ISSN: 1412-033X
E-ISSN: 2085-4722

BIODIVERSITAS

Journal of Biological Diversity

Volume 20 - Number 11 - November 2019

BUKTI KORESPONDENSI BIODIVERSITAS

Jurnal : Biodiversitas

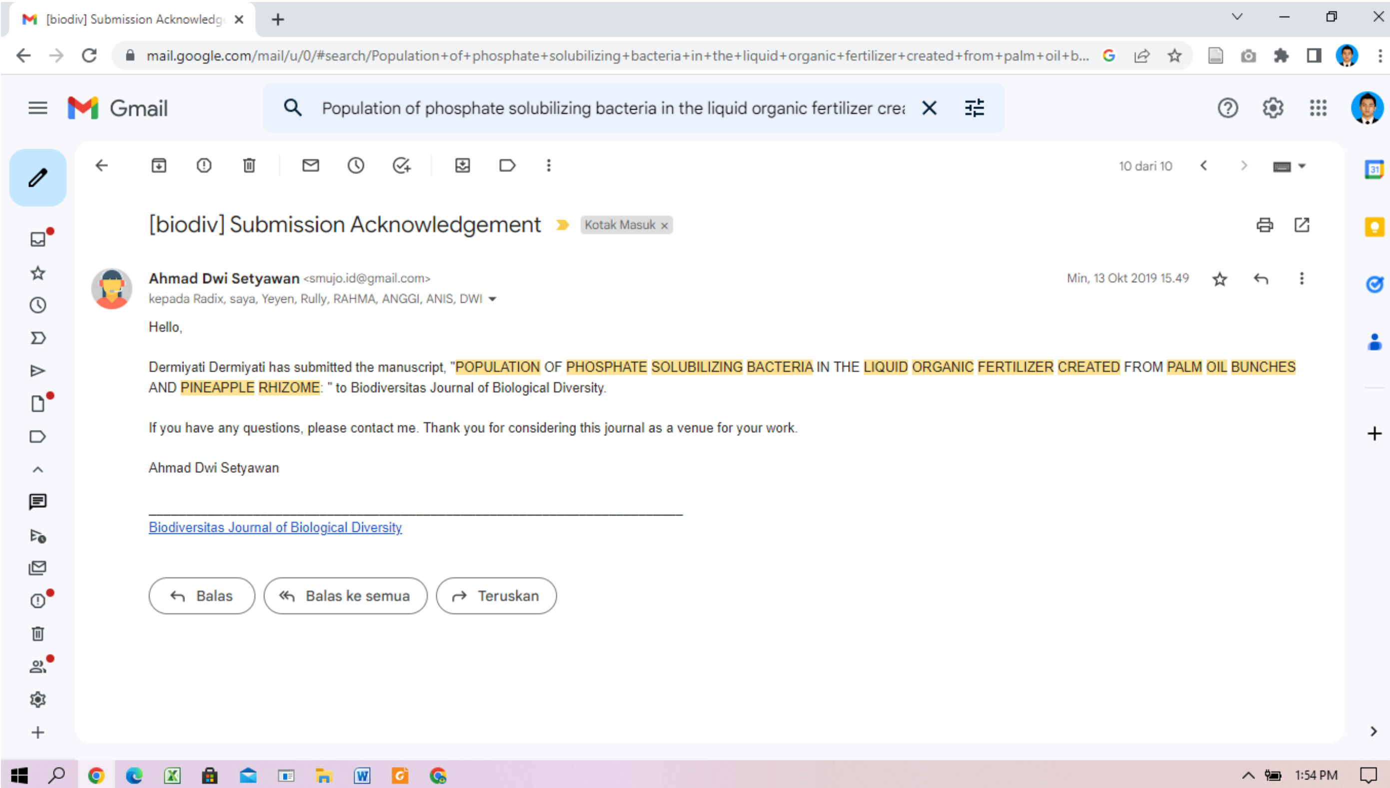
Judul : Population Of Phosphate Solubilizing Bacteria In The Liquid Organic Fertilizer Created From Palm Oil Bunches And Pineapple Rhizome

- SUBMIT (11 Oktober 2019)
- REVISI (23 Agustus 2019)
- ACCEPTED SUBMISSION (26 Oktober 2019)
- TERBIT DI BIODIVERSITAS (11 November 2019)

Daftar Lampiran

1. Submit jurnal
2. Pemberitahuan editor untuk revisi naskah
3. Balasan author bahwa naskah telah direvisi
4. Revisi naskah jurnal
5. Accept submission
6. Proses publish jurnal

SUBMISSION MANUSCRIPT



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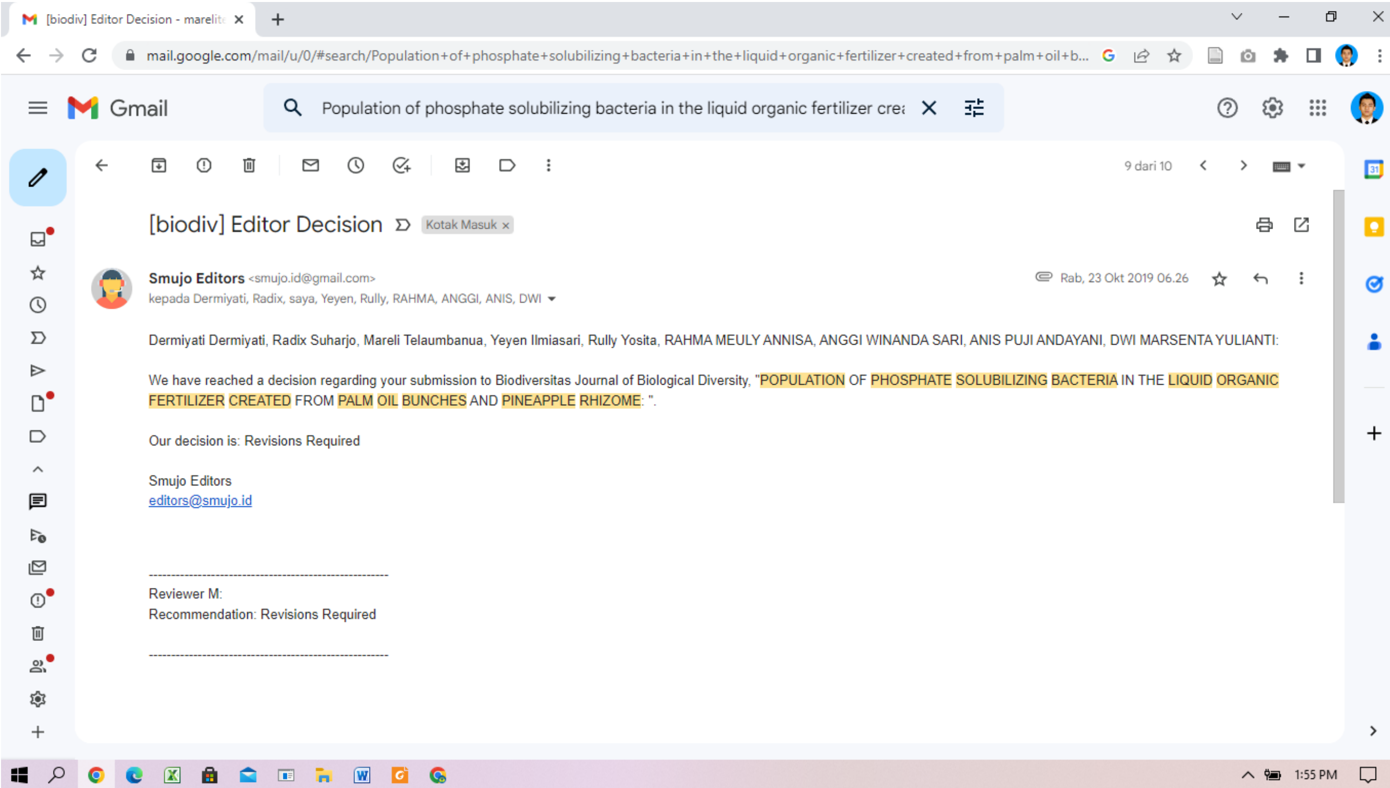

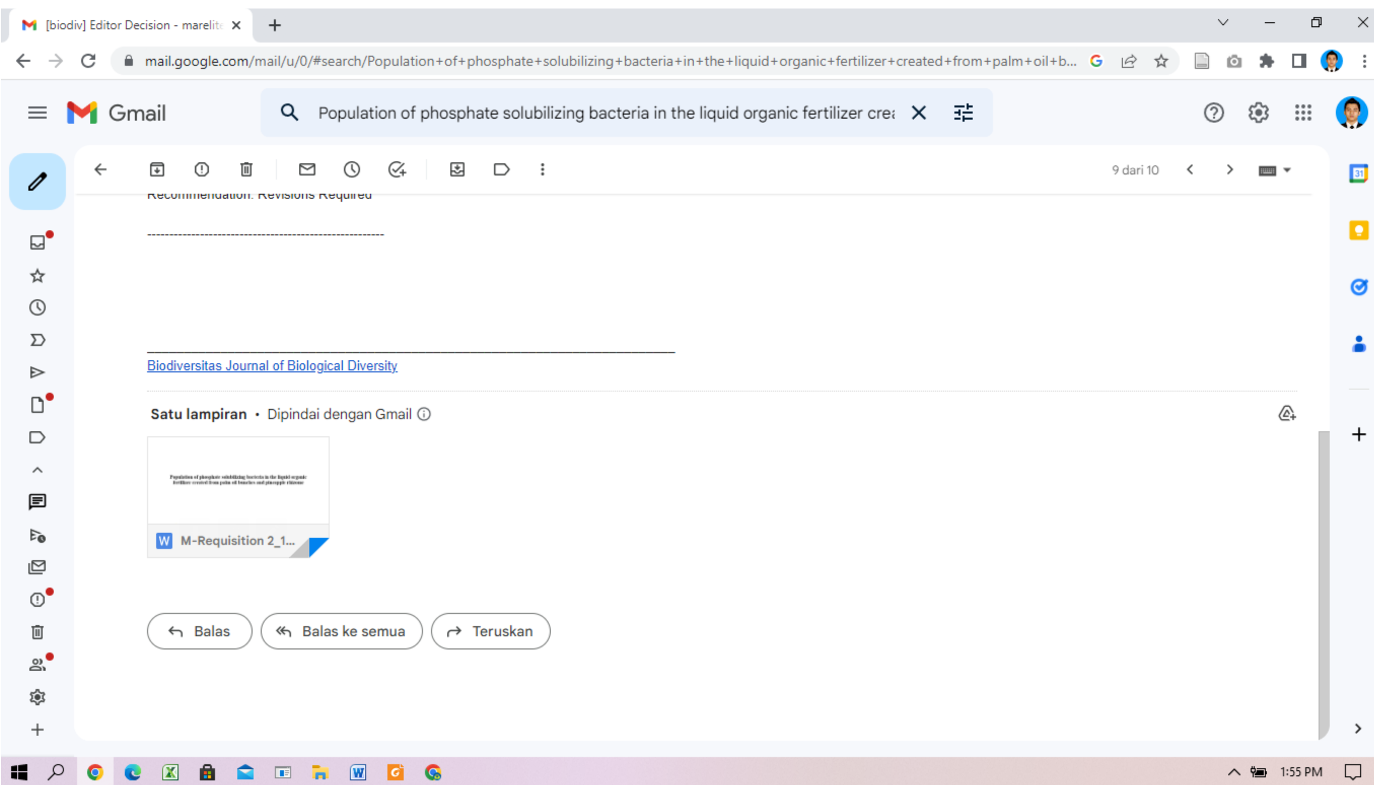

Population of phosphate solubilizing bacteria in the liquid organic fertilizer created from palm oil bunches and pineapple rhizome

Abstract. This study was aimed to reveal microbial population within liquid organic fertilizer (LF) which was developed from palm oil bunches (PB) and pineapple rhizome (PR) and their role as phosphate solubilizing bacteria (PSB). The capability to solubilize phosphate was recorded from the clear zone area around the bacterial colony which was cultivated on Pikovskaya agar medium. The LF was prepared in three kinds of conditions, namely aerobe, semi-aerobe and anaerobe. Isolation was performed every 3 days until 24 days after incubations. The results showed that microbial population in the LF from PR was significantly higher than those developed from PB. Totally, 791 bacterial isolates were obtained from the LF; 490 isolates were achieved from PR (153 isolates from aerobe, 188 isolates from semi aerobe, 149 from anaerobe) and 301 isolates were collected from PB (96 isolates from aerobe, 112 isolates from semi aerobe, 93 isolates from anaerobe). In the case of isolates which were gained from PB, 166 isolate (55.15%) showed capability to degrade phosphate and 135 isolates (44.85%) did not show any capability as PSB. As for isolates obtained from PR, 269 isolates (54.90%) had capability to solubilize phosphate, meanwhile the other 221 isolates (45.10%) did not showed any capability to s olubilize phosphate. Among166 isolates of PSB from PB, 60 isolates had very low capability, 55 isolates had low capability, 34 isolates had medium capability, 10 isolates had high capability and only 7 isolates had very high capability. Meanwhile, from 269 isolates of PSB obtained from PR, 135 isolates had very low capability to solubilize phosphate, 84 isolates had low capability to solubilize phosphate, 32 had medium capability to solubilize phosphate, 14 isolates had high capability to solubilize phosphate and 4 isolates prod uced very high capability as PSB. In total, 11 chosen isolates were selected from LF developed from both PB (7 isolates) and PR (4 isolates).
Please add conclusion

Keywords: aerobic and anerobic bacteria, bacterial population, biofertilizer, biodiversity, organic materials.PR &LF

INTRODUCTION

Lampung Province has many potential agro-industries that play an important role in the economic growth of Indonesia such as sugar, palm oil, rubber, tapioca, mono-sodium glutamate (MSG), ethanol, fruit processing, and canning shrimp. However, agro-industries also produce various kinds of organic waste in large quantities that are harmful to the environment (Muralikrishna and Manickam 2017).

Palm oil bunches waste become an important problem in palm oil plantation worldwide. The large amount of this waste (220-230 kg ton^{-1} of fresh palm oil fruit) and its difficulties to be degraded are the main reasons. Some companies are doing nothing on this waste and the consequence is that this waste become suitable host of some palm oil pest insects and pathogens, especially *Oryctes rhinoceros* and *Ganoderma boninense* (Salmina 2016).

On the other hand, pineapple rhizomes are also important waste found in pineapple plantations. These waste cause problems especially on the planting seasons. If these pineapple rhizomes are not degrading immediately, the stump are still alive and it will grow and occupy the field that will be used to cultivate the seedling. The other problem is that if this waste is laid on the field, it will be host of some pest insects and pathogens.

Converting these both waste into beneficial material is now still being investigated and improved and one of which is converting this waste into liquid organic fertilizer. A liquid organic fertilizer is fertilizer which was made from fermented organic materials such as rice, compost, plant materials including palm oil bunches and pineapple rhizomes. This fermented fertilizer consists of bacterial isolates which has various capability such as phosphate solubilizing bacteria (PSB), chitin degrading bacteria, antagonist and plant growth promoter (Suryanto et al. 2006; Suyanto et al. 2016; Wiswasta et al. 2016; Rina et al. 2017). This fertilizer also can be used as bio activator to decompose other organic materials (Rina et al. 2017).

Phosphate-solubilizing bacteria play a vital role in P solubilization by producing organic acids. The application of organic acids along with PSB enhanced soluble P in the soil solution, improved root growth, and increased plant biomass of aerobic rice seedlings without affecting soil pH. Several bacteria belonging to genera Pseudomonas, Bacillus, Rhizobium, and Enterobacter and also some fungi like Penicillium and Aspergillus are capable of solubilizing P (Whitelaw

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2000) and the bacteria like Bacillus megaterium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous, Pseudomonas striata, and Enterobacter are often most referred as PSB (Subbarao 1988; Kucey et al. 1989). Moreover, PSB can solubilize the fixed phosphorus in the soil because its capacity to convert inorganic unavailable phosphorus form to soluble forms $HPO4^{2-}$ and $H2PO4^{-}$ through the process of organic acid production, chelation and ion exchange reactions and make them available to plants. Therefore, the use of PSB in agricultural practice would reduce the high cost of manufacturing phosphate fertilizers as well as would also mobilize insoluble phosphate in the fertilizers and soils (Zaidi et al. 2010; Banerjee et al. 2010).

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This research was performed in order to examine bacterial population and to do screening of PSB within liquid fertilizer developed from palm oil bunches and pineapple rhizomes. The chosen PSB will further explore to know their possibilities to be used as bio activator to decompose other organic materials, especially palm oil bunches and pineapple rhizomes.

MATERIALS AND METHODS

Development of liquid organic fertilizer (LF)

Liquid organic fertilizer (LF) were developed using two basic ingredients (palm oil bunches and pineapple rhizomes) in three conditions, namely aerobe, anaerobe and semi aerobe. The process of making LF were performed using reactor machine developed by Telaumbanuwa et al. (2019). Pineapple rhizomes was taken from PT. Great Giant Pineapple Co., Terbanggi Besar, Central Lampung, Lampung Province (Figure 1A), while palm oil bunches was taken from PTPN VII, Rejosari, Natar, Lampung Province (Figure 1B).

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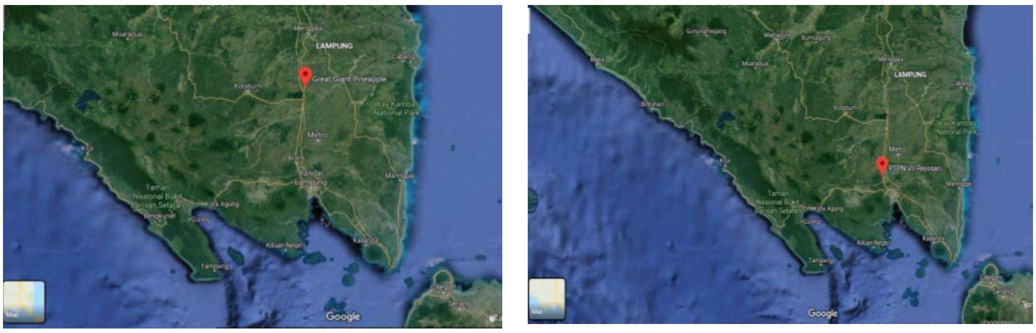
LF from palm oil bunches

Palm oil bunches (2.5 kg) were cut into small pieces and put inside the reactor machine. The other ingredients, namely palm sugar (0.5 kg), coconut water (2.5 L), and water of washing rice (2.5 L) were also put into machine. The sample of LF was collected from the machine 9 times in each condition every 3 days after placing the materials.



A B

Figure 1. Sampling location where organic materials are taken for this study: A. PT Great Giant Pineaple Co. at Terbanggi Besar, Central Lampung, Lampung Province; B. PTPN VII at Rejosari, Natar, Lampung Province (Source: Google Map).

LF from pineapple rhizomes

Pineapple rhizomes (5 kg) were cut into small pieces (2×2 cm) and placed into reactor machine and followed by palm sugar (1 kg), coconut water (5 L) and water of washing rice (5 L). The sample of LF was collected 9 times from the machine in each condition every 3 days after placing the material.

Bacterial isolation and propagation

Bacterial isolation

All the collected sample were diluted at 10^{10}, 10^{12} and 10^{14}. From each dilution series, 50µl and 100µl of the suspension were spread onto sterile plastic petri dish (ϕ 9 cm) containing *Plate Count Agar Peptone* (PCAP) media (plate count agar (PCA; OXOID®; England) 17.4 g, peptone (OXOID®; England) 2.5 g, Agar 2 g and 1000 mL distillate water). Observation was performed every day for 7 days on the total bacterial colonies which were emerged.

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Bacterial propagation

The emerging bacteria were purified by placing onto sterile plastic petridish (ϕ 9 cm) containing Yeast Peptone Agar (YPA) media peptone (OXOID®, England) 10 g, Yeast Extract (HIMEDIA®; India) 5 g, agar 20 g and 1000 mL distillate water). For further analysis, the bacteria were streak into Potato Peptone Glucose Agar (PPGA) slant media.

Capability as phosphate solubilizing bacteria

Investigation was performed by placing the bacteria onto sterile plastic Petri dish containing Pikovskaya (HIMEDIA®; India). Each Petri dish consists of 3 bacterial isolates. Observation was conducted every day for 7 days on the clear zone area around the bacterial colony. The clear zone area was calculated using millimeter block. This clear zone shows capability of the bacterial isolates to solubilize phosphate. Wider clear zone area created by the colony shows higher capability to solubilize phosphate. In this study, capability to solubilize phosphate was divided into 5 level, namely very low capability (clear zone area is in the range of 0.1 – 1.0 cm^2), low capability (clear zone area is in the range of 1.01 – 2.0 cm^2), medium capability (clear zone area is in the range of 2.01 – 3.0 cm^2), high capability (clear zone area is in the range of 3.01 to 4.0 cm^2) and very high capability (clear zone area is more than 4.0 cm^2).

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RESULTS AND DISCUSSION

Three conditions of making liquid organic fertilizer (LF) were used in this study. Those were aerobe, anaerobe and semi aerobe conditions. Two kinds of material were used as basic ingredients, namely palm oil bunches and pineapple rhizomes. The LF processing was made using a machine developed by Telaumbanuwa et al. (2019). The LF was further investigated on its bacterial population and their capability to solubilize phosphate.

Microbial population within liquid organic fertilizer (LF)

The results showed that bacterial population within liquid organic fertilizer in each conditions of making LF. In the case of LF developed from palm oil bunches, at 24 days after processing, the highest population was obtained from aerobe (285.60 x 10^{10} CFU mL^{-1}), followed by anaerobe (230.27 x 10^{10} CFU mL^{-1}) and semi aerobe (175.13 x 10^{10} CFU mL^{-1}) conditions. It appeared that in the aerobe condition, the bacteria will grow and replicate faster than in the anaerobe and semi aerobe conditions (Fig 2a). This was likely that LF developed from palm oil bunches was dominated by aerobic bacteria. Aerobic bacteria are able to use oxygen, whereas anaerobic bacteria can sustain itself without the presence of oxygen.

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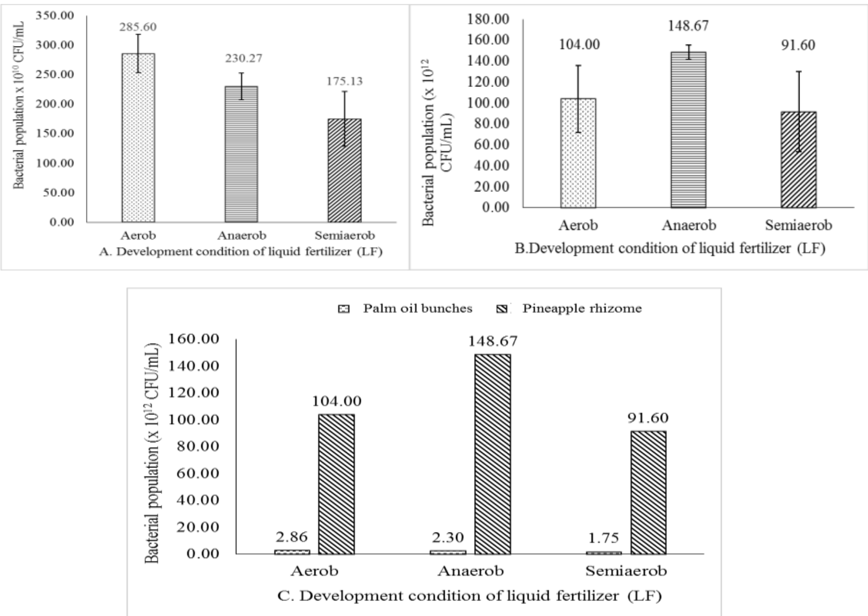

Figure 2. Bacterial population within liquid organic fertilizer. Total bacterial population within liquid organic fertilizer developed from palm oil bunches (A), Total bacterial population within liquid organic fertilizer developed from pineapple rhizomes (B), and Total bacterial population within liquid organic fertilizer developed both from palm oil bunches and pineapple rhizomes (C).

Different result was gained from LF which was developed from pineapple rhizomes. In the same days' isolation as LF from palm oil bunches, the highest bacterial population was resulted by anaerobe (148.67 x 10^{12} CFU mL^{-1}) followed by aerobe (104 x 10^{12} CFU mL^{-1}) and the lowest population was obtained from semi aerobe (91 x 10^{12} CFU mL^{-1}) condition. It was likely that bacteria which was in the anaerobe condition can grow and replicate faster than in the aerobe and semi aerobe conditions (Fig 2b). On the contrary with palm oil bunches with, it was likely that LF developed from pineapple rhizome was dominated by anaerobic bacteria.

Analysis on the total bacterial population within LF which was acquired from palm oil bunches and pineapple rhizomes in every different conditions of making LF revealed that bacterial population within LF from palm oil bunches was significantly lower than those developed from pineapple rhizome (Fig 2c). This may have happened because of the different characteristics between palm oil bunches and pineapple rhizomes. Pineapple rhizomes is more lenient than palm oil bunches, and it will be easier for the bacteria to degrade and to utilize organic materials from pineapple rhizomes for their growth than palm oil bunches.

Total bacteria obtained from LF

Totally, 791 bacterial isolates were successfully obtained from LF developed from both palm oil bunches and pineapple rhizomes. The 301 isolates were successfully obtained from pineapple rhizomes and 490 isolates from pineapple rhizomes. Among the total isolates, 249 isolates were obtained from aerobe condition, 242 isolate from anaerobe condition and 300 isolate from semi aerobe condition. As for LF created from palm oil bunches, from 301 isolates, 96 isolates obtained from aerobe condition, 93 isolates from anaerobe conditions and 112 isolates from semi aerobe conditions. In the case of 490 bacterial isolates acquired from LF developed from pineapple rhizomes, 153 isolate was gained from aerobe condition, 149 isolate from anaerobe condition and 188 isolate from semi aerobe condition (Table 1). Amount of bacteria isolated from aerobe was higher than anerobe conditions, while amount of bacteria from semi aerob condition was higher than both formerly conditions. Environmental conditions affected the growth of those bacteria. Aerobic bacteria are the species of bacteria which require oxygen for their basic survival, growth, and the process of reproduction, on the other hand, anaerobic bacteria are the species of bacteria which do not require oxygen for growth. This can be attributed to the fact that aerobic species have the ability to detoxify oxygen. In contrast, anaerobic species lack the ability to sufficiently break down food molecules like their aerobic counterparts. Between the two, there is semi aerob or facultative bacteria. These species, which carry out aerobic respiration in the presence of oxygen, also have the tendency to switch over to the process of fermentation in the absence of oxygen. In other words, facultative bacteria are capable of adapting to a range of conditions (Pelczar, Chan and Kreig 2001).

Table 1. Bacterial isolates obtained from liquid organic fertilizer

Conditions of making liquid organic fertilizer (LF)	Basic ingredients: Palm oil bunches	Basic ingredients: Pineapple rhizomes	Total bacterial isolates
Aerobe	96	153	249
Anaerobe	93	149	242
Semi aerobe	112	188	300
Total bacterial isolates	301	490	791

Total isolates of phosphate solubilizing bacteria

It was revealed that not all the isolates obtained in this study were phosphate solubilizing bacteria (PSB) (Figure 3 and 4). From 301 isolates of LF developed by palm oil bunches, 166 isolate (55.15%) showed capability to degrade phosphate and 135 isolates (44.85%) did not show any capability as PSB. As for 166 isolates of PSB, 67 isolates were obtained from aerobe condition, 38 isolates from anaerobe condition and 61 isolate from semi aerobe condition. In the case of the 135 isolates which were not PSB, 29 isolates were gained from aerobe condition, 55 isolates from anaerobe condition and 51 isolates from semi aerobe condition (Table 2).

Table 2. Total phosphate solubilizing bacteria

Conditions of making liquid organic fertilizer (LF)	Basic Ingredients			
	Palm oil bunches		Pineapple rhizomes	
	PSB	Non PSB	PSB	Non PSB
Aerobe	67	29	85	68
Anaerobe	38	55	83	66
Semi aerobe	61	51	101	87
Total bacterial isolates	166	135	269	221

As for 490 isolates acquired from pineapple rhizomes, 269 isolates (54.90%) had capability to solubilize phosphate, meanwhile the other 221 isolates (45.10%) did not showed any capability to solubilize phosphate. Among 269 isolates of PSB, 85 isolates originated from aerobe condition, 83 isolates from anaerobe condition and 101 isolates from semi aerobe condition. From 221 isolates which were not PSB, 68 isolates were isolated from aerobe condition, 66 isolates from

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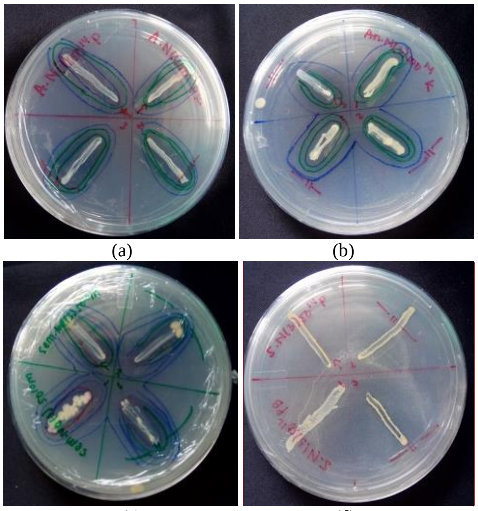
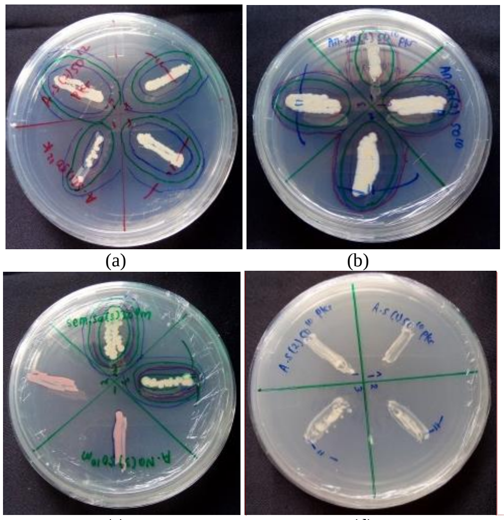
Comment [sajeesh30]: Please adjust like above table anaerobe condition and 87 isolates from semi aerobe condition (Table 2). Total isolates PSB found either in the palm oil bunches or pineaple rhizomes in the semi aerob condition were higher than aerobe or anaerobe condition. It was likely alternate condition between aerobe and anaerobe condition was more favorable for the bacteria to grow properly. Aerobic bacteria make energy with oxygen, while anaerobic bacteria make energy without oxygen. They do this in one of two ways, either through lactic acid or alcoholic fermentation. During lactic acid fermentation, cells use a molecule called NADH to take electrons from glucose. The NADH use the energy stored in the electrons to make ATP, and convert glucose to pyruvate. This process is called glycolysis and is the first step in all forms of cellular respiration. In lactic acid fermentation, the next step is to pyruvate to lactic acid, while, in alcoholic fermentation pyruvate to ethanol (Campbell and Reece 2007).



(a) (b) (c) (d)

Figure 3. Phosphate solubilizing bacteria from the extraction of pineapple rhizome that form clear zones under Aerob (a), Anaerobes (b), Semiaerobes (c) conditions and that do not form clear zones (d) on Pikovskaya media 7 days after inoculation.



(a) (b) (c) (d)

Figure 4. Phosphate solubilizing bacteria from the extraction of palm oil bunches that form clear zones under Aerob (a), Anaerobes (b), Semiaerobes (c) conditions and that do not form clear zones (d) on Pikovskaya media 7 days after inoculation.

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The chosen isolates of phosphate solubilizing bacteria

Among all the isolates obtained from palm oil bunches which were had capability to solubilize phosphate, most of the isolates had very low capability to solubilize phosphate and only small number isolates had high or very high capability to solubilize phosphate. Those were 60 isolates (19 isolates from aerobe condition, 19 isolates from an aerobe condition and 22 isolates from semi aerobe condition) had very low capability, 55 isolates (26 isolates from aerobe condition, 9 isolates from anaerobe condition and 20 isolates from semi aerobe condition) had low capability, 34 isolates (13 isolates from aerobe condition, 7 isolates from anaerobe condition and 14 isolates from semi aerobe condition) had medium capability, 10 isolates (4 isolates from aerobe condition, 3 isolates from anaerobe condition and 3 isolates from semi aerobe condition) had high capability and only 7 isolates (5 isolates from aerobe condition, 2 isolates from semi aerobe condition and none from anaerobe condition) had very high capability (Table 3).

Table 3. Capability of the bacterial isolates to solubilize phosphate

	Palm oil bunches			Pineapple rhizomes		
Phosphate solubilizing capability	**Aerobe**	**Anaerobe**	**Semi aerobe**	**Aerobe**	**Anaerobe**	**Semi aerobe**
very low	19	19	22	36	54	45
low	26	9	20	34	23	27
medium	13	7	14	12	6	14
high	4	3	3	2	0	12
very high	5	0	2	1	0	3
Total bacterial isolates	67	38	61	85	83	101

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In this study, we collected very high capability isolates of PSB as the chosen isolates. In total, 11 chosen isolates were selected from LF developed from both palm oil bunches (7 isolates) and pineapple rhizomes (4 isolates) (Table 4).

Table 4. The chosen phosphate solubilizing bacterial isolates

Name of isolates	**Condition of making liquid organic fertilizer**	**Basic ingredients of liquid organic fertilizer**	**Clear zone area (cm^2)**
A. Sa (1) 50.10 PKr			4.87
A. Sa (3) 50.8 PKr			4.76
A. Sa (2) 50.8 P	Aerobe		4.74
A.S(2)50.8 PKR		Palm oil bunches	4.35
A.S(3)50.8 Pkr			4.27
S.S (2) 50.8 PKr	semi aerobe		4.72
S.S(1)50.8PKR			4.54
A.N(1)100.10PKR	Aerobe		4.19
S.N(3)50.12P		Pineapple rhizomes	5.03
S.N(2)50.12P	semi aerobe		4.24
S.N(1)50.12P			4.08

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As for bacterial isolates gained from pineapple rhizomes which were showed capability as PSB, same as those on palm oil bunches, most of the isolates had very low capability to solubilize phosphate and only small number which produced high or very high capability as PSB. In detail, 135 isolates (36 isolates from aerobe condition, 54 isolates from anaerobe condition and 45 isolates from semi aerobe condition) had very low capability to solubilize phosphate, 84 isolates (34 isolates from aerobe condition, 23 isolates from anaerobe condition and 27 isolates from semi aerobe condition) had low capability to solubilize phosphate, 32 isolates (12 isolates from aerobe, 6 isolates from anaerobe and 14 isolates from semi aerobe) had medium capability to solubilize phosphate, 14 isolates (2 isolates from aerobe condition, 12 isolates from semi aerobe condition and none from anaerobe condition) had high capability to solubilize phosphate, 4 isolates (1 isolate from aerobe condition, 3 isolates from semi aerobe condition and none from an aerobe condition) produced very high capability as PSB (Table 3). The capability as PSB would affect the availability of soil phosphorus for the plant growth. Phosphate Solubilizing Bacteria stimulates plant growth through enhanced P nutrition (Tomar et al. 1993; Whitelaw et al. 1999), increasing the uptake of nitrogen (N), phosphate (P), potassium (K), and iron (Fe) (Biswas et al. 2000).

The isolates from LF created from palm oil bunches produced clear zone area on Pikovskaya media were in the range of 4.27 cm^2 to 4.87 cm^2. Those isolates were A. Sa (1) 50.10 PKr, A. Sa (3) 50.8 PKr, A. Sa (2) 50.8 P, A.S(2)50.8 PKR, A.S(3)50.8 Pkr (obtained from aerobe condition), S.S (2) 50.8 PKr, S.S(1)50.8PKR (obtained from semi aerobe condition). As for isolates from LF developed from pineapple rhizomes, they produced clear zone area on Pikovskaya media were between 4.19 cm^2 to 5.03 cm^2. The isolates were A.N(1)100.10PKR (obtained from aerobe condition), S.N(3)50.12P, S.N(2)50.12P and S.N(1)50.12P (obtained from semi aerobe condition).

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This research findings of isolates of PSB from palm oil bunches and pineapple rhizomes that have high capability to solubilize phosphorus, therefore, it will make a chance for phosphorus that is fixed in the soil to become available to plants. Those PSB will have capacity to convert inorganic unavailable phosphorus form to soluble forms HPO42- and H2PO4- through the process of organic acid production, chelation and ion exchange reactions (Khan et al. 2007). In the future, the use of PSB in agricultural practice would decline the high cost of manufacturing phosphate fertilizers as well as

Comment [sajeesh40]: Same point in line 59 they will also mobilize insoluble phosphorus in the fertilizers and soils (Alori et al. 2017; Zaidi et al 2010; Banerjee et al. 2010). Moreover, the use of PSB is considered as an environmental-friendly alternative to further applications of chemical based P fertilizers.

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CONCLUSIONS

Microbial population in the liquid organic fertilizer developed from palm oil bunches was lower than those from pineapple rhizomes. Totally, 791 bacterial isolates were obtained from the liquid organic fertilizer; 301 isolates were collected from palm oil bunches and 490 isolates were achieved from pineapple rhizomes. In the case of isolates which were gained from palm oil bunches, 166 isolate (55.15%) showed capability to degrade phosphate and 135 isolates (44.85%) did not show any capability as PSB. Among166 isolates of PSB from palm oil bunches, 60 isolates had very low capability, 55 isolates had low capability, 34 isolates had medium capability, 10 isolates had high capability and only 7 isolates had very high capability as PSB. Meanwhile, from 269 isolates of PSB obtained from pineapple rhizomes, 135 isolates had very low capability, 84 isolates had low, 32 had medium capability, 14 isolates had high capability and 4 isolates produced very high capability as PSB. In total, 11 chosen isolates were selected from LF developed from both palm oil bunches (7 isolates) and pineapple rhizomes (4 isolates).

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ACKNOWLEDGEMENTS

We would like to thank the University of Lampung (Unila) through LPPM Unila Indonesia for providing "Hibah Professor" DIPA BLU funds year 2018 as well as Faculty of Agriculture Unila for the permission to use research facilities. Thanks also to PT. GGPC (Great Giant Pineaple Company) for supplying pineaple rhizomes and PTPN VII for supplying palm oil bunches.

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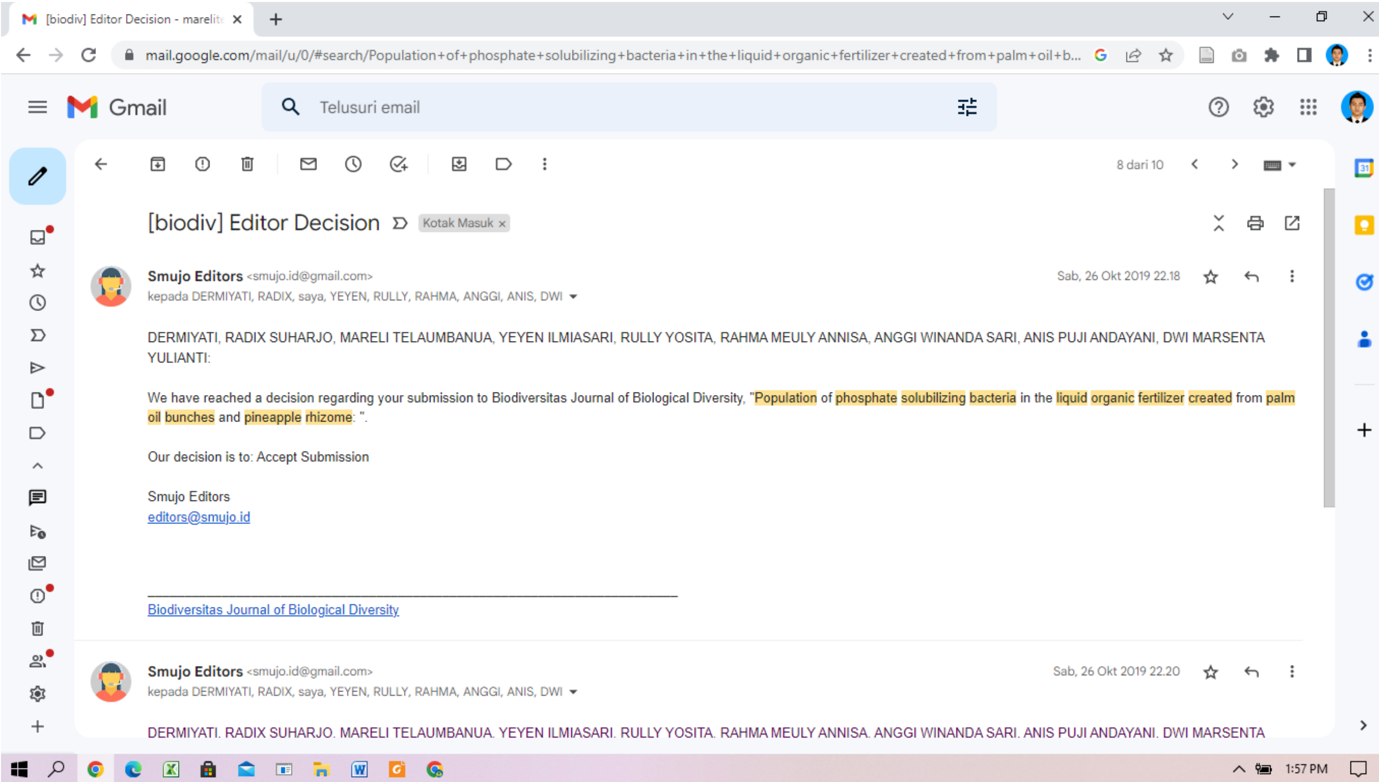

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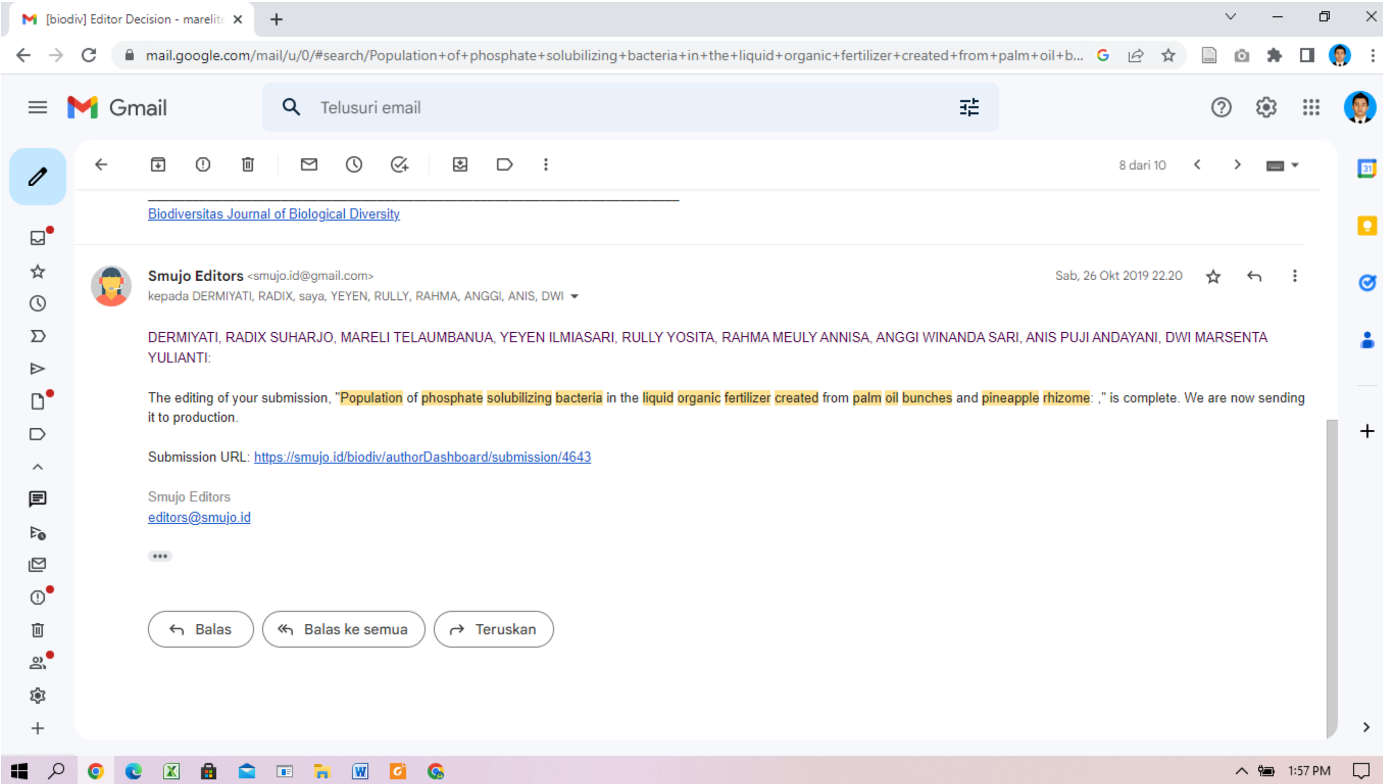

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BIODIVERSITAS
Volume 20, Number 11, November 2019
Pages: 3315-3321

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d201126

Population of phosphate solubilizing bacteria in the liquid organic fertilizer created from palm oil bunches and pineapple rhizome

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Manuscript received: 13 October 2019. Revision accepted: 25 October 2019.

Abstract. *Dermiyati, Suharjo R, Telaumbanua M, Ilmiasari Y, Yosita R, Annisa RM, Sari AW, Andayani AP, Yulianti DM. 2019. Population of phosphate solubilizing bacteria in the liquid organic fertilizer created from palm oil bunches and pineapple rhizome. Biodiversitas 20: 3315-3321.* Palm oil bunches and pineapple rhizomes are abundant in Indonesia, especially in Lampung Province due to widespread agro-industrial company and they are potential to be used as sources for liquid organic fertilizer. This study was aimed to reveal microbial population within liquid organic fertilizer (LF) which was developed from palm oil bunches (PB) and pineapple rhizome (PR) and their role as phosphate solubilizing bacteria (PSB). The capability to solubilize phosphate was recorded from the clear zone area around the bacterial colony which was cultivated on Pikovskaya's agar medium. The LF was prepared in three kinds of conditions, namely aerobic, anaerobic, and facultative an aerobic. Isolation was performed every 3 days until 24 days after incubations. The results showed that microbial population in the LF from PR was significantly higher than those developed from PB. Totally, 791 bacterial isolates were obtained from the LF; 490 isolates were achieved from PR (153 isolates from aerobic, 188 isolates from facultative anaerobic, 149 from anaerobic conditions) and 301 isolates were collected from PB (96 isolates from aerobic, 112 isolates from facultative anaerobic, 93 isolates from anaerobic conditions). In the case of isolates that were gained from PB, 166 isolates (55.15%) showed capability to degrade phosphate and 135 isolates (44.85%) did not show any capability as PSB. As for isolates obtained from PR, 269 isolates (54.90%) had capability to solubilize phosphate, meanwhile, the other 221 isolates (45.10%) did not show any capability to solubilize phosphate. Among166 isolates of PSB from PB, 60 isolates had very low capability, 55 isolates had low capability, 34 isolates had medium capability, 10 isolates had high capability and only 7 isolates had very high capability. Meanwhile, from 269 isolates of PSB obtained from PR, 135 isolates had very low capability to solubilize phosphate, 84 isolates had low capability to solubilize phosphate, 32 had medium capability to solubilize phosphate, 14 isolates had high capability to solubilize phosphate and 4 isolates produced very high capability as PSB. In total, 11 chosen isolates were selected from LF developed from both PB (7 isolates) and PR (4 isolates). Therefore, palm oil bunches and pineapple rhizomes contained PSB that can be used as LF.

Keywords: Aerobic and anaerobic bacteria, population, biodiversity, liquid organic fertilizer, palm oil bunches, pineapple rhizomes

INTRODUCTION

Lampung Province has many potential agro-industries that play an important role in the economic growth of Indonesia such as sugar, palm oil, rubber, tapioca, monosodium glutamate (MSG), ethanol, fruit processing, and canning shrimp. However, agro-industries also produce various kinds of organic waste in large quantities that are harmful to the environment (Muralikrishna and Manickam 2017).

Palm oil bunches waste become an important problem in palm oil plantation worldwide. A large amount of this waste (220-230 kg ton^{-1} of fresh palm oil fruit) and its difficulties to be degraded are the main reasons. Some companies are doing nothing on this waste and the consequence is that this waste becomes suitable host of some palm oil pest insects and pathogens, especially *Oryctes rhinoceros* and *Ganoderma boninense* (Salmina 2016).

On the other hand, pineapple rhizomes are also important waste found in pineapple plantations. This waste cause problems especially on the planting seasons. If these pineapple rhizomes are not degrading immediately, the stump is still alive and it will grow and occupy the field that will be used to cultivate the seedling. The other problem is that if this waste is laid on the field, it will be host of some pest insects and pathogens (de Kogel 2019).

Converting these both waste into beneficial material is now still being investigated and improved and one of which is converting this waste into liquid organic fertilizer. Liquid organic fertilizer is fertilizer which was made from fermented organic materials such as rice, compost, plant materials including palm oil bunches and pineapple rhizomes. This fermented fertilizer consists of bacterial isolates which have various capacities such as phosphate solubilizing bacteria (PSB), chitin degrading bacteria, antagonist and plant growth promoter (Alori et al. 2017; Ingle and Padole 2017; Rina et al. 2017). This fertilizer also can be used as bioactivator to decompose other organic materials (Rina et al. 2017).

Phosphate-solubilizing bacteria play a vital role in P solubilization by producing organic acids. The application of organic acids along with PSB enhanced soluble P in the soil solution, improved root growth, and increased plant biomass of aerobic rice seedlings without affecting soil pH (Biswas et al. 2000). Several bacteria belonging to genera Pseudomonas, Bacillus, Rhizobium, and Enterobacter and also some fungi like *Penicillium* and *Aspergillus* are capable of solubilizing P (Whitelaw 2000) and the bacteria like *Bacillus megaterium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous, Pseudomonas striata,* and *Enterobacter* are often most referred as PSB (Subba Rao 1993; Kucey et al. 1989). Moreover, PSB can solubilize the fixed phosphorus in the soil because of their capacity to convert inorganic unavailable phosphorus form to soluble forms HPO_4^{2-} and $H_2PO_4^-$ through the process of organic acid production, chelation and ion exchange reactions and make them available to plants. Therefore, the use of PSB in agricultural practice would reduce the high cost of manufacturing phosphate fertilizers as well as would also mobilize insoluble phosphate in the fertilizers and soils (Zaidi et al. 2010; Banerjee et al. 2010).

This research was performed in order to examine bacterial population and to do a screening of PSB within liquid fertilizer developed from palm oil bunches and pineapple rhizomes. The chosen PSB will further explore to know their possibilities to be used as bioactivator to decompose other organic materials, especially palm oil bunches and pineapple rhizomes.

MATERIALS AND METHODS

Development of liquid organic fertilizer (LF)

Liquid organic fertilizer (LF) was developed using two basic ingredients (palm oil bunches and pineapple rhizomes) in three conditions, namely aerobic, anaerobic, and facultative anaerobic. The process of making LF was performed using reactor machine developed by Telaumbanuwa et al. (2019). Pineapple rhizomes were taken from Perseroan Terbatas (PT) Great Giant Pineapple Co., Terbanggi Besar, Central Lampung, Lampung Province (Figure 1.A), while palm oil bunches were taken from PT Perkebunan Nusantara (PTPN) VII, Rejosari, Natar, Lampung Province (Figure 1.B).

LF from palm oil bunches

Palm oil bunches (2.5 kg) were cut into small pieces and put inside the reactor machine. The other ingredients, namely palm sugar (0.5 kg), coconut water (2.5 L), and water of washing rice (2.5 L) were also put into machine. The sample of LF was collected from the machine 9 times in each condition every 3 days after placing the materials.

LF from pineapple rhizomes

Pineapple rhizomes (5 kg) were cut into small pieces (2×2 cm) and placed into reactor machine and followed by palm sugar (1 kg), coconut water (5 L) and water of washing rice (5 L). The sample of LF was collected 9 times from the machine in each condition every 3 days after placing the material.

Figure 1. Sampling location where organic materials are taken for this study in Lampung Province, Indonesia: 1. PT Great Giant Pineapple Co. at Terbanggi Besar, Central Lampung, Lampung Province; 2. PTPN VII at Rejosari, Natar, Lampung Province (Source: Google Map & Wikipedia)

Bacterial isolation and propagation

Bacterial isolation

All the collected samples were diluted at 10^{-10}, 10^{-12} and 10^{-14}. From each dilution series, 50µl and 100µl of the suspension were spread onto sterile plastic petri dish (diameter 9 cm) containing Plate Count Agar Peptone (PCAP) media (plate count agar (PCA; OXOID®; England) 17.4 g, peptone (OXOID®; England) 2.5 g, Agar 2 g and 1000 mL distillate water). Observation was performed every day for 7 days on the total bacterial colonies which were emerged.

Bacterial propagation

The emerging bacteria were purified by placing onto sterile plastic petridish (diameter 9 cm) containing Yeast Peptone Agar (YPA) media peptone (OXOID®, England) 10 g, Yeast Extract (HIMEDIA®; India) 5 g, agar 20 g, and 1000 mL distillate water). For further analysis, the bacteria were streak into Potato Peptone Glucose Agar (PPGA) slant media.

Capability as phosphate solubilizing bacteria

Investigation was performed by placing the bacteria onto sterile plastic Petri dish containing Pikovskaya (HIMEDIA®; India) (Nguyen et al. 1992; Seshadri et al. 2002). Each Petri dish consists of 3 bacterial isolates. Observation was conducted every day for 7 days on the clear zone area around the bacterial colony. The clear zone area was calculated using millimeter block. This clear zone shows capability of the bacterial isolates to solubilize phosphate. Wider clear zone area created by the colony shows higher capability to solubilize phosphate. In this study, capability to solubilize phosphate was categorised as index phosphate solubilization which was divided into 5 level, namely very low capability (clear zone area is in the range of 0.1-1.0), low capability (clear zone area is in the range of 1.01-2.0), medium capability (clear zone area is in the range of 2.01-3.0), high capability (clear zone area is in the range of 3.01 to 4.0) and very high capability (clear zone area is more than 4.0) (Matos et al. 2017).

RESULTS AND DISCUSSION

Three conditions of making liquid organic fertilizer (LF) were used in this study. Those were aerobic, anaerobic and facultative anaerobic conditions. Two kinds of material were used as basic ingredients, namely palm oil bunches and pineapple rhizomes. The LF processing was made using a machine developed by Telaumbanuwa et al. (2019) which created the aerobic, anaerobic, or facultative anaerobic conditions. The LF was further investigated on its bacterial population and its capability to solubilize phosphate.

Microbial population within liquid organic fertilizer (LF)

Microbial population in the LF was counted by the amount of bacterial grown in the Plate Count Agar Peptone (PCAP) media which was modified from PCA media (Atlas 2010). The results showed that bacterial population within liquid organic fertilizer in each aerobic, anaerobic, facultative anaerobic conditions of making LF by using reactor machine developed by Telaumbanua et al. (2019). In the case of LF developed from palm oil bunches, at 24 days after processing, the highest population was obtained from aerobic (285.60 x 10^{10} CFU mL^{-1}), followed by anaerobic (230.27 x 10^{10} CFU mL^{-1}) and facultative anaerobic (175.13 x 10^{10} CFU mL^{-1}) conditions. It appeared that in the aerobic condition, the bacteria will grow and replicate faster than in the anaerobic and the facultative anaerobic conditions (Fig 2a). This was likely that LF developed from palm oil bunches was dominated by aerobic bacteria. Aerobic bacteria are able to use oxygen, whereas anaerobic bacteria can sustain itself without the presence of oxygen.

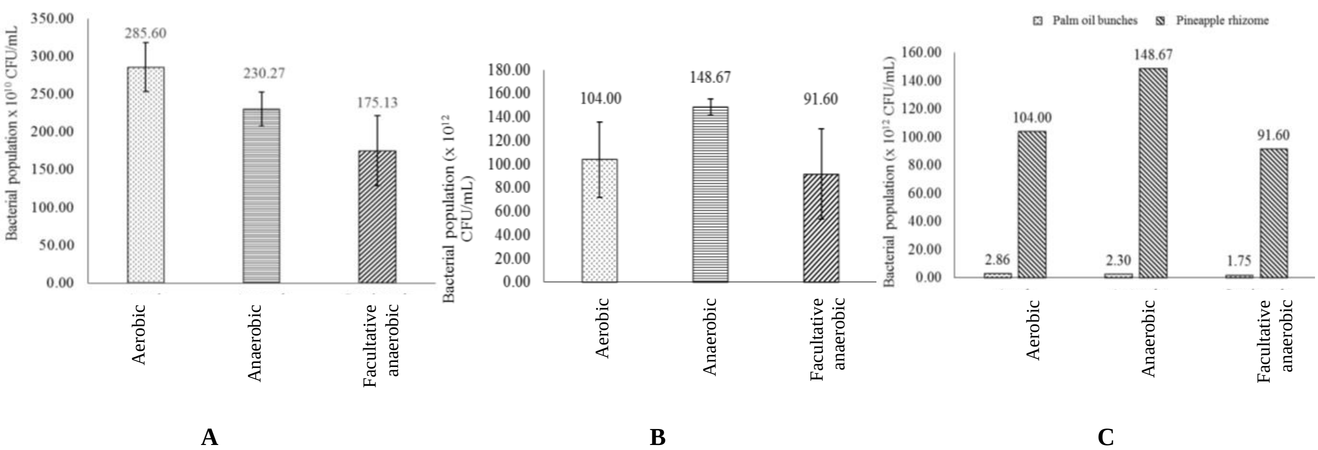

Figure 2. Bacterial population within liquid organic fertilizer. A. Total bacterial population within liquid organic fertilizer developed from palm oil bunches. B. Total bacterial population within liquid organic fertilizer developed from pineapple rhizomes. C. Total bacterial population within liquid organic fertilizer developed both from palm oil bunches and pineapple rhizomes

Different result was gained from LF which was developed from pineapple rhizomes. In the same days' isolation as LF from palm oil bunches, the highest bacterial population was resulted by anaerobic (148.67 x 10^{12} CFU mL^{-1}) followed by aerobic (104 x 10^{12} CFU mL^{-1}) and the lowest population was obtained from facultative anaerobic (91 x 10^{12} CFU mL^{-1}) conditions. It was likely that bacteria which was in the an aerobic condition can grow and replicate faster than in the aerobic and facultative anaerobic conditions (Fig 2b). On the contrary with palm oil bunches, it was likely that LF developed from pineapple rhizome was dominated by anaerobic bacteria.

Analysis of the total bacterial population within LF which was acquired from palm oil bunches and pineapple rhizomes in every different condition of making LF revealed that bacterial population within LF from palm oil bunches was lower than those developed from pineapple rhizome (Fig 2c). This may have happened because of the different characteristics of palm oil bunches and pineapple rhizomes. Pineapple rhizomes are more lenient than palm oil bunches, and it will be easier for the bacteria to degrade and to utilize organic materials from pineapple rhizomes for their growth than palm oil bunches.

Total bacteria obtained from LF

Totally, 791 bacterial isolates were successfully obtained from LF developed from both palm oil bunches and pineapple rhizomes. The 301 isolates were successfully obtained from palm oil bunches and 490 isolates from pineapple rhizomes. Among the total isolates, 249 isolates were obtained from aerobic conditions, 242 isolates from anaerobic conditions, and 300 isolates from facultative anaerobic conditions. As for LF created from palm oil bunches, from 301 isolates, 96 isolates obtained from aerobic conditions, 93 isolates from anaerobic conditions and 112 isolates from facultative anaerobic conditions. In the case of 490 bacterial isolates acquired from LF developed from pineapple rhizomes, 153 isolates was gained from aerobic condition, 149 isolates from an aerobic condition and 188 isolates from facultative anaerobic condition (Table 1). Amount of bacteria isolated from aerobic conditions was higher than anaerobic conditions, while amount of bacteria from facultative anaerobic conditions was higher than both formerly conditions. Environmental conditions affected the growth of those bacteria. Aerobic bacteria are the species of bacteria which require oxygen for their basic survival, growth, and the process of reproduction, on the other hand, anaerobic bacteria are the species of bacteria which do not require oxygen for growth. This can be attributed to the fact that aerobic species have the ability to detoxify oxygen. In contrast, anaerobic species lack the ability to sufficiently break down food molecules like their aerobic counterparts. Between the two, there is facultative anaerobic bacteria. These species, which carry out aerobic respiration in the presence of oxygen, also have the tendency to switch over to the process of fermentation in the absence of oxygen. In other words, facultative bacteria are capable of adapting to a range of conditions (Pelczar et al. 2001).

Total isolates of phosphate solubilizing bacteria

According to the study, phosphate solubilizing bacteria (PSB) was found in the isolates (Figures 3 and 4). From 301 isolates of LF developed by palm oil bunches, 166 isolate (55.15%) showed capability to solubilize phosphate and 135 isolates (44.85%) did not show any capability as PSB. As for 166 isolates of PSB, 67 isolates were obtained from aerobic conditions, 38 isolates from anaerobic conditions, and 61 isolates from facultative anaerobic conditions. In the case of the 135 isolates which were not PSB, 29 isolates were gained from aerobic conditions, 55 isolates from anaerobic conditions and 51 isolates from facultative anaerobic conditions (Table 2). Media used to grow PSB was Pikovskaya's agar.

As for 490 isolates acquired from pineapple rhizomes, 269 isolates (54.90%) had capability to solubilize phosphate, meanwhile, the other 221 isolates (45.10%) did not show any capability to solubilize phosphate. Among 269 isolates of PSB, 85 isolates originated from aerobic conditions, 83 isolates from anaerobic conditions, and 101 isolates from facultative anaerobic conditions. From 221 isolates which were not PSB, 68 isolates were isolated from aerobic condition, 66 isolates from anaerobic conditions and 87 isolates from facultative anaerobic conditions (Table 2). Total isolates PSB found either in the palm oil bunches or pineapple rhizomes in the facultative anaerobic condition were higher than aerobic or anaerobic condition. It was likely alternate condition between aerobic and an aerobic was more favorable for the bacteria to grow properly. Aerobic bacteria make energy with oxygen, while anaerobic bacteria make energy without oxygen. They do this in one of two ways, either through lactic acid or alcoholic fermentation. During lactic acid fermentation, cells use a molecule called NADH to take electrons from glucose. The NADH uses the energy stored in the electrons to make ATP, and convert glucose to pyruvate. This process is called glycolysis and is the first step in all forms of cellular respiration. In lactic acid fermentation, the next step is to pyruvate to lactic acid, while, in alcoholic fermentation pyruvate to ethanol (Campbell and Reece 2007).

Table 1. Bacterial isolates obtained from liquid organic fertilizer

Conditions of making liquid organic fertilizer (LF)	Basic ingredients		Total bacterial isolates
	Palm oil bunches	Pineapple rhizomes	
Aerobic	96	153	249
Anaerobic	93	149	242
Facultative anaerobic	112	188	300
Total bacterial isolates	301	490	791

Table 2. Total phosphate solubilizing bacteria

Conditions of making liquid organic fertilizer (LF)	Basic Ingredients			
	Palm oil bunches		Pineapple rhizomes	
	PSB	Non PSB	PSB	Non PSB
Aerobic	67	29	85	68
Anaerobic	38	55	83	66
Facultative anaerobic	61	51	101	87
Total bacterial isolates	166	135	269	221

Figure 3. Phosphate solubilizing bacteria from the extraction of pineapple rhizome that form clear zones under: A. Aerobic, B. Anaerobic, C. Facultative anaerobic conditions, D. Do not form clear zones on Pikovskaya's media 7 days after inoculation

Figure 4. Phosphate solubilizing bacteria from the extraction of palm oil bunches that form clear zones under: A. Aerobic, B. Anaerobic, C. Facultative anaerobic conditions, D. Do not form clear zones on Pikovskaya's media 7 days after inoculations

Table 3. Capability of the bacterial isolates to solubilize phosphate

Phosphate solubilizing capability	Palm oil bunches			Pineapple rhizomes		
	Aerobic	Anaerobic	Facultative anaerobic	Aerobic	Anaerobic	Facultative anaerobic
Very low	19	19	22	36	54	45
Low	26	9	20	34	23	27
Medium	13	7	14	12	6	14
High	4	3	3	2	0	12
Very high	5	0	2	1	0	3
Total bacterial isolates	67	38	61	85	83	101

The chosen isolates of phosphate solubilizing bacteria

Among all the isolates obtained from palm oil bunches which were had capability to solubilize phosphate (P), most of the isolates had very low capability to solubilize P and only small number isolates had high or very high capability to solubilize P. Those were 60 isolates (19 isolates from aerobic condition, 19 isolates from anaerobic condition and 22 isolates from facultative anaerobic condition) had very low capability, 55 isolates (26 isolates from aerobic condition, 9 isolates from anaerobic condition and 20 isolates from facultative anaerobic condition) had low capability, 34 isolates (13 isolates from aerobic condition, 7 isolates from anaerobic condition and 14 isolates from facultative anaerobic condition) had medium capability, 10 isolates (4 isolates from aerobic condition, 3 isolates from anaerobic condition and 3 isolates from facultative anaerobic condition) had high capability and only 7 isolates (5 isolates from aerobic condition, 2 isolates from facultative anaerobic condition and none from anaerobic condition) had very high capability to solubilize P (Table 3).

In this study, we collected very high capability isolates of PSB as the chosen isolates. In total, 11 chosen isolates were selected from LF developed from both palm oil bunches (7 isolates) and pineapple rhizomes (4 isolates) (Table 4).

Table 4. The chosen phosphate solubilizing bacterial isolates

Name of isolates	Condition of making liquid organic fertilizer	Basic ingredients of liquid organic fertilizer	Index capability to solubilized phosphate
A. Sa (1) 50.10 PKr	Aerobic	Palm oil bunches	4.87
A. Sa (3) 50.8 PKr			4.76
A. Sa (2) 50.8 P			4.74
A.S(2)50.8 PKR			4.35
A.S(3)50.8 Pkr			4.27
S.S (2) 50.8 PKr	Facultative anaerobic		4.72
S.S(1)50.8PKR			4.54
A.N(1)100.10PKR	Aerobic	Pineapple rhizomes	4.19
S.N(3)50.12P	Facultative anaerobic		5.03
S.N(2)50.12P			4.24
S.N(1)50.12P			4.08

As for bacterial isolates gained from pineapple rhizomes which were showed capability as PSB, same as those on palm oil bunches, most of the isolates had very low capability to solubilize phosphate and only small number which produced high or very high capability as PSB. In detail, 135 isolates (36 isolates from aerobic condition, 54 isolates from anaerobic condition and 45 isolates from facultative anaerobic condition) had very low capability to solubilize P, 84 isolates (34 isolates from aerobic condition, 23 isolates from anaerobic condition and 27 isolates from facultative anaerobic condition) had low capability to solubilize P, 32 isolates (12 isolates from aerobic, 6 isolates from anaerobic and 14 isolates from facultative anaerobic conditions) had medium capability to solubilize P, 14 isolates (2 isolates from aerobic condition, 12 isolates from facultative anaerobic conditions and none from anaerobic condition) had high capability to solubilize phosphate, 4 isolates (1 isolate from aerobic condition, 3 isolates from facultative anaerobic condition and none from anaerobic condition) produced very high capability as PSB (Table 3). The capability as PSB would affect the availability of soil phosphorus for plant growth. Phosphate Solubilizing Bacteria stimulates plant growth through enhanced P nutrition (Tomar et al. 1993; Whitelaw et al. 1999), increasing the uptake of nitrogen (N), phosphate (P), potassium (K), and iron (Fe) (Biswas et al. 2000).

The isolates from LF created from palm oil bunches produced clear zone area on Pikovskaya's media were in the index range of 4.27 to 4.87. Those isolates were A. Sa (1) 50.10 PKr, A. Sa (3) 50.8 PKr, A. Sa (2) 50.8 P, A.S(2)50.8 PKR, A.S(3)50.8 Pkr (obtained from aerobic condition), S.S (2) 50.8 PKr, S.S(1)50.8 PKR (obtained from facultative anaerobic condition). As for isolates from LF developed from pineapple rhizomes, they produced clear zone area on Pikovskaya's media were between 4.19 cm^2 to 5.03 cm^2. The isolates were A.N(1)100.10PKR (obtained from aerobic condition), S.N(3)50.12P, S.N(2)50.12P and S.N(1)50.12P (obtained from facultative anaerobic condition).

This research findings of isolates of PSB from palm oil bunches and pineapple rhizomes that have high capability to solubilize P, therefore, it will make a chance for phosphorus that is fixed in the soil to become available to plants. Those PSB will have capacity to convert inorganic unavailable phosphorus form to soluble forms $HPO_4^{2-}$and $H_2PO_4^-$through the process of organic acid production, chelation and ion exchange reactions (Khan et al. 2007). In the future, the use of PSB in agricultural practice would decline the high cost of manufacturing phosphate fertilizers as well as they will also mobilize insoluble phosphorus in the fertilizers and soils (Alori et al. 2017; Zaidi et al. 2010; Banerjee et al. 2010). Moreover, the use of PSB is considered as an environmental-friendly alternative to further applications of chemical-based P fertilizers (Alori et al. 2017).

In conclusion, microbial population in the liquid organic fertilizer (LF) developed from palm oil bunches (PB) was lower than those from pineapple rhizomes (PR). Similarly, collected isolates from PB has lower index capability as PSB compared to PR. Although, some isolates either from PB or PR did not have capability as PSB. From the study, 11 chosen isolates (from PB was 7 isolates and from PR was 4 isolates) were selected as PSB and will be used for further study to develop LF from PB and PR.

ACKNOWLEDGEMENTS

We would like to thank the University of Lampung (Unila), Indonesia through LPPM Unila for providing *Hibah Professor* DIPA BLU funds year 2018 on behalf of Prof. Dr. Dermiyati, Dr. Radix Suharjo, and Dr. Mareli Telaumbanua as well as Faculty of Agriculture, Unila for the permission to use research facilities. Thanks also to PT. GGPC (Great Giant Pineapple Co.) for supplying pineapple rhizomes and PTPN VII for supplying palm oil bunches.

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