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Characterization of Soil Microbial Diversity in Relation to Disease Incidences of Basal Stem Rot

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Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* remains one of the most devastating diseases of oil palm industry in South East Asia. To date, there is no remedy in controlling this disease effectively. In this study, soil microbial diversity in different oil palm estates with high and low BSR incidences was characterized and analyzed as a possible approach in managing the BSR disease. Soil samples were collected from two different estates in Sabah namely; Warisan Gagah, Tawau and Kam Cheong Plantations, Sandakan which incorporate different disease management and agronomic practices. Community Level Physiological Profiling (CLPP) using Biolog Ecoplate was carried out to analyze the soil metabolic activity and catabolic diversity. The results showed soil samples with low BSR disease showed higher metabolic activity and catabolic diversity. Carbohydrates, polymers and amines were the most utilized substrates by microbes in all of the soil samples. The utilization ratios of these carbon sources were found higher in low BSR incidences soil. There was no significant difference in soil microbial diversity, neither between the high BSR nor low BSR incidences soil in both estates. The study shows the presence of *Ganoderma* in the soil may have an impact on soil microbial community.

Keywords: basal stem rot; oil palm; soil microbial diversity; metabolic activity; catabolic diversity

I. INTRODUCTION

Oil palm is one of the most significant commodity crops in Malaysia. The increase in demand of palm oil has made an important contribution to the economics of Malaysia. Despite the rapid growth of the industry, oil palm plantation remains threatened by the Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* (Liu *et al.*, 2014). BSR disease is lethal and incurable although it has been long discovered. It is a soil-borne disease which can spread directly to the host plants; root-to-root contact, basidiospore and from free secondary inoculum in the soil (Khunaw *et al.*, 2017). This disease infection can cause numerous yield losses and ultimately result in destruction of basal tissue hence palm death which resulted losses up to RM 1.5 billion a year in Malaysia and about RM 3 billion a year in Indonesia. To date, there is no conclusive method of treatment although numerous attempts in early detection and controlling the disease have been reported (Chong *et al.*, 2016). A reliable view on the soil microbial community structure is necessary as soil contains enormous microbial diversity which forms important ecosystem for nutrient and biogeochemical cycles (Frac *et al.*, 2012). Understanding of soil physique, chemical element and microbial community changes that contribute to soil fertility could possibly improve agricultural practices in handling BSR disease for oil palm plantation. Despite the understanding of the significant of microbes in sustaining soil ecosystem, there is limited study on the possibility of soil microbial diversity in suppressing BSR disease of oil palm. Previous study of Khunaw *et al.* (2017) suggested that soil microbial community affects the development of *G. boninense* in plant roots. Hence, this study characterized soil microbial diversity in oil palm areas with high and low BSR and outlined the relation between soil microbial diversity and BSR incidences.

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II. MATERIALS AND METHOD

A. Sites Selection

Study was conducted in two plantation sites in Sabah, Malaysia. The estates are located at Sandakan and Tawau, namely Kam Cheong Plantation and Warisan Gagah respectively. Both estates incorporate different disease management and agronomic practices. Kam Cheong Plantation applied organic acids while Warisan Gagah applied microbial products to manage BSR disease. A total of 50 soil samples were randomly collected from the surrounding palms which represents 0.01% of the total palms in the study area. Two plots which are high and low BSR disease were collected from each estate. The chosen plots are within 30 to 40 hectare and the palm density is 136 trees per hectare. BSR incidences data in the study area was provided by both participating estates.

B. Soil Sampling and Preparation

Soil samples were collected at three different zones around the palm trees to represent spatial heterogeneity. The three zones are harvest path, circle and windrow. Harvest path is the zone compacted by the passage of plantation workers, circle is a circular zone with a radius of 1.8 m directly around the palm trunk and windrow is the zone where pruned palm fronds are placed on the ground around the tree during harvest. Soil in the plot of high and low BSR disease were taken using an auger (15 cm in depth), kept in zip-locked plastic bag and transported to laboratory. Soil samples from the disease free palms were also collected for comparison. For each plot, soil samples collected from harvest path, circle and windrow were thoroughly mixed to obtain a single representative. Samples were air dried for five days, grinded using mortar and pestle and sieved through 2 mm mesh before keeping in refrigerator for further analysis.

C. Inoculation of Soil Samples into Biolog Ecoplate

Soil samples (5 g) and 45 mL sterile Phosphate Buffered Saline (PBS) (NaCI, 0.08 gL^{-1}; KCI, 0.002 gL^{-1}; Na_2HPO_4, 0.015 gL^{-1}; KH_2PO_4, 0.002 gL^{-1}; pH=7.4) were added into a sterile 50 mL Falcon tube. Mixture was vortexed at 10,000 x g for 10 min and incubated at 28 ˚C for 30 min. A one-tenth serial dilution was made by transferring 5 mL of the supernatant into 45 mL PBS that created a 10^{-1} dilution. The dilution was repeated until 10^{-3} and 100 µL of the solution was inoculated into each well of Biolog Ecoplate (Hayward, CA, USA) before incubating at 28 ˚C. Biolog Microstation® was used to measure the absorbance of the incubated plate at 590 nm for 0, 24, 48 and 72 h.

D. Community Level Physiological Profile (CLPP) of the Soil Samples

Different soil microbial communities were determined using Biolog Ecoplate analysis. Each plate contains 31 carbon sources that subdivided into five groups which are amines, amino acids, carbohydrate, carboxylic/ketone acids and polymers (Table 1). Utilization rate of carbon sources was determined by the tetrazolium violet redox dye that changed from colourless to purple when added microorganism utilized the substrate. Microbial activity was expressed as Average Well Colour Development (AWCD), Shannon diversity index (H), richness (S) and evenness (E) as described by previous study (Table 2) (Garland and Mill, 1991).

E. Statistical Analysis

AWCD, H, S and E were analyzed using T-Test at $p < 0.05$. All statistical analyses were performed with the software Statistical Package for the Social Sciences (SPSS) version 22.

Table 1. Carbon sources in Biolog EcoPlate wells. Each well represent triplicate in real configuration.

Biolog EcoPlate	1	2	3	4
A	Water	ß-Methyl-D-Glucoside	D-Galactonic Acid ϒ-Lactone	L-arginine
B	Pyruvic acid methyl ester	D-xylose	D-galacturonic acid	L-asparagine
C	Tween 40	i-erythritol	2-hydroxy benzoic acid	L-phenylalanine
D	Tween 80	D-mannitol	4-hydroxy benzoic acid	L-serine
E	α-Cyclodextrin	N-acetyl-D-glucosamine	ϒ-Hydroxybutyric Acid	L-threonine
F	Glycogen	D-glucosamic acid	Itaconic acid	Glycyl-L-glutamic acid
G	D-cellobiose	Glucose-1-phosphate	α-Ketobutyric Acid	Phenylethyl amine
H	α-D-Lactose	D,L-α-Glycerol Phosphate	D-mallic acid	Putrescine

Amines
Carbohydrates
Carboxylic/ ketonic acids
Amino ac□ds
Polymers
Water (blank)

Table 2. Calculation formulae

Index	Formulae	Descriptions
Average well colour development	AWCD=$\sum OD_i$/ 31	Optical density (OD_i) value from each well was corrected by subtracting the blank well value from each plate well.
Shannon diversity index	H= -$\sum$pi(lnpi)	Pi= proportional colour development of the well over total colour development of all wells of a plate
Shannon evenness	E= H/ lnS	S= number of well with colour development

III. RESULTS AND DISCUSSION

Oil palm industry has been under threat of BSR disease for decades. With no remedy to date, the industry is still in search for the most effective way in controlling the disease. Soil is the key element of successful agriculture. Soil microorganisms play essential role in functioning the terrestrial ecosystem.

Microbial activity is in charge for biogeochemical cycle that keeps soil healthy and supports the growth of crops. *Ganoderma* is a soil-borne fungus. It may destroy some poor competitor microbes in soil which are beneficial to the growth of oil palm. Thus, monitoring soil microbial community in the soil is crucial to allow comprehensive approach in managing *Ganoderma* activity. CLPP using Biolog Ecoplate is a rapid and convenient tool to study functional diversity of microbial communities (Frac et al.,2012). Biolog analysis is based on the premise that microorganims vary in the pattern and the rate at which they utilize carbon sources (Xue et al., 2008). The carbon utlization patterns are used as a measure of microbial community structure and functional potential. The AWCD values that characterize the catabolic activity of soil microbial community increased along the incubation time (Figure 1). It was shown that low BSR incidences soil in both estates had greater AWCD values compared to high

BSR incidences soil. This implied that microbial communities in low BSR incidences soil had stronger metabolic activity to utilize the carbon substrate. Soil collected from disease free oil plam areas had the highest AWCD value compared to all BSR incidences soils, indicating that presence of *Ganoderma* insoil may have affect the catabolic activity of the microbial communities.

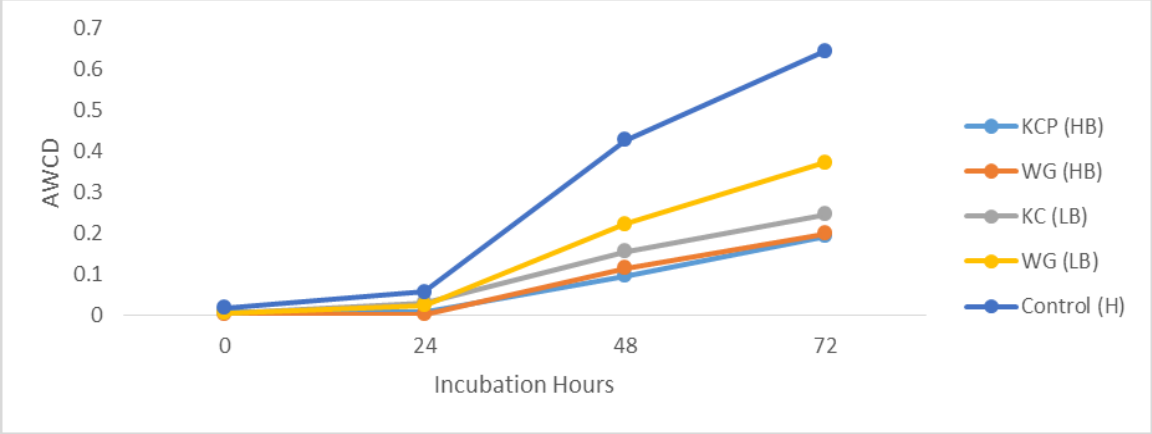

Figure 1. Average Well Colour Development (AWCD) of metabolized substrates in Biolog Ecoplate after 72h of incubation. KCP= Kam Cheong Plantation, WG=Warisan Gagah, (HB)= High basal stem rot disease , (LB)= Low basal stem rot disease, (H)= Disease free

To further compare the catabolic diversity, H, S and E were calculated (Table 3). Shanon is a diveristy index where the relative abundance of species is weighted by eveness (Tripathi et al., 2016).Increase in eveness is associated with significant increase in microbial diversity. The result revealed that richness for all of the soils were within the range of 2.184 to 2.456. The diversity and eveness in the low BSR disease site are greater than high BSR disease site. Equitability takes value E=1 being complete eveness. However, all of the E values were less than 0.4, indicating individuals in the community were not distributed equitably among the species.igh microbial counts and diversity are usually attributed to rich soils. Thus, the tight correlation between the biological activity of microorganisms and organic matter content in soil were investigated (Dobrovolskaya et al., 2007).

Table 3. Shannon diversity index (H), richness (S) and evenness (E) for respective samples after 72 h of incubation.

Soil samples	Shannon Index (H)	Richness (S)	Eveness (E)
KCP (HB)	0.385 ± 0.068	2.456 ± 0.029	0.158 ± 0.030
WG (HB)	0.392 ± 0.020	2.184 ± 0.119	0.183 ± 0.011
KCP (LB)	0.512 ± 0.023	2.395 ± 0.053	0.214 ± 0.009
WG (LB)	0.654 ± 0.018	2.395 ± 0.053	0.274 ± 0.014
Control (H)	1.158 ± 0.048	2.883 ± 0.089	0.403 ± 0.027

Note: KCP= Kam Cheong Plantation, WG=Warisan Gagah, (HB)= High basal stem rot disease, (LB)= Low basal stem rot disease, (H)= Disease free; Significant difference at $p>0.05$ within interval; Mean ± Standard error, $n=3$.

The utilization ratio of carbon sources in Biolog Ecoplate is shown in Figure 2. The carbon sources were subdivided into six main groups which are carbohydrates, polymers, carboxylic/ketone acids, amino acids and amines. The results showed carbohydrates, polymers and amines are the most utilized substrates by microbes in all of the soil.

The utilization ratios of these carbon sources were found higher in disease free and low BSR incidences soil. Lower carbon availability in high BSR incidences soil was possibility related to the presence of *Ganoderma* as previous study of Widiastuti et al. (2018) reported that *Ganoderma* is less aggressive in soil with high carbon content. It was noticeable that disease free soil exhibited highest levels consumption of carbon sources (Almendras et al., 2018), indicating higher microbial metabolism compared to the other soil samples.

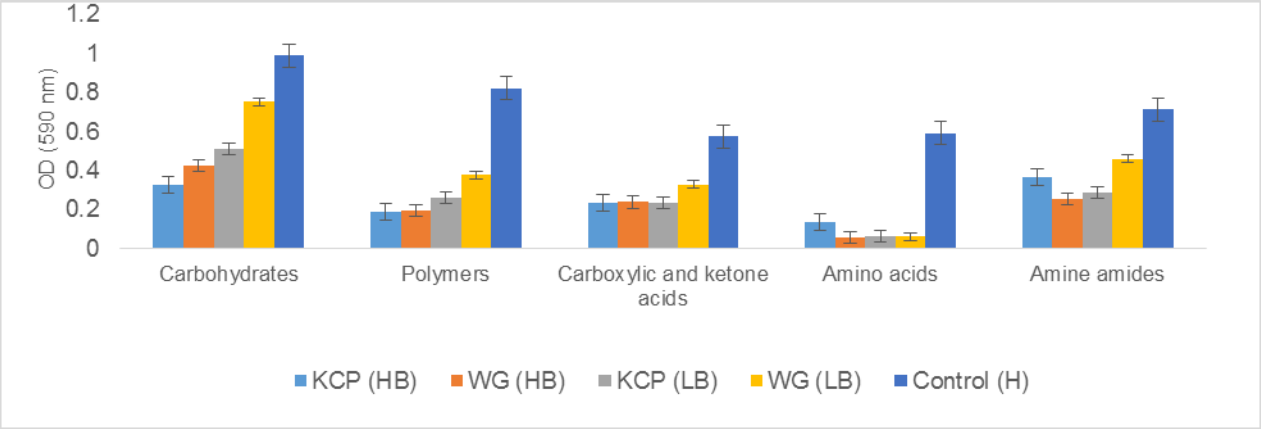

Figure 2. Relative utilization ratios (%) of five group of carbon sources in Biolog Ecoplate.
Note: KCP= Kam Cheong Plantation, WG=Warisan Gagah, (HB)= High basal stem rot disease, (LB)= Low basal stem rot disease, (H)= Disease free

IV. CONCLUSION

In conclusion, Community Level Physiological Profile (CLPP) in this study provides a reference that reflects presence of *Ganoderma* in soil may have an impact on the microbial community. The findings revealed potential metabolic diversity was significantly enhanced in the disease free and low BSR incidences soil as shown by AWCD values and S index. The utilization ratios of carbon sources were also found higher in disease free and low BSR incidences soil. Interestingly, there was no significant difference in soil microbial diversity, neither between the high BSR nor low BSR incidences soil in both estates although different disease management and agronomic practices were applied. Further experimental studies are required to better define the structural and functional information of the specific microbial communities in suppressing development of *Ganoderma*.

V. REFERENCES

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