Potential test of non-symbiotic nitrogen-fixing bacteria in increasing nitrogen in Andisol soil

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Abstract. Nitrogen (N) is one of the elements most needed by plants and widely distributed in nature. Nitrogen fixation can occur symbiotic or non-symbiotic by soil microbes. This research aimed to examine the potential of N-fixing microbes in increasing the availability of N in the soil. This research was conducted at the Soil Biology Laboratory of Faculty of Agriculture, Universitas Sumatera Utara. The results showed that the ability of microbes to increase the availability of N in the soil was varied. The population of N-fixing bacteria increased during 4 weeks of incubation. All bacterial isolates which incubated on Andisol soil were able to increase soil N-total content by around 9.4% -15.6%. The isolate with the most potential to increase soil N-total content was B6 isolate with an increase in total N content by 15.6%.

1. Introduction
Nitrogen in Andisol soil is an essential nutrient with higher content compared to other soils. From the total nitrogen content data analyzed from Andisol soil in several places in Java and Sumatra, the average nitrogen content was classified as moderate (0.33%) with a range between 0.11 to 0.76%. The n-total content in Andisol soil is determined by the soil c-organic content [1]. With less carbon available, the bacterial population decreases, and the free soil nitrogen that is absorbed is reduced as well [2]. The results of research by [3-6] stated that the pH of Andisol soil around Mount Sinabung ranges from 4 to 5.6. Low soil pH can disrupt the activity of soil organisms and reduce the population of microorganisms in the soil [7,8]. The analysis results by [9-11] reported that the C-organic content ranges from 5.74% and the N total of Andisol soil is 0.56% - 0.61%.

Nitrogen has always been a limiting nutrient for plant growth in most soils, including Andisols. Andisol soil always accumulates large amounts of organic matter which always contains organic nitrogen. Therefore, volcanic ash soil can supply large amounts of N-minerals to plants. According to data from several Andisol soils cultivated in Japan, the average N-organic content in overlay was 0.44% in paddy fields and 0.37% in the dry land. The percentage of N-organic mineralization is rather small in volcanic ash soil (Andisols). This indicates that organic N in volcanic ash soil is highly resistant to microbial decomposition [12].

There are large amounts of N in Andisol soil, but N is easily leached and evaporates, hence its presence is a limiting factor for plant growth. Increasing the amount of N in the soil can be done by applying N-fixing microbes. Bacteria that capable of fixing N non-symbiitically include Azospirillum, Azotobacter, Beijerinckia, Bacillus, Enterobacter, and others [13]. Based on the description above, this research aimed to examine the potential of N-fixing microbes in increasing the availability of N in the soil.
2. Materials and methods
This research was conducted at the Soil Biology Laboratory of the Agrotechnology Study Programme, Faculty of Agriculture, Universitas Sumatera Utara, Medan. After isolation, the N-fixing bacteria isolates were tested for their potential (potential test) on Andisol soil. 50 g of soil was provided in Erlenmeyer and then inoculated as much as 1 mL (10^8) of the N-Fixing Bacteria inoculant on Andisol soil. The soil was incubated for 30 days at room temperature and kept moist. After the incubation process is complete, observations are made on pH (electrometry), N-Total (Kjeldahl), and total microbes (Total Plate Count Method). Bacteria potential test on Andisol soil had the isolate codes as follows: B0 = Control (without bacterial isolates application) B1, B2, B3, B4, B5, B6 and B7. The bacterial isolate used was from the results of previous research, namely the initial activity of isolating Nitrogen-fixing microbes from Sinabung Andisol Soil.

3. Results and discussion
The final soil analysis with soil pH, total microbial, and N-total soil parameters after incubation for 1 month on Andisols are presented in the following table:

| Isolate | pH   | N-Total (%) |
|---------|------|-------------|
| B0      | 5.21b| 0.64b       |
| B1      | 5.42a| 0.70a       |
| B2      | 5.52a| 0.73a       |
| B3      | 5.71a| 0.72a       |
| B4      | 5.61a| 0.72a       |
| B5      | 5.63a| 0.73a       |
| B6      | 5.71a| 0.74a       |
| B7      | 5.52a| 0.73a       |

Note: Numbers followed by the same letter are not significantly different (5%) according to the DMRT.

Based on table 1, the soil pH parameter showed that B1 treatment is significantly different from B0 treatment but is not significantly different from B2 to B7 treatments. Nitrogen-fixing bacteria application can increase soil pH. The highest soil pH was 5.17, which is soil pH with incubation of bacterial isolates B3 and B6. This happens because bacteria can produce organic acids such as Humic Acid and Fulvic Acid. Organic acids have functional groups that can react with the aluminum metal to form a chelate. This is following the literature of [14] stated that the humic acid functional groups (carboxyl (COO⁻) and phenolics (OH⁻) groups) form complex compounds or Al chelates hence some Al cannot be hydrolyzed. If Al is not hydrolyzed, the hydrogen ion which causes soil acidity will decrease. As a result, the soil pH will increase. Soil N-Total parameter showed that the B1 - B7 treatments are significantly different from B0 treatment. The bacteria with the most potential to increase soil N-total were B6 isolates by 0.74% in which there was an increase of 15.6% compared to B0 treatment, and isolate bacteria with low potential to increase N-total was B1 isolates by 0.70% in which there was only an increase of 9.4% compared to B0 treatment. This indicates that in the soil which applied with Non-symbiotic nitrogen-fixing bacteria can fix various free-nitrogen. Based on the results of research by [15] stated that non-symbiotic nitrogen-fixing bacteria could increase N-total contents in the soil by 3.2%.

Based on figure 1, the microbial population increases from the 1st week to the 4th week. The bacterial population at week 4 showed that B5 treatment is significantly different from B0 and B1 treatments, but not significantly different from B2, B3, B4, B6, and B7 treatments. The bacteria that were most effective in increasing the population of nitrogen-fixing bacteria were those with isolate code B5 with a population of 62 x 10⁸ isolates, and the less effective in increasing the population of
non-symbiotic nitrogen-fixing bacteria was isolate code B1 with a bacterial population of 20 x 10^8. This indicates that all types of non-symbiotic nitrogen-fixing bacteria can increase the bacterial population but have different abilities. The increase in the number of the bacterial population is not too high, because there is no application of organic matter in the soil. Microorganisms use organic matter as a source of metabolism. This is under the literature of [16] which stated that the high total microorganisms are due to the accumulation of organic matter from the land above. Lands that have a high percentage of organic matter will have a larger number of soil microorganisms.

Based on the observations, the growth of each bacterial isolate was different. This occurs because bacterial growth is influenced by several factors, including soil pH, carbon availability (C), oxygen availability, nitrogen availability, humidity, and temperature. Research results from [17,18] found that the lower the pH, the soil moisture will reduce the population of microorganisms in the soil. This is under the literature of [19] which stated that the factors that influence N2 fixation in the soil include: (1) soil pH, most bacteria are sensitive to soil pH, but the ability of each bacterium is different to soil acidity. Rhizobia cannot live below a pH of 4.3 due to Al poisoning. Azotobacter is not able to live at a pH below 6.0. (2) The availability of carbon (C), non-symbiotic N2 fixation will be disrupted if the availability of carbon in the rhizosphere is limited. (3) Oxygen availability, nitrogenase enzyme activity is disrupted by the presence of oxygen, but at the same time, this enzyme activity depends on the availability of ATP which is formed through bacterial oxidative phosphorylation. (4) Nitrogen availability, due to the large amount of energy required for N2 fixation, diazotrophs have a regulatory system that ensures organisms can use low inorganic N sources such as NH4+ and NO3- when available in the soil. The observations of various metabolic results indicate the possibility of some non-symbiotic nitrogen-fixing bacteria can live in various types of habitats and soil conditions.

4. Conclusions
All bacterial isolates incubated on Andisol soil were able to increase soil N-total content by around 9.4% -15.6%. The isolate with the most potential to increase soil N-total content was B6 isolate with an increase in total N content by 15.6%.
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