Amount of soil phosphate solubilizing bacteria in the Reservoir of ITERA and its environmental conditions.

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Abstract. The aim of this study to examine the total number of phosphate solubilizing bacteria in the soil around the reservoir C and F and the surrounding environmental conditions. Calculation of the amount of phosphate solubilizing bacteria using pikovskaya medium. The number of bacteria that have a clear zone is calculated based on the amount of dilution. The results showed that the amount of soil phosphate solubilizing bacteria from C and F Reservoir amounted to $36.5 \times 10^4$ and $14.2 \times 10^4$ cfu/g, respectively. An Environmental condition such as rain rates, air temperature and humidity around ITERA do not support an increase in the amount of phosphate solubilizing bacteria. It can be seen from the data on environmental conditions that are not in accordance with the conditions of soil microbial growth.

1. Introduction

Institut Teknologi Sumatera is an area that has soil conditions derived from weathering of rocks and former rubber plantations areas that have limited nutrient availability. ITERA has several reservoirs which are used as a way to increase water availability in the ITERA region. The phosphate content in C and F ITERA pond water is quite high, each of (0.049 ppm and 0.051 ppm), respectively [1]. In addition, in December 2017, 17 goldfish in C reservoir was found dead, this was thought to be an increase in phosphate in the water, causing fish deaths. This shows that the soil under the reservoir of ITERA has high phosphate content and has not been utilized properly. The presence of phosphate in this pond has a correlation with phosphate in the soil around the ITERA Reservoir, which is the location for growing plants. The presence of phosphate in the soil around the reservoir C and F has a relationship to environmental conditions such as rainfall, air humidity and air temperature. Environmental factors usually have a considerable contribution to soil conditions (soil fertility). The soil is a unit of a substance that is interrelated between types of climate, physical-chemical properties, variations in soil microbes and vegetation that grows and develops in the area [2]. Variable pH and soil fertility levels, vegetation diversity allows population numbers and microbial diversity, especially phosphate solubilizing bacteria [3]. Phosphate solubilizing bacteria play an important role in the ecosystem as a decreasing of organic materials, providing organic materials that can be used by plants.
Crop productivity depends on plant nutrients which are mostly taken from the soil, one of them is phosphorus [4]. Soil generally contains a lot of phosphorus, but only a few available to plants. Plants are only able to absorb mono or dibasic phosphate, organic phosphate or phosphate forms that are not dissolved must be mineralized or dissolved by microorganisms [5], if the soil does not contain phosphate solubilizing bacteria then only a few phosphates can be absorbed by the soil and plants, resulting in infertile land [6]. So far the population of phosphate solubilizing bacteria and environmental conditions in ITERA has never been studied, so this study aims to examine the total number of phosphate solubilizing bacteria in the soil around the reservoir C and F and the surrounding environmental conditions.

2. Materials and methods

2.1. Sample collection

Soil sampling was carried out using a purposive random sampling method. Soil samples randomly from 2 points (Soil Reservoir C and F) at the soil surface layer between 0-15 cm. Soil samples were taken from the soil near the reservoir location C and F. Determination of this location was based on the analysis of the phosphate content in the pond water that had been carried out previously by (Nufutomo, 2017) [1], so that the current study carried out soil sampling in Reservoir with high phosphate content that is Reservoir C (1) and Reservoir F (2). The coordinates of the soil sampling points are:

| No | Location          | Coordinate                   |
|----|-------------------|------------------------------|
| 1  | Soil Reservoir 1  | 5°21'35.10" LS, 105°18'46.95" BT |
| 2  | Soil Reservoir 2  | 5°22'5.99" LS, 105°19'12.67" BT |
2.2. Isolation and Enumeration of Phosphate Solubilizing Bacteria

A total of 3 grams of soil samples were put into 27 ml of 0.85% physiological saline (NaCl) and incubated in a shaking incubator at a speed of 120 rpm for 4 hours at room temperature, then made serial dilution until $10^{-4}$. 0.1 ml of $10^{-2}$ to $10^{-4}$ dilution was spread on the surface of Pikovskaya agar medium consisting of 10 g $C_6H_{12}O_6$, 5 g Ca$_3$(PO$_4$)$_2$, 0.5 g (NH$_4$)$_2$SO$_4$, 0.2 g KCl, 0.1 g MgSO$_4$.7H$_2$O , 0.002 g MnSO$_4$.7H$_2$O, 0.002 g FeSO$_4$.7H$_2$O, 0.1 g NaCl, 0.5 g yeast extract, 20 g agar in 1000 ml with pH 7 and incubated for 7 days at room temperature. The growth of P solubile bacterial colonies is characterized by the formation of clear zones around the colonies. The number of bacteria that have a clear zone is calculated based on the amount of dilution.

2.3. Soil pH Measurement

A total of 10 g of soil sample was divided into 2 bottles, added with 50 ml of aquadest in the bottle I (pH H2O), and 50 mL of KCl 1 M into Bottle I. Samples were shaken for 30 minutes. Then the soil susceptibility is measured by a pH meter. Soil pH measurement uses 1:5 ratio.

2.4. Collecting of Environmental Conditions Data

Environmental conditions data include rain rate, air temperature, air humidity and air pressure obtained from the results of monitoring the UPT MKG ITERA data. The data was taken from February to July and analyzed using simple Ms Excel.

3. Results and discussion

3.1. Enumeration of Phosphate Solubilizing Bacteria

| No | Soil Sample | Amount (cfu/gram) | Average of total bacteria |
|----|-------------|------------------|--------------------------|
| 1  | 1           | $20.5 \times 10^2$ – $15 \times 10^3$ | $36.5 \times 10^4$ |
| 2  | 2           | $62 \times 10^3$ – $15 \times 10^4$ | $14.2 \times 10^4$ |
The population of P solubilizing bacteria in ITERA in two locations showed a fairly good amount (> 10,000 cfu/g soil), with a range of cfu/g soil 36.5 x 10^4 cfu/gram soil and 14.2 x 10^4 (Table 1). There is a tendency to decrease the population of P solubilizing bacteria in a more acidic soil pH. In the more acidic Reservoir 2 soil samples (pH 4.09), P solubilizing bacterial population was lower than P solubilizing bacterial population in Reservoir 2 soil samples. Less influence on soil pH on P solubilizing bacterial population was in line with the results of [7] who reported that pH, P-available and P-total soil were not significantly correlated with P. solubilizing bacterial populations. In contrast [8] reported that the bacterial community of P solubilizing in the soil decreased with increasing soil fertility. This was thought to be related to the increase in P levels in more fertile soils because the presence of high P levels could inhibit P dissolution activity. The effectiveness of P dissolution was indicated by the formation of clear zones around the colonies of isolates grown on Pikovskaya agar media. According to [9], the color change in the zone around the colony becomes transparent indicating a decrease in pH in the area and media immersion is related to the dissolution process of P.

The soil samples that have been taken are analyzed for soil chemistry as a parameter of soil sample conditions and their implications for bacterial diversity and soil fertility. The results of soil chemical analysis showed that both soil types were categorized in acid soils with pH 5.4 (Reservoir 1) and 4.09 (Reservoir 2). The acid soils used in this study are categorized in the type of ultisol soil. The results of acid soil analysis have a pH of 5.4 and 4.09 with the P content still very low and containing Al. According to [10] Ultisol soils have characteristics, the soil material is acid sedimentary rocks and is sensitive to erosion. Ultisols are generally brownish to red in color. The ultisol soil reaction is generally acidic to very acidic (pH 5-3.10). According to [11] ultisol acid soils are known to be Al and Fe poisoning. Soil fertility is the quality of soil for planting, which is determined by the interaction of a number of physical, chemical and biological properties of the soil body parts which become the habitat of active roots of plants. The soil sample used has a reaction (pH) of acid with a pH of 4.09 - 5.4. The availability of P depends largely on the pH of the soil with an optimal pH range of 6.5 - 7.5 [12]. In acid soils, the presence of high Al, Fe and Mn cations can fix P to Al-P, Fe-P and Mn-P which are insoluble so that P is not available for plants. Even though plants are fertilized with high amounts of P, they will settle with iron (Fe) or aluminum (Al) [13]. Acid is usually carried out to increase pH and precipitate Al as insoluble Al hydroxide, but lime is generally needed in large quantities 2-10 t / ha [14].

3.2. Environmental Factors that Affect the Number of Phosphate Solubilizing Bacteria
The composition of the population and microbial activity of the soil will vary due to differences in soil physical-chemical properties, climate and vegetation [2]. The amount of phosphate solubilizing bacteria in the soil around the C and F reservoir is not too significant. This may be due to soil conditions and not diverse of vegetation so that microbial activity is not too high. The total population of phosphate solubilizing bacteria is generally higher on the forest floor [3]. The presence of these bacteria is caused by the activity/metabolism that is more active on the forest floor than the former fields. The existence of soil microorganisms is the role key in the biogeochemical cycle, the way bacteria respond to changes in time and rain rate is a very important factor in predicting changes that occur in the ecosystem process. Very fast changes in humidity are things that trigger stress on microbes. Microbes must release energy to regulate osmotic pressure to their habitat environment [15].

![Rain rate for six month](image)

**Figure 3.** Rain rate for six month

The amount of rainfall has a correlation to soil moisture thus affecting the amount of soil respiration, thus affecting the overall response of the microbial community in it [16]. The number of phosphate solubilizing microbial populations that are not high enough, may be caused by several factors such as environmental temperature, soil acidity, humidity, the temperature in the area. The diversity and dominance of microbial populations in the rhizosphere depends on biotic and abiotic factors that apply specifically to ecological niches [17]. Usually, the amount of phosphate solubilizing bacteria in type A of climate with rain rate between 4000-6000 mm with an average of air temperature of 18-26 °C is quite high ranging from 10^6-10^7 cfu/g of soil. While the number of bacteria found in C and F Reservoir amounted to 10^4 cfu/g of soil. Seeing the high fluctuations of rain rate conditions in the ITERA region, which is between 102.8 - 5723.4 mm (Figure 3) affects the amount of phosphate solubilizing bacteria around ITERA. This is also affected by sampling conducted in July which in the previous month was the lowest rain rate condition around the sampling location.
Rain rate also affects the level of humidity and air temperature. These two things also have a correlation to the microbial population. ITERA’s air temperature is always above > 26 °C (Figure 4), so it contributes to the number of microbial populations on the ground. The ability of several bacteria such as \textit{Pseudomonas} sp., \textit{Acinetobacter calcoaceticus, coryneform, Staphylococcus} sp., can survive at 4, 15 and 25 °C on dry soil [18]. ITERA's air humidity is at 44.79-86.65% (Figure 5). Bacterial could tolerance to environmental humidity at 0%, 34% and 75% [18]. This shows that ITERA's air condition is not sufficiently supportive to survive. Microbe has the product can be released into environmental. Products released by microbes interact with temperature and humidity can increase bacterial attachment to the substrate [19]. In this case, it can be connected that phosphatase produced by phosphate solubilizing bacteria and supported by temperature and humidity can increase the ability of phosphate solubilizing bacteria to stay to the soil substrate.
4. Conclusions

The amount of soil phosphate solubilizing bacteria from C and F Reservoir amounted to 36.5x $10^4$ and 14.2 $10^4$ cfu/g, respectively. This amount is affected by environmental factors such as rain rate, air temperature and humidity. Based on data of Environmental conditions, ITERA is not very supportive in terms of increasing the number of phosphate solubilizing bacteria.

Acknowledgements

The authors thank the financial support from Institut Teknologi Sumatera (ITERA) for research grant with the scheme of Penelitian Dasar Hibah Mandiri No. 236/IT9.A/SP/PP/2018.

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