AGRIVITA Journal of Agricultural Science. 2017. 39(3): 252-256

Quantification of BRIS Soil Bacteria at Tembila, Besut Terengganu

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Received: February 13, 2017 /Accepted: August 3, 2017

ABSTRACT

A study was made to determine total number of bacteria living in the rhizosphere of three common plants at BRIS soil namely *Acacia mangium*, *Melaleuca cajuputi* Powell and *Eleusine indica* that grow on field. Soil samples were collected from each plant's rhizosphere at three different locations of UniSZA Apiary, AGROPOLIS Research Farm and FBIM Teaching Farm at UniSZA, Besut Campus on May and November 2015. Enumeration of total bacteria by standard plate counts found that Acacia's rhizosphere at AGROPOLIS Research Farm showed the highest CFU g^{-1} in November 2015 for aerobic (6.14 x 10^6) and anaerobic (3.97 x 10^6) bacteria. Acacia's rhizosphere from UniSZA Apiary showed the highest CFU g^{-1} value in November 2015 for nitrogen fixing bacteria (aerobic; 1.87 x 10^6, anaerobic; 1.5 x 10^6) and phosphate solubilization bacteria (aerobic; 1.4 x 10^6, anaerobic; 1.41 x 10^6). The rainy season on November gave a higher bacterial CFU g^{-1} number (20-44 %) compared to the dry season on May for all different plants and locations. The finding showed that although BRIS soil is the problematic and unfertile soil, it has a large number of bacterial colony that might be beneficial to be exploited for enhancing plant growth and soil fertility.

Keywords: bacteria; BRIS soil; CFU; N-fixing; P-soluble

INTRODUCTION

Beach Ridges Interspersed with Swales (BRIS) soil is the sandy soil that has a weak structure and a relatively high soil temperature, low water retention capacity, nutrient deficiency and has limited ability to support plant growth (Roslan, Shamshuddin, Fauziah, & Anuar, 2011). The soil has 95 – 98 % sand fractions with silt and clay contents below 4 %. High soil contents caused excessive drainage that consequently resulting in low moisture and nutrient content at this soil. This soil has a coarse sand component that originates from the undulating processes during the monsoon seasons. The processes caused accumulations of sediment and sand from the sea which made up the ridges and swales (Roslan, Shamshuddin, Fauziah, & Anuar, 2011; Ishaq, Umara, Armanto, & Adzemi, 2014). In Peninsular Malaysia, this soil is distributed along the east coast of Kelantan (17806.2 hectares), Terengganu (67582.61 hectares), Pahang (36017.17 hectares) and west coast of Johor (Ekhwan Hj Toriman, Mokhtar, Gazim, & Azlina Abd Aziz, 2009).

BRIS soil is considered as problematic soil and not well utilized for crop production due to its low physical and chemical characteristics. During the hot day, the temperature of the soil can rise to about 43 °C and dropping to 25 °C at night. The soil is very dry and lack of nutrient because of the high temperature and low water retention capacity. This situation can seriously affect plants as its leaf will scorch and the plant will wilt thus hinders the plants growth at this area. The lack of plants growing on BRIS soil, in turn, causes the temperature of the soil to remain high. As such, this high soil temperature causes the speedy vaporization of moisture and also nitrogen on soil surfaces that seriously affect plants' growth. In addition, the constant leaching process also reduces the moisture content of the soil.

The land areas of BRIS soil are mostly covered by grass and small shrubs. It is found that most untouched areas are dominated by the Acacia tree (*Acacia mangium*) and Gelam tree (*Melaleuca cajuputi* Powell) while the field area are covered by the Goose grass (*Eleusine indica*) and Mission grass (*Pennisetum polystachion*). Plant's health is affected by numerous biotic and abiotic factors in

Cite this as: Mustapha, Z., Mat, N., Othman, R., & Zakaria, Abd. J. (2017). Quantification of BRIS soil bacteria at Tembila, Besut Terengganu. *AGRIVITA Journal of Agricultural Science*, *39*(3), 252–256. http://doi.org/10.17503/agrivita.v39i3.1292
Accredited: SK No. 60/E/KPT/2016

soil, atmosphere and root surfaces. Nitrogen is the most important and limiting nutrient for plant growth. The atmospheric nitrogen needs to be converted to ammonia to make it accessible to plants. This enzymatic process mediated by diazotrophs or bacteria that has the ability to fix atmospheric nitrogen. Besides nitrogen, phosphorus is another major nutrient for plant growth and development. It is important for energy storage and transfer for cell division and enlargement and in the process of photosynthesis and respiration (Senthil Kumar & Bharathi, 2015). This mineral remains in insoluble form in the soil. It needs to be released to soluble and fixed form to be enabled to plant. The process is usually mediated by the phosphate solubilizing bacteria.

Soil is a complex habitat for many types of microorganisms such as yeast, fungi, actinomycetes, and bacteria. Among them, fungi and bacteria are recorded as the main decomposers and produced the largest part of soil biomass (Logeswaran, Prabagaran, & Ramesh, 2014). Other than that, microorganisms are also important for various functions in nature. It is including degradation of complex organic matter to a simple form, improve soil biological and physicochemical properties, supply fertilizers to plant as well as improve plants resistant to pest and diseases.

This study was conducted to determine the presence of microorganism especially the bacteria that has both abilities to fix nitrogen and solubilize phosphate that contribute to the soil fertility for plant growth and development. This type of bacteria might be the cause that boosts certain plants to grow well in the BRIS soil area. The physicochemical properties of BRIS soil in Tembila, Besut Terengganu were also studied to determine the soil fertility at this area. This study revealed the richness of BRIS soil effective microorganisms that could be isolated and further used in biotechnological advances to enhance soil fertility as well as plant growth and yield.

MATERIALS AND METHODS

Soil Sampling

Soil samples were collected in May and November 2015 from the rhizosphere of *Acacia mangium* (Acacia), *Melaleuca cajuputi* Powell (Gelam) and *Eleusine indica* (Goose grass) from three different sites; Universiti Sultan Zainal Abidin (UniSZA) Apiary, Agricultural Production and Food Innovation Research Institute (AGROPOLIS) Research Farm, and Faculty Bioresources and Food Industry (FBIM) Teaching Farm of Tembila, Besut Campus of UniSZA. Five random samples from each plant's rhizosphere were withdrawn from 10-15 cm depth. The soil samples were air dried and passed through a 2 mm sieve before being mixed into a single composite sample for further analysis (Tallapragada & Seshachala, 2012).

Physicochemical Properties of Soil

The collected soil samples were subjected to standard physicochemical analyses in the laboratory. The pH and temperature were determined using the digital pH meter and thermometer. The total organic carbon was measured using the method as described by Nelson and Sommers (1996), total nitrogen (Kjeldahl method) and available phosphate (Bray & Kurtz, 1945). Cation exchange capacity (CEC) was determined using 1 M Ammonia acetate (NH_4OAc) buffered at pH 7. The basic cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) in the NH_4OAc extract were determined by atomic absorption spectrophotometer (Perkin Elmer 5980 AAS Instrument).

Isolation of Rhizospheric Bacteria

From each sample, 10 g of soil was suspended in 90 ml sterile saline water, rotary shaking at 150 rpm for one hour at room temperature (28 ± 2 °C) and serially diluted. The dilutions were plated on nutrient agar for enumeration of total bacteria, Pikovskaya's (PVK) medium to isolate phosphate solubilizing bacteria (Tallapragada & Seshachala, 2012) and Burk's medium to isolate nitrogen-fixing bacteria (Stella & Suhaimi, 2010). The colonies that appeared on Burk's medium were considered as N-fixing bacteria and the colonies surrounded by with a halo zone on PVK medium were considered as P-solubilize bacteria. On the other hand, one ml of dilution was also aliquoted in 99 ml of Burk's broth medium for three days prior plated on PVK medium for 7 days at room temperature (28 ± 2 °C) for enumeration of bacteria that has both abilities to fix nitrogen and solubilize phosphate. The enumeration was done for both aerobic and anaerobic bacteria in the anaerobic jar supplied with GasPak. Each colony that appears on the plate will be considered as one Colony Forming Unit (CFU) (Sutton, 2011) and the number of living bacteria were determined using the formula:

$$\text{CFU g}^{-1} = \frac{\text{Number of colonies per g plated}}{\text{Total dilution factor}}$$

RESULTS AND DISCUSSION

The BRIS soil at Tembila, Besut Terengganu are from the Rudua series. Based on the results, all the three locations do not show much difference in physicochemical properties (Table 1) and considered as low (weak) fertility soil. The average temperature of BRIS soil at Tembila on a daylight is around 31.2 – 33 °C and a medium acidic pH around 5.2 – 5.47. The low values of total organic carbon are correlated to the low values of soil's CEC at that three different places in Tembila. This means that the source of many plant nutrients is also low that contributed to the poor plant diversity at this type of soil. However, the value of total nitrogen is very high (5.34 - 5.39 %). It was observed that the Acacia and Gelam trees are growing well on this soil. It is possible that there are the valuable N-fixing and P-solubilize bacteria at this plant's rhizosphere. It is because, the physicochemical properties of soil could give an impact on micro-organism's growth (Jasuja, Saxena, Chandra, & Joshi, 2013).

The enumeration results (Table 2 and Table 3) showed that there are a large number of bacteria at the Acacia, Gelam and Goose grass that has the N-fixing, P-solubilize and both able to fix nitrogen and solubilize phosphate. From the results, it was found that the value of bacterial CFU g^{-1} for both aerobic and anaerobic were higher in November compared to May. In East Coast of Peninsular Malaysia, November is the raining season that caused the soil temperature to decrease and soil moisture and humidity to increase compared to the hot season in May. Since this situation is favorable to microbial growth, the CFU g^{-1} value for total bacteria, N-fixing and P-solubilize are higher (20-44 %) compared to the month of May.

Table 1. Physicochemical properties of The BRIS soil in Tembila, Terengganu

Location	Series	pH	Temp (°C)	Corg (%)	N (%)	C/N ratio	Avail P (ppm)	CEC (meq.100 g^{-1})			
								Ca^{2+}	Mg^{2+}	K^+	Na^+
UniSZA Apiary	Rudua	5.2	31.2	1.2	5.39	0.22	2.214	1.07	0.89	0.07	0.14
AGROPOLIS Research Farm	Rudua	5.6	33	1.47	5.34	0.28	2.17	1.05	1.04	0.07	0.15
FBIM Teaching Farm	Rudua	5.4	32.4	1.32	5.35	0.25	2.19	1.06	0.92	0.06	0.15

Table 2. Average bacterial counts (CFU g^{-1}) of BRIS soil at different location, condition and type of plant on May 2015

Location	Condition	Plant	Colony count (CFU g^{-1})			
			NA medium	BM medium	PVK medium	BM and PVK medium
Apiary	Aerobic	Acacia	3.12 x 106	1.36 x 106	1.21 x 106	8.44 x 105
		Gelam	2.7 x 106	1.02 x 106	8.33 x 105	6.11 x 105
		Grass	5.44 x 105	1.56 x 105	1.22 x 105	1.11 x 105
	Anaerobic	Acacia	2.37 x 106	1.17 x 106	1.08 x 106	7.88 x 105
		Gelam	1.56 x 106	5.11 x 105	4.78 x 105	4.44 x 105
		Grass	3.11 x 105	1.22 x 105	1.11 x 105	1.11 x 105
AGROPOLIS Research Farm	Aerobic	Acacia	4.14 x 106	1.21 x 106	1.11 x 106	8.44 x 105
		Gelam	2.96 x 106	9.11 x 105	8.78 x 105	6.00 x 105
		Grass	4.44 x 105	1.44 x 105	1.11 x 105	1.00 x 105
	Anaerobic	Acacia	2.86 x 106	1.12 x 106	1.03 x 106	6.44 x 105
		Gelam	1.58 x 106	4.67 x 105	4.56 x 105	4.33 x 105
		Grass	3.00 x 105	1.33 x 105	1.11 x 105	1.11 x 105
FBIM Teaching Farm	Aerobic	Acacia	2.94 x 106	1.18 x 106	9.56 x 105	6.33 x 105
		Gelam	2.54 x 106	8.56 x 105	7.67 x 105	5.44 x 105
		Grass	3.56 x 105	1.22 x 105	1.22 x 105	1.11 x 105
	Anaerobic	Acacia	2.28 x 106	1.09 x 106	1.00 x 106	6.22 x 105
		Gelam	1.26 x 106	3.67 x 105	3.22 x 105	3.44 x 105
		Grass	2.78 x 105	1.22 x 105	1.11 x 105	8.88 x 104

Table 3. Average bacterial counts (CFUg^{-1}) of BRIS soil at different location, condition and type of plant on November 2015

Location	Condition	Plant	Colony count (CFU g^{-1})			
			NA medium	BM medium	PVK medium	BM and PVK medium
Apiary	Aerobic	Acacia	4.02 x 106	1.87 x 106	1.40 x 106	1.06 x 106
		Gelam	3.37 x 106	1.16 x 106	9.89 x 105	9.00 x 105
		Grass	8.22 x 105	2.44 x 105	2.56 x 105	2.11 x 105
	Anaerobic	Acacia	3.17 x 106	1.50 x 106	1.41 x 106	1.16 x 106
		Gelam	1.98 x 106	7.89 x 105	7.00 x 105	6.00 x 105
		Grass	6.78 x 105	1.89 x 105	1.78 x 105	1.56 x 105
AGROPOLIS Researh Farm	Aerobic	Acacia	6.14 x 106	1.53 x 106	1.23 x 106	9.67 x 105
		Gelam	3.47 x 106	1.18 x 106	9.78 x 105	9.00 x 105
		Grass	7.11 x 105	2.89 x 105	2.22 x 105	1.89 x 105
	Anaerobic	Acacia	3.97 x 106	1.46 x 106	1.34 x 106	1.01 x 106
		Gelam	1.92 x 106	7.44 x 105	6.78 x 105	5.22 x 105
		Grass	4.89 x 105	1.56 x 105	1.44 x 105	1.22 x 105
FBIM Teaching Farm	Aerobic	Acacia	3.95 x 106	1.27 x 106	1.07 x 106	8.00 x 105
		Gelam	3.27 x 106	1.01 x 106	1.01 x 106	6.67 x 105
		Grass	5.89 x 105	2.22 x 105	2.11 x 105	1.78 x 105
	Anaerobic	Acacia	3.06 x 106	1.31 x 106	1.21 x 106	7.00 x 105
		Gelam	1.86 x 106	6.44 x 105	6.00 x 105	5.33 x 105
		Grass	5.00 x 105	1.56 x 105	1.22 x 105	1.22 x 105

Soil properties variations are also among the important factors that affect the composition of soil bacterial populations. BRIS soil has shown low physicochemical properties and low CFU g^{-1} value of bacteria compared to other places in the world. The CFU g^{-1} at BRIS soil only reach a maximum value of 10^6 compare to other places that can reach 10^9. The low value of bacteria at BRIS soil might be one of the reasons of low plant diversity in this area because bacteria is one of the micro-organism that contributes to the process of plant growth and development.

The abiotic factors such as temperature, pH, humidity, and moisture can influence the bacterial population density. Disturbance of forestry can alter the soil properties and characteristics. It also caused the bacterial community to shift between natural and disturbed forest soils (Lin, Jangid, Whitman, Coleman, & Chiu, 2011). The undisturbed forest of UniSZA Apiary showed the highest bacterial CFU g^{-1} followed by AGROPOLIS Research Farm and FBIM Teaching Farm. Previously, AGROPOLIS Research Farm is a place that is developed for agriculture purpose while FBIM Teaching Farm is the place that is developed for modernization such as for agriculture, housing and transportation. This could be the reason why the CFU g^{-1} value of bacteria at these places are lower compared to the UniSZA Apiary.

It was also found that the bacterial community at the field area (under the Goose grass) was lower compared to the rhizosphere of Acacia and Gelam. The field area at BRIS soil is too open without plant's shading that caused higher temperature and low humidity that is not favorable to bacterial growth. The Acacia tree showed the highest CFU value at all locations. It was observed that the Acacia tree grow close to each other to form cluster compared to the Gelam tree that grows single and apart from other plants. This kind of nature make the environment at the Acacia bush more humid, higher moisture, lower temperature and favorable to the bacterial growth compare to the other places.

CONCLUSION

Soil is the habitat for a various microorganism. Even though BRIS soil is categorized as the sandy and nutrient deficient soil and showed low physicochemical properties, it still has plenty of microorganisms such as bacteria that live in the plant's rhizosphere. The Acacia's rhizosphere from AGROPOLIS Research Farm in Tembila has shown highest total bacteria CFU g^{-1} value in November (6.14 x 10^6) compare to May (4.14 x 10^6) 2015. The Acacia' rhizosphere from UniSZA Apiary showed the highest CFU g^{-1} value of bacteria (1.16 x 10^6) that could both fix nitrogen and solubilize phosphate. This kind of bacteria that can live in this such area might be beneficial for plant growth and yield and also to enhance soil fertility. There are so many things regarding to BRIS soil bacteria could be exploited. The most dominant colony in the bacterial enumeration was isolated, purified and cultured on slant agar and glycerol stocks and maintained for further studies.

ACKNOWLEDGEMENT

The authors are thankful to the Institute of Agricultural Production and Food Innovation (AGROPOLIS) UniSZA, Faculty of Bioresources and Food Industry (FBIM), UniSZA and Ministry of Higher Education Malaysia for the Knowledge Transfer Program- KTP Community grant KTP/Bil 003/16 (KTP-R5).

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