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Comparison of fermented fruit juice as a carrier for isolation phosphate-solubilizing bacteria from rice root

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Abstract

Fermented fruit juice is the material use to increase the soil microbial activity as it made up from fermented fruit in the container for a several time before it became a liquid compose fertilizer. It contains a lot of substances such as an organic compound which is one of the important nutrient source for bacteria in soil. By applying the fermented fruit juice (FFJ) in soil, it will help to increase the soil fertility itself. The aim of the present study is investigate the targeted soil bacteria which is phosphate-solubilizing bacteria inside the soil paddy root. This study was also revealed type of fruit that been used for fermented fruit juice namely corn cobs, coconut, and fruit waste. The statistical analysis has been conducted by using IBM SPSS version 2.0 for the bacteria population calculation. The findings of the present study showed there is significant different between the bacteria population among the treatments and fermented fruit juice can be used as carrier for phosphate solubilizing bacteria and one of the effective biofertilizer to increase soil fertility.

1. INTRODUCTION

The amount of bacteria that will be present in the soil is determined by the soil condition itself either it suitable for the soil microbe live inside it. Soil microbe are one of the major component that should be present in the soil by providing the sufficient nutrient for plant growth. As an example, the amount of nutrient that cannot be taken the plant are larger without the present of soil microbe because soil microbe helps to change the characteristic of the nutrient compound by solubilizing, nitrification and many more to make it easier for plant uptake. The presentation of large amount of organic matter can help improves the amount of soil microbe within that area. This is because soil microbes are needed to feed the organic matter as a food resources. Fermented fruit juice (FFJ) is the material use to increase the soil microbial activity as it contains a lot of substances such as an organic compound which is one of the important food sources for bacteria in soil. By applying the fermented fruit juice (FFJ) on soil it will help to increase the soil fertility itself. This study was conducted to determine the effective of phosphorus solubilizing bacteria that will present in the soil with the application of the fermented fruit juice (FFJ).

2. MATERIALS AND METHODS

2.1 Sample Preparation

The soil sample was collected from the Share Farm UiTM Melaka Campus Jasin. phosphate-solubilizing bacteria were isolated from the root of paddy plant. One gram of soil sample was mix in 9 mL of autoclaved distilled water and was thoroughly shaken. 1 mL of the above solution was again transferred to 9ml of sterile distilled water to form 10-fold dilution. 0.1ml of each dilution was spread on National Botanical Research Institute's (NBRIP) medium which contained the following ingredients glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $MgCl_2$ $6H_2O$, 5 g; $MgSO_4$ $7H_2O$, 0.25g KCl, 0.2 g and $(NH_4)_2SO_4$, 0.1 g. the mixture then incubated at 27 – 30ºC for 7 days. For studying the bacteria colony morphology, the halozone produce colony was subculture by using NBRIP media.

2.2 Preparation of NBIRP Media

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According to Islam et al. (2006), the medium was prepared by mixing 16 g of agar powder, 10 g of 1 -1 glucose, 5 g of magnesium chloride hexahydrate ($MgCl_2$, $6H_20$), 5 g of calcium phosphate Ca_3PO_4, 0.2 g of potassium chloride (KCl), 0.25 g of magnesium sulphate heptahydrate ($MgSO_4$, $7H_20$) and 0.1 g of ammonium sulphate $(NH_4)_2SO_4$ with 1L of distilled water in a scoot bottle.

2.3 Gram Staining Observation

Gram staining method was conducted to differetiate positive or negative bacteria. The bacteria were isolated from the NBRIP media. The smear is taken on the glass slide until it dried. After that the drying process is helped with heat using Bunsen burner. The crystal violet was added and waited for 30 seconds to make sure it dry. Distilled water is used to wash the glass slide and same waited until it dried. Gram iodine is added around 60 second and been washed with 95% Ethyl alcohol. 30 seconds needed to wait before washed it using distilled water. The sample was dried and observed it using the microscope. The pink colonies showed the gram negative while the purple colonies showed the gram positive. The halo surrounding was measured using the solubilizing index (SI) formula: SI = (Colony diameter X Halo diameter) / Colony diameter.

2.4 Fermented fruit juice preparation

The fermented fruit juice was prepared using the starched fruit which are banana. Two sample was prepared one with molasses added with it and the other one without molasses. Phosphate - solubilizing bacteria is added to both of the fermented fruit juice. The mixture of raw materials was placed in a plastic bucket only 4/5 full and the space above the raw materials was covered with a water filled plastic bag to help to produce anaerobic conditions (Duangporn.K ,2009). After that it was incubated at the temperature 28^{o}C to 30 ^{o}C in 1 week.

2.5 Inoculant of phosphate solubilizing bacteria.

After that the nutrient broth then introduced with phosphate solubilizing bacteria by using the loop with every 200ml of nutrient broth introduced with one colony of phosphate solubilizing bacteria. The media was put on the shaker with 180 rpm for 24 hours until it turns to cloudy. Phosphate solubilizing bacteria was added into the 200ml of fermented fruit juice that with and without molasses.

2.6 Bacterial population calculation in fermented fruit juice

The analysis of the fermented fruit juice was calculated by the population of the bacteria inside the fermented fruit juice.

2.7 Statistical analysis

The data recorded during investigation was subjected to t−test using the IBM SPSS version 20 and the mean of treatment is compared with the p − value ($p \leq 0.05$) using ANOVA.

2.8 Secondary data collection related to phosphate-solubilizing bacteria and fermented fruit juice as their carrier

Based on the data collected from the paper that has been reviewed, the fermented fruit juice was prepared using the starched fruit which are banana. Regarding to (Adriano, 2012). Every 7 days the aerated fermented fruit juice should be turn over until it reaches the stabilisation around 12 weeks' interval.

From the second review paper from preparation and utilization of phosphate bio fertilizer using agricultural waste (Wang, 2015) which are the method that has been collected was it used the corns cobs as the carrier for phosphate-solubilizing bacteria. The next paper review from Combined application of bio-organic phosphate and phosphorus solubilizing bacteria *Bacillus* strain MWT 14 improve the performance of bread wheat with low fertilizer input under an arid climate (Muhammad, 2018). Lastly, the reviewed paper from Optimisation of Parameters for Fermentation Conditions of Phosphate Solubilising Bacteria (Sonia, 2016)

3 RESULTS AND DISCUSSION

Bacterial isolates were characterized as PSB based on their appearance, by formation of visible dissolution halos on NBRIP agar. Dilution of factor 10^4 has large number of colonies on plate while 10^6 dilution has few colonies that present on plate after it has been counted. The dilutions were plated onto 3 replications of NBRIP media. The solubilisation index was based on the colony diameter and halo zone for PSB isolates is presented in Table 3.1. The result show only two colonies of PSB on a plate (Figure 1) that been inoculate from the serial dilution isolation produces halo within 6 days of incubation.

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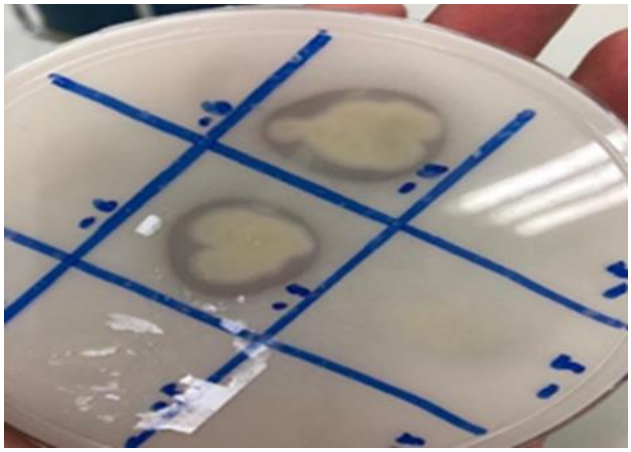

Figure 1: There are two colonies that showed clear halo zones

Table 1: Phosphate Solubilisation Index

Colony Diameter (cm)	Halo Diameter (cm)	Phosphate Solubilisation Index (cm)
1.4	0.2	1.14[a]
1.2	0.3	1.25[b]

Table 1 showed that two colonies of phosphate solubilizing index. The second colony has the highest compared to the first colony. It showed that the second colony has 0.3 cm halo diameter wider than the first at $p<0.05$.

3.2 Color Morphology

The colour of them mostly off yellow and off-white. It shows in Figure 2. The colony morphology of most strains presented circular, wet texture, and entire edge except for *Bacillus* strain, and most of them exhibited off-yellow when it grows on nutrient agar NA. It possibly identified as the phosphate-solubilizing bacteria.

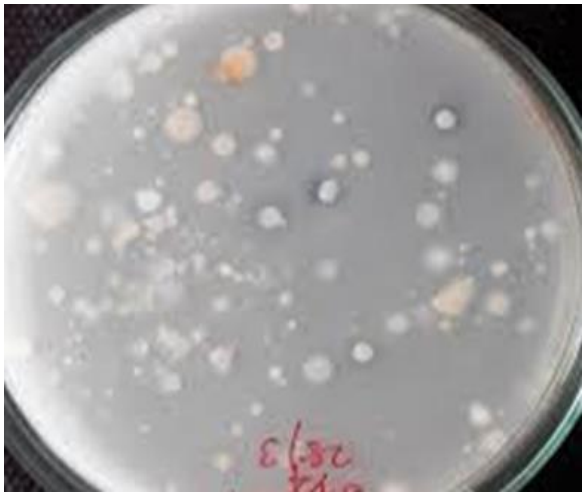

Figure 2: Colonies on NBRIP medium that been isolated from serial dilution

3.3 Percentage of bacteria shape

Bacillus shape of bacteria was identified as the PSB. From the bar chart in Figure 3, it has been clarified that it shows bacillus have the higher percentage with 70% of them from 10^4 serial dilutions. While the other shape such as cocci and rod also presented inside the colony of bacteria. Cocci and rod shape, both have the 13% and 12%. *Bacillus* shape is dominant due to the high level of organic acid inside the media.

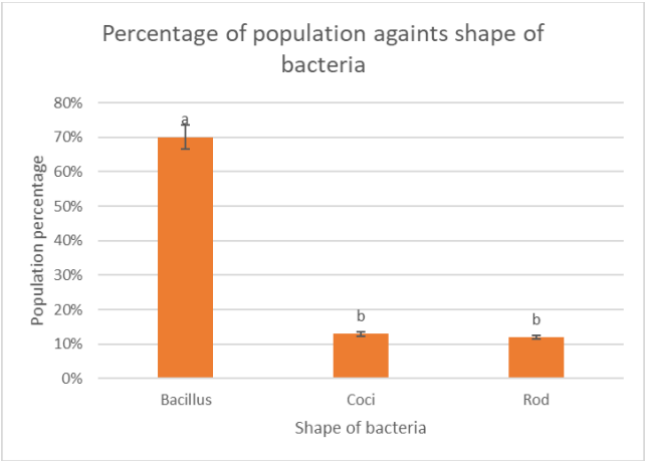

Figure 3: Graph show the percentage of the population against shape of bacteria

3.4 Gram staining

From the figure 4, result shows that the Gram-negative bacteria have the highest amount of percentage with 66.67% while the Gram-positive bacteria are at remaining balance which is 33.33%. This determined that the pink colour is dominantly performed on the glass slide under the microscopic observation.

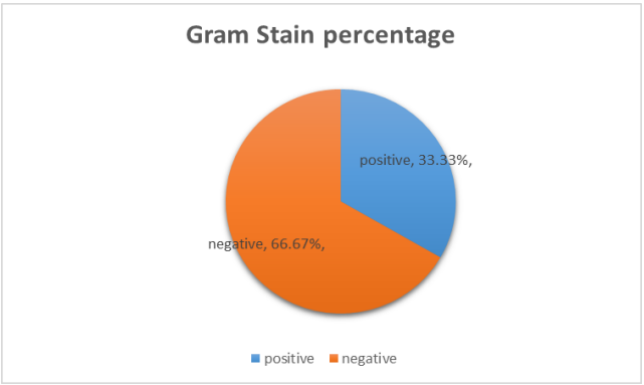

Figure 4: The total percentage of Gram negative and Gram-positive stain

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3.5 Comparison of secondary data with on-site study

In the study of Wang (2015) revealed the highest availability of phosphate-solubilizing bacteria availability were inside the corn cobs with 20% of perlite (CCP) wheat husk with 20% of perlite (WHP) after 7 month of storage at 4ºC temperature. In addition, corn cobs and wheat husk were a better material to support the growth and maintain the population of phosphate-solubilizing bacteria. This is because the water contains and high-water holding capacity presented inside this materials (Kiriya). The result showed that the compost cattle manure were not suitable materials in order to make bio fertilizer and not available to maintain the phosphate-solubilizing bacteria inside. For the second paper reviewed from (Muhammad, 2018). The result indicates that the effectiveness of phosphate solubilizing bacteria was at the booting stage which is 60-70 day after sowing of the wheat crop. From this result we can conclude that the most effective time of phosphate-solubilizing bacteria to show it highest availability is at 2-3 month of storage. For the next paper review by Duangporn,(2009) showed storage period about 2 month were done for fermentation of noni fruit. The result show that for the gram stain identification it produce around 80% of gram negative gram which are resulted in the present of bacillus megatarium shape of bacteria. The result for application of fermented fruit juice that contain PSB is significance difference between rate of application and the height of plant. The other paper review obtained from (Tanuwat, 2009) stated the result of effectiveness microbial activity inside the liquid fertilizer made from starch fruit is significance with the duration of storage period. For the last resulted stated from reviewed paper from (Sonia, 2016) showed the data shows that the coconut cake is the second highest number of phosphate-solubilizing bacteria with 0.46 ug/mL of simple phosphorus. The isolated showed a big halo zone on the plate of PVK.

4. CONCLUSION

We successfully isolated the phosphate-solubilizing bacteria from paddy field. A clear halo zone was evident surrounding the colony on the NBRIP medium. In the basis morphological characteristics such as bacillus shaped and Gram-negative characteristic, and the isolates was confirmed as phosphate-solubilizing bacteria. From the comparison of literature search, we can conclude that the most effective time of phosphate-solubilizing bacteria to show it highest availability is at 2-3 month of storage

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