to have round cells and are generally gram-negative types. In general, round and gram-negative cell shape is one of the common characteristics found in Nitrogen-fixing bacteria. This is in accordance with the results of previous studies by [11] reported there were 3 bacterial isolates that can tether N freely namely Azotobacter, Azospirillum and Pseudomonas. The isolates obtained have morphological characteristics of round cells and gram-negative cells.

4. Conclusion
The difference in ash thickness affects environmental conditions, which can affect the number of Non-symbiotic Nitrogen-Fixing Bacteria in the soil. The number of non-symbiotic Nitrogen-fixing Bacteria
isolates found in Andisol soil at Location I (ash thickness 0 cm) is 2 isolates, location II (ash thickness <2 cm) is 2 isolates, Location III (ash thickness 2-5 cm), is 2 isolates, and Location IV (Ash thickness >5 cm), is 1 isolate.

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