Soil biology characteristics of oil palm land endemic to *Ganoderma* after four years conversion to sugarcane

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Abstract. Basal stem rot disease is the primary disease in oil palm production. One of the control methods to keep the disease severity low is through crop rotation by planting non-host crops for a certain period to reduce the pathogen population. This study aimed to evaluate the biological and molecular characteristics of *Ganoderma* endemic land after converted to sugarcane for four years. Results showed that the total abundance of bacteria in the land ranged from 3.5 × 102–6.1 × 108 CFU/g, whereas phosphates solubilizing bacteria, was in the range of 101–1.0 × 106 CFU/g and N fixing bacteria was 101–2.4 × 104 CFU/g. The genus dominated mycorrhizal fungi from Glomus and Acaulospora with spore population of 1 to 135 spores per 25 g of soil. Another soil biota analyzed was Trichoderma sp. that had population range between 102 to 105 CFU/g. Based on PCR analyses using specific primers Gan2 and Gan3, *Ganoderma* fungi were still detected in selected soil samples and the remaining roots in the field. Nevertheless, the productivity of sugarcane up to 4 ratoons remained high, i.e. more than 90 t/ha.

Keywords: endemic *Ganoderma* land, crop rotation, sugarcane, control technique, biological and molecular characters.

1. Introduction

Basal stem rot (BSR) caused by *Ganoderma* fungi is a significant disease in palm oil cultivation. Various techniques have been done to control the disease, including the use of chemical pesticides, digging holes around infected plants, genetic engineering and application of moderate or tolerant planting material. Nevertheless, there are no effective control methods for severely diseased plants. Alternative methods to break the *Ganoderma* life cycle is through replanting by a special technique [1] and by crop rotation with non-host plants.

Crop rotation can be interpreted as an effort to control pathogen based on the management of inoculum by breaking the life cycle of soil pathogens [2–5]. On the other hand, Partey et al. [6] suggested that crop rotation aims to eradicate inoculum sources which are infested in the land. Furthermore, Zhou et al. [7] suggested that crop rotation can increase crop productivity because of improved soil fertility through the increased level of soil biota population and diversity.

Crop rotation to control *Ganoderma* sp. has not been previously reported. This technique is expected to suppress *Ganoderma*, especially at severe disease level. This research is to study the soil biology characteristics of *Ganoderma* endemic land that have been converted from palm oil to

sugarcane plantation. The data collected can be used to determine the effect of rotation on population and diversity of soil microbes as an instrument not only to control *Ganoderma* sp. but also to restore soil fertility.

2. Materials and methods

2.1. Plant disease history of the land

The experiment was conducted at palm oil plantation at Unit Bekri Plantation, Central Lampung. The land has been planted with oil palm for four generations or approximately 100 years. In that area, the death rate of palm oil has reached more than 30% population or about 70–100 trees per hectare. Production of fresh fruit bunches in productive age crop is also reduced. In endemic block, production has been decreased to ±9,557 kg/ha. Several techniques have been applied to control the disease such as eradication of *Ganoderma* colonized tissue, raising soil bed surrounding basal stem and application of organic materials, such as ash and sludge or application of antagonistic microbes. However, these treatments were not consistently done, leading to the increased of *Ganoderma* disease severity level.

Table 1. Soil microbe population in soil samples taken from 36 blocks of sugarcane cultivation area in ex-oil palm area endemic to *Ganoderma.*

Block no.	Total bacteria (CFU/g)	*Ganoderma* sp. (propagule/g)	*Trichoderma* sp. (propagule/g)	Phosphate solubilizing bacteria (CFU/g)	Nitrogen fixing bacteria (MPN/g)	Lignolytic fungi (propagule/g)	Mycorrhiza (spore/25 g)
02	8.5×10^7	50	1.0×10^5	1.0×10^5	6.1×10^2	2.5×10^2	36
03A	4.0×10^7	10	6.0×10^4	<10	9.4×10^2	1.0×10^2	54
04	4.0×10^7	0	<102	10×10^5	<10	$<10^2$	21
05A	2.6×10^7	20	10×10^4	2×10^5	2.9×10^3	1.5×10^2	19
05C	3.1×10^5	7.3	2.0×10^3	1.8×10^4	7.5×10^3	8.0×10^2	32
05D	3.1×10^6	8.5	<102	1.0×10^4	2.4×10^4	3.0×10^2	22
05F	2.7×10^5	5.5	1.0 x 10^2	1.1×10^4	9.3×10^3	1.5×10^3	13
06A	3.1×10^7	1	1.0 x 10^3	<10	4.6×10^3	1.7×10^2	38
06A.1	3.8×10^8	0	<10	4.5×10^3	3.6×10^2	1.0×10^3	18
06B	3.2×10^6	2	1.5×10^3	<10	1.5×10^3	7.5×10^2	20
06C	3.8×10^8	3	1.5×10^3	<10	2.3×10^3	4.5×10^2	20
07A	1.4×10^7	1	3.5×10^2	7.2×10^3	3.6×10^2	3.0×10^2	4
07B	3.5×10^2	0	$<10^2$	<10	<10	$<10^2$	3
07C	7.0×10^6	6	$<10^2$	1.1×10^3	9.3×10^2	2.5×10^2	7
07D	6.5×10^6	8	$<10^2$	1.6×10^3	<10	1.0×10^2	8
09	6.5×10^7	0	$<10^2$	2.0×10^4	<10	$<10^2$	19
10A	9.2×10^6	10	$<10^2$	2.0×10^4	<10	$<10^2$	21
11A	1.0×10^7	0	0	1.0×10^5	3.6×10^2	$<10^2$	68
12A	1.0×10^7	11	$<10^2$	1.0×10^5	<10	1.5×10^2	18
15A	4.3×10^7	2.5	2.5×10^3	1.2×10^4	<10	4.0×10^2	18
16	3.1×10^7	5.5	2.0×10^3	9.5×10^3	<10	1.0×10^3	1
17	9.5×10^7	1	3.0×10^2	3.0×10^3	<10	1.0×10^2	3
18	5.1×10^8	0	1.5×10^3	6.0×10^3	4.6×10^3	$<10^2$	2
19	6.5×10^6	8	1×10^4	3×10^3	<10	3.0×10^2	1
20	8.0×10^6	10.5	2.5×10^3	1.0×10^2	<10	7.5×10^2	23
36	4.0×10^6	4.5	1.5×10^3	5×10^3	<10	1.5×10^3	23
47A	1.6×10^5	2	$<10^2$	1.8×10^3	<10	1.0×10^2	135
504A	1.1×10^7	2.5	1.5×10^3	1×10^3	3.6×10^2	1.5×10^2	1
545	4.0×10^6	4	1.5×10^3	$<10^2$	4.6×10^3	6.0×10^2	3
585	1.5×10^6	3	1.5×10^3	1.5×10^3	2.9×10^2	3.5×10^2	1
586	1.5×10^7	5.5	1.5×10^3	1.0×10^2	$<10^2$	$<10^2$	31
663	5.5×10^5	0	1.5×10^3	7.0×10^3	7.5×10^2	$<10^2$	9
698	4.3×10^6	0	7.5×10^3	7.5×10^3	$1,1 \times 10^2$	$<10^2$	24
859	1.1×10^6	0	1.5×10^3	$<10^2$	2.1×10^3	$<10^2$	10
981	6.1×10^8	5	3.5×10^2	$<10^2$	3.6×10^2	$<10^2$	2

2.2. Palm oil rotation to sugarcane

Sugarcane varieties planted for crop rotation in *Ganoderma* endemic land in 2012 were PS 881 and KK/BM 9605 (Kidang Kencana). Land preparation was started with intensive soil tillage twice to a depth of 50 cm, soil ploughing twice and ground levelling once. *Ganoderma* biological controlling agent, *Trichoderma* sp. was given once at the beginning of the crop rotation. Sugarcane planting practice was done by the standard method.

After four periods of planting or about four years, composite soil samples and non-decomposed palm oil roots which were still found on land were taken to be analyzed in a laboratory. The total sampling area was 739 ha. About 500 g of soil sample each from 180 sampling points were taken from rhizosphere at a depth of 30–40 cm. These samples represented 32 blocks of sugarcane cultivation area (±699 ha) and 4 blocks of existing palm oil (±40 ha) (Table 1). The soil samples were pooled to make up 36 soil samples. These samples were analyzed for soil biological properties including the abundance of total bacteria, phosphate solubilizing bacteria (PSB), nitrogen-fixing bacteria (NFB), arbuscular mycorrhizal fungi (AMF), *Trichoderma* sp. and *Ganoderma* sp. Also the ligninolytic microbial population was also explored. Bacterial population analysis was done by the standard method using a specific medium. Molecular analysis of *Ganoderma* sp. was performed with Polymerase Chain Reaction (PCR) using Gan2 specific primers [8] and Gan3 [9].

2.3. Analysis of ligninolytic microbial population

Ligninolytic microbe population in soil samples and the remaining palm oil roots that have not decomposed on the land were analyzed using a specific medium containing guaiacol. In this selective agar medium colonies of ligninolytic fungus, including *Ganoderma*, produces brownish halo [10,11]. Soil samples were diluted up to 108, and then 1 ml of each dilution was dispersed on agar media in petri dishes. Tissue samples were directly plated on petri dishes containing the same medium.

2.4. Molecular detection of suspected Ganoderma sp. fungus

DNA isolation was performed on 20 isolates of suspected *Ganoderma* fungus using the ExgeneTM Cell SV DNA Kit, following the protocol in the GeneAll ExgeneTM Protocol Handbook. DNA was amplified using a PCR machine with *Ganoderma* specific primers Gan2 [8] and Gan3 [9]. Amplicons were electrophoresed along with a standard 1 Kb ladder [9].

3. Results and discussion

3.1. Rotation of oil palm to sugarcane

Sugarcane planted in ex-palm oil field endemic to *Ganoderma* showed average growth with high productivity during four planting periods (Table 2). Kidang Kencana variety planted in this area was capable of producing 84.32% of its yield potential, which are 112.5±32.5 t/ha [12]. Therefore, the land is highly supportive of sugarcane growth after crop rotation.

Table 2. The productivity of sugarcane after four years of conversion of oil palm area endemic to *Ganoderma*.

Rotation periods	Average yield (t/ha)
1	101.6
2	99.7
3	91.6
4	93.8
5	87.0

3.2. Analysis of soil biology

Analysis of soil samples showed that the abundance of soil microorganisms varied (Table 3, Figure 2). The data showed that total soil bacteria, PSB, NFB and ligninolytic fungi populations ranged from 102–108 CFU/g, 101–106 CFU/g, 101–104 CFU/g and 102–103 propagules/g soil, respectively. Total soil bacteria population is affected by the type and soil content. Previous research reported a higher population level of total soil bacteria, i.e. 105–106 [13] and 108–109 [14]. Accordingly, the PSB population found in the soil was generally 105–106 CFU/g soil [15]. Marista et al. [16] reported that PSB bacteria found in alluvial soil, peat and red-yellow podzolic (RYP) generally belonged to *Acetobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Azotobacter*, *Pseudomonas*, *Staphylococcus*, *Escherichia*, and *Paracoccus*.

Table 3. Soil microbe population in soil samples taken from ex-oil palm area endemic to *Ganoderma* after four years conversion to sugarcane.

Analysis	Population range
Bacteria total	3.5×10^2–6.1×10^8 CFU/g
Phosphate solubilizing bacteria	<10–10×10^5 CFU/g
N fixing bacteria	<10–2.4×10^4 CFU/g
Lignolytic fungi	<10^2 –1.5×10^3 propagules per g
Arbuscular mycorrizal fungi	1–135 spore/ 25 g soil
Trichoderma sp.	<10^2–1.0×10^5 CFU per g
Ganoderma sp.	0–50 propagules per g

Varying levels of spore population of mycorrhiza were also observed, ranging from 1 to 135 spores/25 g soil. Meanwhile, the abundance of *Trichoderma* sp. ranged from 102–105 CFU/g soil. The fungus that was taken directly from the soil and plated on *Ganoderma* selective medium (GSM) was confirmed as *Ganoderma* and had a population ranging from 0 to 50 propagules/g soil (Table 3).

Bacteria that grow on nitrogen-free agar medium formed pellicle, a characteristic of NFB. NFB population is relatively low when compared to total bacteria population. While other research reported that NFB function as endophytic microbes such as *Gluconacetobacter diazotrophicus* is found in roots, stems and leaves in a reasonably high population (about 103–107 CFU/g) in various sugarcane plantations in Brazil, Mexico, Cuba and Australia.

The population of AMF spores on soil samples varied, ranging from 1 to 135 spores/25 g soil. This value was lower than the regular rate, which is 35–124 spore/10 g soil [17]. Mycorrhizal species on red-yellow podsolic soil Bekri's crop rotation area was dominated by *Acaulospora* sp. and *Glomus* sp. (Figure 1). AMF form mutualistic symbioses with almost 90% of higher plants; they are capable of improving plant health, soil structure, nutrient uptake and aiding plants to stand dry conditions [18]. Their highest population was observed in soil was 135 spores/25 g soil in sample block 47A, while the lowest was found in block 585 (1 spore/25 g soil sample).

Trichoderma could be found in most of the soil samples, but most of them were in lower a population compared to that reported by Sariah et al. [19], which is 2.1 × 103–2.1 × 104 CFU/g soil. The highest population was obtained from block 02 with population of 105 CFU/g while in block 06 there was no *Trichoderma* found. It seemed that the organic compounds in block 02 were more abundant compared to another block, so that *Trichoderma* best survive.

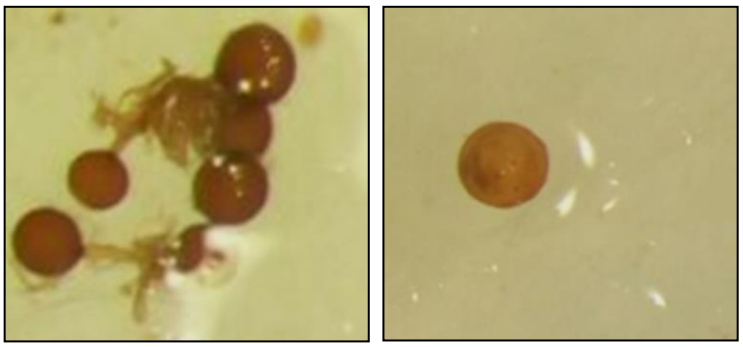

Figure 1. Spores of arbuscular mycorrhizal fungi *Glomus sp.* (left panel) and *Acaulospora sp*. (right panel).

Ligninolytic microbial analysis found ligninolytic microbes in selected soil samples and oil palm root fragments. The rest of the remaining root fragments root can become the food source of ligninolytic microbes which use carbon (C). The range of ligninolytic fungi found was between 102–103 propagules/g soils. *Ganoderma* was simply detected in selective agar medium which contains guaiacol. In this medium, brownish halo appeared surrounding its colony due to the use of guaiacol as the food sources (Figure 2D). There were only 17% of the total soil samples that contained *Ganoderma* of >10–50 propagules/g, 58% had less than ten propagules/g soil, and the remaining 25% did not contain *Ganoderma* propagules.

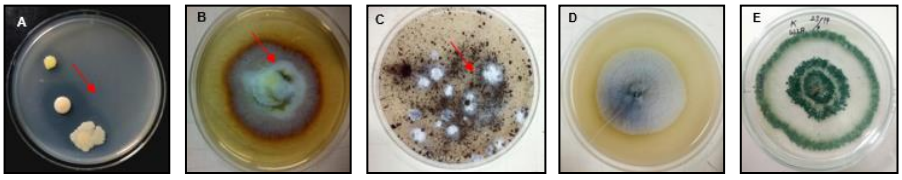

Figure 2. Colonies of fungus isolated from rhizosphere soil samples of sugarcane crops planted in ex-oil palm area endemic to *Ganoderma*. (A) Phosphate solubilizing bacteria. (B) Ligninolytic fungi. (C) Lignin-degrading fungi. (D) Suspected *Ganoderma* on selective agar medium containing guaiacol. (E) *Trichoderma* colony on PDA medium.

3.3. Molecular analysis of suspected Ganoderma fungus

PCR results of DNA isolated from suspected *Ganoderma* grew selective media is shown in Figure 3–5. Based on the molecular identification we found that PCR products of two isolates obtained from soil samples (05b and 545) and one isolate (47) found in the remaining root tissue have the same band size with positive control *Ganoderma* (260 and 454 bp). Meanwhile, isolates 02 and 859 showed negative results on all primers, which indicate that they are not *Ganoderma*. Meanwhile, isolates 05B.1, 17, 19 and 05B.2-3 obtained from non-decomposed oil palm roots tissue showed positive results as *Ganoderma* when identified molecularly. Other fungal isolates suspected to be *Ganoderma* derived from the soil that was positively identified, including isolates 663, 10A, 9, 03A and 36, whereas five other isolates (20, 504A, 12A, 11A and 10A.1) obtained from soil were not identified as *Ganoderma* (Table 4). These results showed that *Ganoderma* could persist in the soil during four years of rotation where they survived well in the remaining oil palm roots.

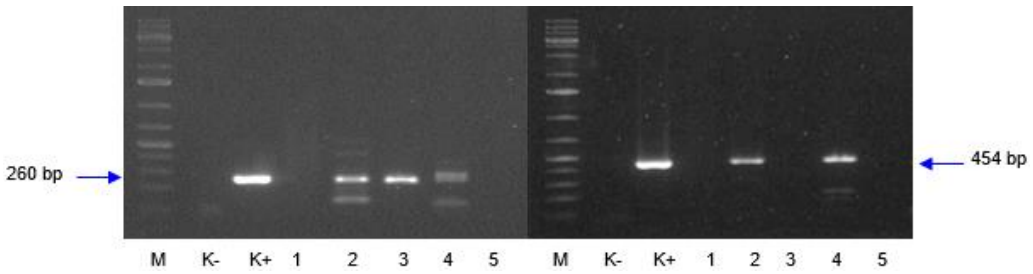

Figure 3. Electrophoregram of PCR results of suspected *Ganoderma* fungus with Gan2 primer (260 bp, left panel) and Gan3 (454 bp, right panel). Negative control (water), positive control (*G. lucidum*), PCR product of isolate 02, isolate 05B, isolate 47, isolate 545 and isolate 859 were loaded in the lanes K-, K+, 1, 2, 3, 4 and 5, respectively.

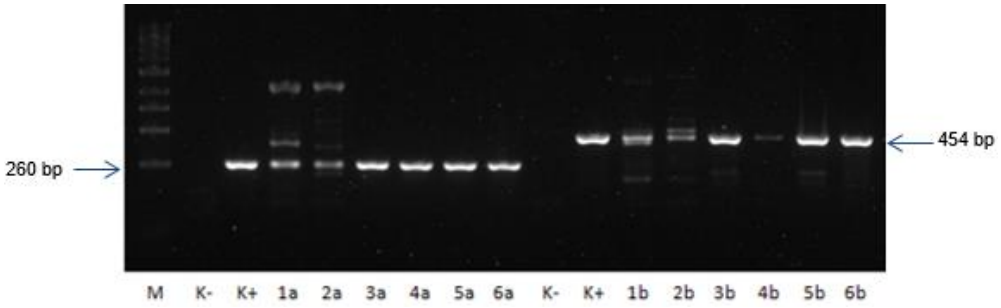

Figure 4. Electrophoregram of PCR results of suspected *Ganoderma* fungus with Gan2 primer (260 bp, left panel) and Gan3 (454 bp, right panel). PCR products of negative control (water), positive control (*G. lucidum*), isolate 663, isolate 09, isolate 019, isolate 017, isolate 05B.1 and isolate 10A were loaded in the lanes K-, K+, 1, 2, 3, 4, 5 and 6, respectively.

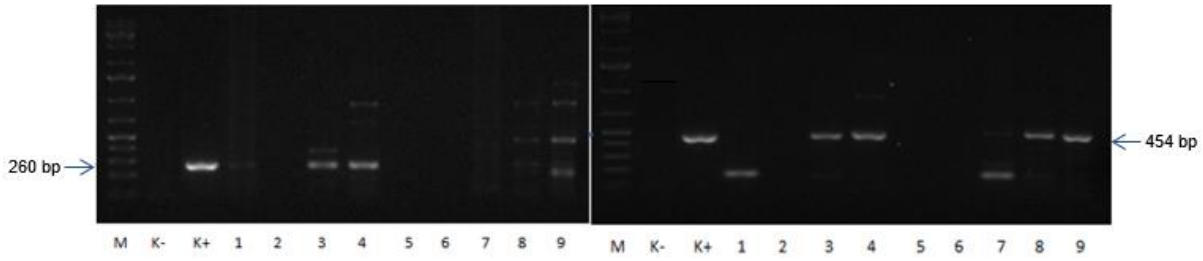

Figure 5. Electrophoregram of PCR results of suspected *Ganoderma* fungus with Gan2 primer (260 bp, left panel) and Gan3 (454 bp, right panel). PCR products of negative control (water), positive control (*G. lucidum*), isolate 12A, isolate 12A.1, isolate 05B.2, isolate 05B.3, isolate 504, isolate 11A, isolate 10A, isolate 03A and isolate 36 were loaded in the lanes K-, K+, 1, 2, 3, 4, 5, 6, 7, 8 and 9, respectively.

Table 4. PCR result of 20 isolates suspected as *Ganoderma* using Gan2 and Gan3 primers.

Isolates code	Origin of isolates	PCR result
03A	Soil	Positive
05B	Soil	Positive
9	Soil	Positive
10A	Soil	Positive
10A.1	Soil	Negative
11A	Soil	Negative
12A	Soil	Negative
20	Soil	Negative
36	Soil	Positive
545	Soil	Positive
859	Soil	Negative
663	Soil	Positive
504A	Soil	Negative
02	Root tissue remains	Negative
05B.1	Root tissue remains	Positive
05B.2	Root tissue remains	Positive
05B.3	Root tissue remains	Positive
17	Root tissue remains	Positive
19	Root tissue remains	Positive
47A	Root tissue remains	Positive

Ganoderma has a saprophytic phase in its life cycle by using C source in organic matter in soil and able to form the resting stage structure, pseudosclerotium [20]. This characteristic enables *Ganoderma* to survive for several years in the soil. Early colonization of oil palm by *Ganoderma* is through saprophytic way rather than pathogenic way [21]. It is recommended to eradicate oil palm organic matter that is contaminated by *Ganoderma* to suppress disease incidence.

4. Conclusions

Sugarcane productivity in *Ganoderma* endemic land after four years of crop rotation or up to ratoon four still high. Soil bacteria, such as phosphate solubilizing bacteria and N fixing bacteria, and soil fungi, such as ligninolytic fungi and arbuscular mycorrhizal fungi, were highly abundant in ex-oil palm land planted with sugarcane. Meanwhile, the population of other soil fungi such as *Trichoderma* sp. was rather low, ranged from 102 to 105 CFU/g soil. The results of the molecular analysis showed that after four years of crop rotation, *Ganoderma* was still found on some soil samples.

5. References

[1] Darmono T W, Widiastuti H, Siregar A, Prakoso H and Eris D 2017 *Standard Operating Procedure for Replanting Free Ganoderma* (Bogor)
[2] Abawi G S and Widmer T L 2000 Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops *Appl. Soil Ecol.* **15** 37–47
[3] Bailey K and Lazarovits G 2003 Suppressing soil-borne diseases with residue management and

organic amendments *Soil Till. Res.* **72** 169–80
[4] Larkin R 2008 Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne diseases of potato *Soil Biol. Biochem.* **40** 1341–51
[5] Larkin R P, Griffin T S, Honeycutt C W and States U 2010 Rotation and cover crop effects on soilborne potato diseases, tuber yield, and soil microbial communities *Plant Dis.* **94** 1491–502
[6] Partey S, Taylor P, Saito K, Preziosi R F and Robson G 2016 Biochar use in a legume–rice rotation system: effects on soil fertility and crop performance *Arch. Agron. Soil Sci.* **62** 199–215
[7] Zhou H, Liu B, Weeks D, Spalding M and Yang B 2014 Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice *Nucleic Acids Res.* **42** 10903–14
[8] Karthikeyan M, Bhaskaran R, Radhika K, Mathiyazhagan S, Jayakumar V, Sandosskumar R and Velazhahan R 2008 Development and comparison of ELISA and PCR methods for the early detection of *Ganoderma* disease of coconut *Arch. Phytopathol. Plant Prot.* **41** 396–406
[9] Purnamasari M, Prihatna C, Gunawan A and Suwanto A 2012 Isolasi dan identifikasi secara molekuler *Ganoderma* spp. yang berasosiasi dengan penyakit busuk pangkal batang di kelapa sawit *J. Fitopatol. Indones.* **8** 9–15
[10] Darus A and Seman A 1991 A selective medium for the isolation of *Ganoderma* from diseased tissues *PORIM International Oil Palm Conference*
[11] Alhidayatullah, Sudirmani L and Dharmaputra O 2014 Kemampuan jamur pelapuk kayu isolat JPA dan *Trichoderma* sp. S2-2 dalam mendegradasi tandan kosong kelapa sawit untuk menghasilkan selulosa *Menara Perkeb.* **82** 51–6
[12] Ministry I A 2018 Kidang Kencana sugarcane variety
[13] Pambudi A, Noriko N and Sari E 2016 Isolasi dan karakterisasi bakteri tanah sawah di kecamatan Medan Satria dan Bekasi Utara, kota Bekasi, Jawa Barat *J. Al-Azhar Indones. Seri Sains dan Teknol.* **3** 187–95
[14] Bhattarai A, Bhattarai B and Pandey S 2015 Variation of soil microbial population in different soil horizons *J. Microbiol. Exp.* **2** 75–8
[15] Hameeda B, Harini G, Rupela O, Wani S and Reddy G 2008 Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna *Microbiol. Res.* **163** 234–42
[16] Marista E, Khotimah S and Linda R 2013 Bakteri pelarut fosfat hasil isolasi dari tiga jenis tanah rizosfer tanaman pisang nipah (*Musa paradisiaca* var. Nipah) di kota Singkawang *J. Protobiont* **2** 93–101
[17] Rasyid A, Lapanjang I and Barus H 2016 Density and diversity of arbuscular mycorrhizal fungi in maize (*Zea mays* L.) plantation *J. Agrol.* **23** 141–8
[18] Aprilia D and Purwani K 2013 Pengaruh pemberian mikoriza *Glomus fasciculatum* terhadap akumulasi logam timbal (Pb) pada tanaman *Euphorbia milii J. Sains dan Seni Pomits* **2** 2337–3520
[19] Sariah M, Choo C, Zakaria H and Norihan M 2005 Quantification and characterisation of *Trichoderma* spp. from different ecosystems *Mycopathologia* **159** 113–7
[20] Adedeji G, Aiyeloja A and Omokhua G 2014 Occurrence and severity of *Ganoderma lucidum* (Fr.) P. Karst. on *Azadirachta indica* trees in University of Port Harcourt, Nigeria: implications for sustainable harvesting and replacement *Nat. Sci.* **12** 123–8
[21] Gorea E 2016 *Microscopic Analysis of Oil Palm (Elaeis guineensis) Infection by Ganoderma boninense* (The University of Queensland)