ITB Graduate School Conference (IGSC)
December 21, 2021
ISSN: 2963-718X

A Comparative Metagenomic Analysis of Rhizospheric Bacterial Communities between Suppressive and Conducive Soil towards *Ganoderma boninense* Infection in Oil Palm (*Elaeis guineensis* Jacq.)

Hana Verdiani[1], Dinda Syafira[1], Safira Alethia Loebis[1] & *Adi Pancoro[1]

[1] School of Life Sciences and Technology
Email: hana_verdiani@students.itb.ac.id, dindasyafira@students.itb.ac.id,
fialoebis@students.itb.ac.id, *adi@sith.itb.ac.id

Abstract. The oil palm (*Elaeis guineensis* Jacq.) yields high economic value. Indonesia, as one of palm oil's major producers, is inseparable from the threat of basal stem rot disease by *Ganoderma boninense*. This disease causes severe declines in the plant's productivity. *Ganoderma* suppressive soil is known to suppress the development of pathogens, as the microbes present can improve the plant's health, modulate the immune system, and compete with pathogens. This study aims to compare the community structure of suppressive and conducive soil-forming bacteria at topsoil (0-20 cm) and subsoil (20-40 cm) and their correlation with edaphic factors. Research includes performing a metagenomic analysis using 16S rRNA sequencing data. The research flow includes sequence quality control (FastQC), data analysis (QIIME2), taxonomic classification and gene function prediction (PICRISt2), and a Principal Component Analysis with edaphic factors (XLSTAT). Suppressive soils had highest alpha diversity. Potential suppressive-soil-forming agents include *Acidothermus*, *Burkholderia*, *Reyranella*, *Rokubacter*, *Streptomycetaceae*, *Bryobacter*, *Alphaproteobacteria*, *Rhizobiales*, and *Bradyrhizobium*. Predicted gene functions include the synthesis of antibiotics, antifungals, and plant defense enzymes. pH and clay percentage play a role in increasing the incidence of disease. It was concluded that suppressive-soil-forming bacterial community had higher diversity than conducive soil and was not affected by soil depth.

Keywords: *edaphic factors; conducive and suppressive soils; community structure; Ganoderma boninense*

1 Introduction

Oil palm (*Elaesis guinensis* Jacq.) is a palm oil commodity with high economic value. With such a high oil yield per land size, the palm oil industry continues to grow, especially in the Southeast Asian (SEA) region. The contribution of palm oil to the world's vegetable oil reaches a sizeable percentage of 36%, with only 6% land use [1]. Indonesia is currently one of the world's largest palm oil producers, producing up to 40.57 million tonnes of crude oil with a total plantation area of 6.78 million ha [2]. The rapid growth of the palm oil industry is a result of an ever-increasing demand for the commodity, where 68% of palm oil needs are allocated to the food sector, 27% to the industrial application sector and 5% to the bioenergy sector [2].

The main problem that threatens the productivity of Indonesia's oil palm plantations in Indonesia is basal stem rot disease (BSR) caused by infection of the fungus *Ganoderma boninense*. This disease causes decay and damage to root-stem tissue, disrupting the water and nutrient transport system. Eventually, there is a severe decline in fruit production [3], leading to a substantial oil yield decline of up to 50 - 60% [4]. BSR is commonplace in oil palm plantations in SEA, especially in mature oil palms yielding high fruit productivity [3]. Total losses are estimated at up to 500 million US dollars annually, proving the gravity of the situation [5].

Until now, there has been no sustainable solution to prevent *Ganoderma* infection. The common application of fungicides, for example, significantly reduces the incidence of BSR, but can negatively impact the environment in the long term [5]. A more novel and sustainable alternative is the formation of suppressive soil. Suppressive soil can suppress the development of pathogens. This inhibition is by the presence of bacteria that can improve plant health, modulate the immune system and compete with pathogens through various antagonistic mechanisms to *Ganoderma* [6].

Edaphic factors, microbial diversity and plant roots are the main factors responsible for determining a soil's health status and plant productivity. The interaction between plant roots and suppressive-soil-forming microbes can have a positive effect on plant growth and development by facilitating nutrient availability, inducing its tolerance to environmental stress, and increasing its disease resistance [7]. The edaphic characteristics of the soil are also determinants of the microbial and pathogenic community composition around the plant's roots. Soil edaphic parameters that correlate with the growth of fungal pathogens include texture, pH, nitrogen content, phosphorus, electrical conductivity and organic content [8]. Soil depth also affects microbial diversity. Based on Tailliez's (1971) study, the quantity of root biomass varies at varying soil depths. The highest root biomass in oil palm is at a depth of 20-60 cm or subsoil where most of the absorption of water and nutrients occurs [9]. The increase in root biomass at this depth will increase the availability of substrates beneficial to suppressive bacterial activity in the soil [10].

Although studies of suppressive soil bacteria are not new, studies concerning the suppressive soil formation in oil palm soils are still limited. This study aims to utilise metagenomic analysis at topsoil and subsoil to determine oil palm soil bacteria that can act as suppressive-soil-forming agents and contribute to the long-term control of *Ganoderma*, determine the predictive gene functions of the suppressive and conducive soil-forming bacteria found, and determine the correlation of these bacteria with edaphic factors.

2 Research Methods

2.1 Data Collection

The study was conducted in silico using secondary data consisting of 16S rRNA gene sequencing data. The study also used edaphic data extracted from suppressive soil and conducive soil samples from topsoil (0-20 cm) and subsoil (20-40 cm). Samples originate from the palm oil plantations on the islands of Sumatra, Kalimantan and Sulawesi in 2015. 24 samples are grouped based on these 3 locations and soil sample depth.

2.2 Sequence Quality Control

Sequence quality checks for each sample were carried out using the FastQC and compiled as a report using MultiQC [11]. Faulty sequences will result in faulty results and interpretation of data. Sequence Filtering and denoising were performed by QIIME2-DADA2 [12]. The output of this process is in the form of representative sequences and feature tables that can later be analysed.

2.3 Taxonomic Classification

Taxonomy classification was performed by the QIIME2 feature-classifier plugin. Representative sequences were compared with the SILVA 138 database [13] to produce a table of identified sequences, known as the taxonomy table.

2.4 Community Structure Analysis and Gene Function Prediction

The community structure was visualized using Genepiper to be analysed. Here, feature tables, taxonomy tables, and phylogenetic trees in Newick format are required to generate results. The outputs of this stage are alpha diversity, beta diversity, a Venn operational taxonomic unit (OTU) diagram, a phylogenetic tree, and a bacterial abundance heatmap [14]. Gene function prediction was carried out by QIIME2-PICRUSt2, based on two databases: the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologs (KOs) and Enzyme Commission (EC) numbers [15]. The results are visualized as a functional heatmap using Genepiper.

2.5 Edaphic Factor Analysis

Principle Component Analysis (PCA) was performed on edaphic factors (pH, percentage of sand, organic carbon content, nitrogen, phosphorus, calcium, magnesium, sodium and CEC) with XLSTAT [16].

3 Results and Discussion

3.1 Sequence Read Quality

The screening and denoising process left 55.216% or as many as 140971 sequences from the initial total of 271224 sequences. Higher percentages are preferred as it is able to provide more information downstream. Data normalization involves the process of standardizing the total number of informative sequences so that each sample has the same number of sequence libraries [17]. Based on the results of data normalization, 4225 sequences were selected from each sample for further analysis. This number is capable of accurately representing the diversity of bacterial community structures.

3.2 Community Structure Analysis

A bacterial community's diversity can be determined by analysing its alpha diversity, represented by the Shannon diversity index, which accounts for species abundance and evenness [18].

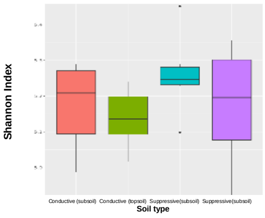

Figure 1 Alpha diversity boxplot for soil samples of Sumatra, Sulawesi and Kalimantan

The results showed that diversity (Figure 1) was highest in suppressive subsoil (p-value > 0.05). This phenomenon can occur due to high root biomass density at the subsoil depth, leading to a higher exudate and abundance of rhizosphere bacteria [10]. The high alpha diversity in suppressive soils correlated with a reduced incidence of BSR in the oil palm plants, explained by the fact that a high diversity can increase the potential for the formation of bacterial interactions that can suppress infection [19].

Differences in bacterial community structure between soil samples can be identified through beta diversity analysis, using the Bray-Curtis dissimilarity index and the Weighted Unifrac index. The Bray-Curtis dissimilarity index defines differences in bacterial community structure based on relative abundance alone, while the Weighted Unifrac index also considers the phylogenetic distance between bacteria [20], [21]. Figure 2 shows that both indices exhibit a grouping of soil samples based on the sample's location (island), indicating the similarity in community structure. Those of different islands show dissimilarity. This observation is presumed to be because the community structure is shaped by the characteristics of habitat it occupies [22].

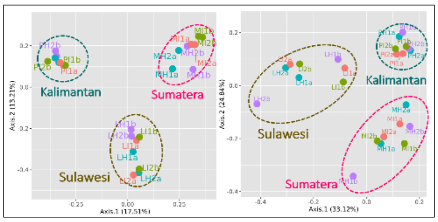

Figure 2 Beta diversity shown by the Bray-Curtis index (left) and the Weighted Unifrac index (right)

Unique bacteria can also be identified by analyzing the OTU Venn diagram, representing the number of the same (intersecting areas) and unique (non-intersecting areas) OTUs in each sample [23]. Based on Figure 3, the highest unique OTU count was found in the suppressive subsoil sample (1471 OTUs). These OTU have the potential to act as a suppressive soil-forming agent because its presence are not found in conducive soil. Subsoil samples show the potential to have a higher number of suppressive soil-forming agents than topsoil samples, though the count difference in unique OTUs is not too distinct.

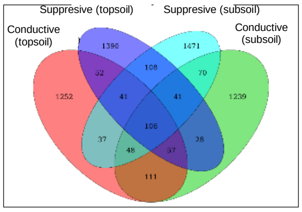

Figure 3 OTU Venn Diagram of all samples

To further determine the bacteria to potentially act as suppressive-soil-forming agents, the relative abundance of bacteria in soil samples was compared. Differentiation between topsoil and subsoil samples was not included due to the similarities of OTU count regardless of depth. The relative abundance of bacteria is visualized by a heatmap, following the colour scale.

On the island of Sumatra (Figure 4), a relatively high abundance of *Acidothermus*, *Burkholderia*, *Reyranella*, and *Streptomycetaceae* bacteria was found in the suppressive soil samples. *Acidothermus* is known to stimulate the presence of other microbes that have antagonistic activity against pathogens and produce antimicrobial compounds that can suppress pathogen development [23]. *Burkholderia* can produce antimicrobial compounds that can potentially interfere with the growth of pathogens and induce systemic resistance systems in plant [24]. *Streptomycetaceae* are known to suppress the presence of fungal pathogens that cause disease in roots by producing various secondary metabolites, including compounds that act antifungal, antibacterial and antiparasitic [25]. *Reyranella* aid in nitrogen fixation that is beneficial for growth [26].

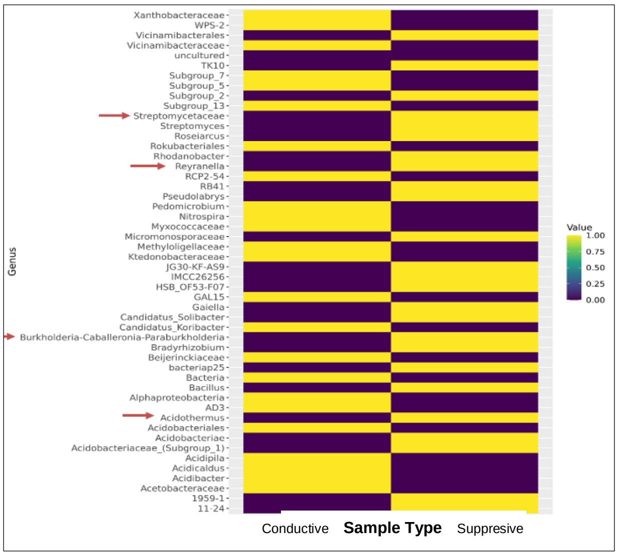

Figure 4 Relative abundance of genera heatmap of the Sumatra sample

Sulawesi (Figure 5) shows high abundances of *Alphaproteobacteria*, *Bryobacter*, and *Rokubacter* in its suppressive soil samples. *Alphaproteobacteria* and *Rokubacter* are capable of protecting plants from pathogen attacks by producing antimicrobial compounds [27], [28]. *Bryobacter* is known to play a key role in maintaining the balance of the ecosystem in areas of restoration [29].

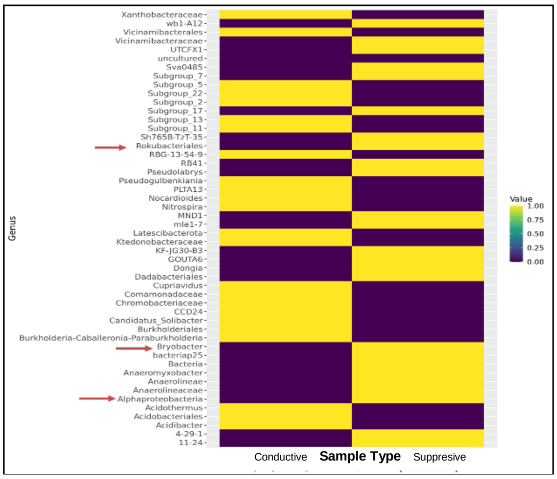

Figure 5 Relative abundance of genera heatmap of the Sulawesi sample

Borneo (Figure 6) shows a high abundance of *Rhizobiales* and *Bradyrhizobium* bacteria in its suppressive soil sample. *Rhizobiales* have a symbiotic relationship with roots, increasing plant resistance through stimulation of plant growth [29]. Bradyrhizobium can suppress pathogen infection through the mechanism of nutrient competition by producing siderophores (iron mineral chelating compounds) in soil limited iron and minerals [30]. In conducive soil, a high abundance of bacteria related to the formation of conducive soil was found, namely *Roseiaecus*. This genus is known to have a symbiotic relationship with the fungus *Russula griseocarnosa* and plays a role in increasing fungal growth [31].

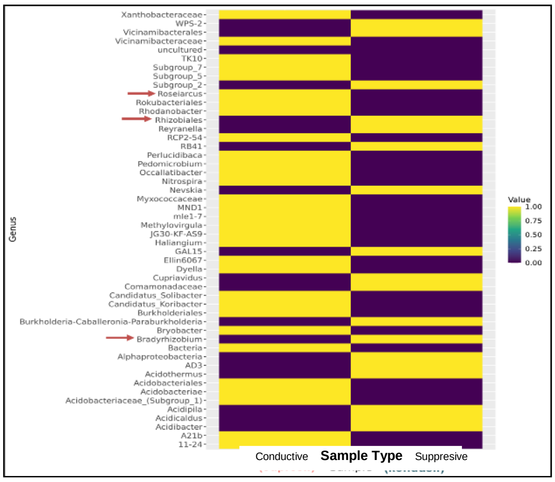

Figure 6 Relative abundance of genera heatmap of the Kalimantan sample

3.3 Gene Function Prediction Analysis

The high relative abundance of suppressive-soil-forming bacteria is supported by several potential metabolic activities obtained. The results of the KEGG Orthologs (KO) gene function prediction of Sumatra samples (Figure 7) show that there are 12 pathways related to the formation of suppressive soils and 1 pathway related to the formation of conducive soils. Sulawesi samples (Figure 8) and Kalimantan samples (Figure 9) show 10 pathways and 9 pathways found respectively related to the formation of suppressive soils. The functions found include relations to quorum sensing, secondary metabolite biosynthesis, xenobiotic metabolism, and the synthesis of ansamycin antibiotics.

Quorum sensing is a form of communication between bacteria that aims to induce a defined gene that can only be active when it reaches a defined density or quorum. The presence of quorum-sensing bacteria on the root surface can help to protect plants from soil-borne pathogens through systems that regulate the production of antibiotics or other secondary metabolites [32]. Gardener and Pal's (2006) study found a quorum-sensing pathway that regulated the induction of genes related to the synthesis of antibiotics, phenazine, rhamnolipids and carbapenems. This discovery, related to the synthesis of many antibiotics, can help inhibit the growth of various pathogenic bacteria or fungi [33]. The synthesis of beta-lactam antimicrobial compounds can suppress the growth of pathogens by disrupting cell permeability and causing lysis [34].

Xenobiotic compounds are toxic to plants and soil microorganisms but are common to soils with high agricultural activity. The persistence of xenobiotics in the soil is disruptive to agriculture due to its lengthy degradation [35]. The discovery of bacterias with the predicted gene function to metabolize xenobiotics in suppressive soil is beneficial for oil palm plantations because it can help improve soil, plant and microbial health.

The results of the predicted gene functions based on the EC Numbers database of samples in Sumatra (Figure 7), Sulawesi (Figure 8), and Kalimantan (Figure 9) showed significant pathways related to suppressive soil formation, including the biosynthesis of phenylalanine, phenazine, phenylpropanoids, O-antigens, and butanoate metabolism. Phenylalanine and phenylpropanoid are enzymes produced by plants that play a role in the defense of pathogenic attacks. Phenylalanine is also a crucial enzyme in the synthesis of phenolic compounds and lignin. Both enzymes also block the initial penetration of pathogens [36]. O-antigen synthesis is also known to contribute to microbial-plant interactions and is a potential precursor to induce systemic resistance [37]. This pathway may have a role in the formation of *Ganoderma* suppressive soil. Butanoate is also an antifungal compound with the potential to suppress the presence of *Ganoderma* in oil palm plantation soils [38].

In addition to the potential for suppressive-soil-forming metabolites, Aflatoxin synthesis was another found pathway related to the formation of conducive soil. Aflatoxin is a soil contaminant that can interfere with plant growth, synthesised by several types of fungi [39]. This pathway, therefore, has the potential to induce *Ganoderma* growth.

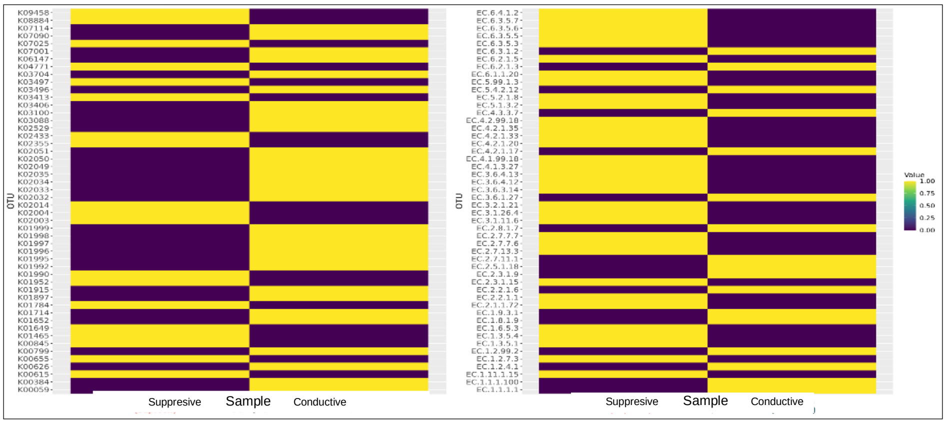

Figure 7 KEGG Orthologs (left) and EC Numbers function heatmap for Sumatera samples

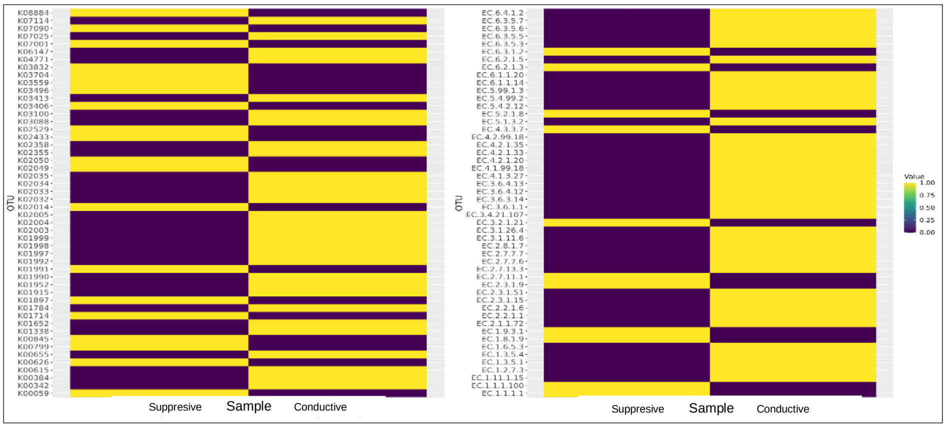

Figure 8 KEGG Orthologs (left) and EC Numbers function heatmap for Sulawesi samples

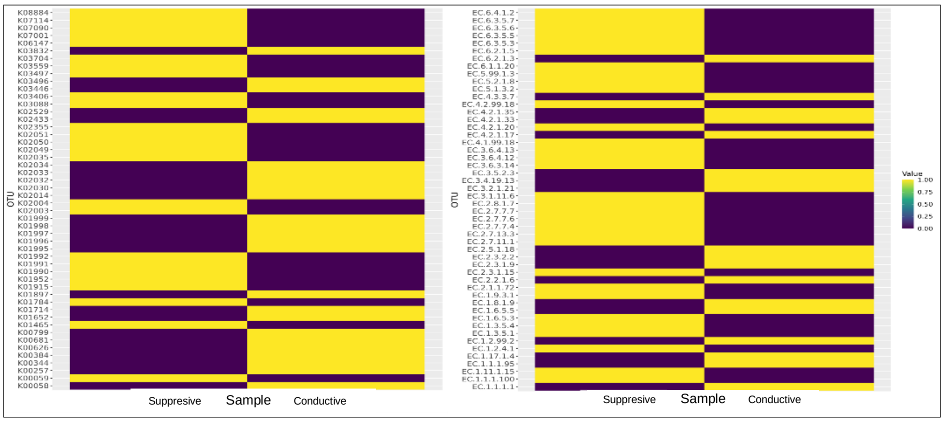

Figure 9 KEGG Orthologs (left) and EC Numbers function heatmap for Kalimantan samples

3.4 Edaphic Factor Analysis

Soil plays a prominent role in determining the bacterial community composition and relative abundance. Edaphic factors such as pH, salinity, electrical conductivity, and soil texture are associated with an increased incidence of BSR disease in oil palm [40]. To show the difference in edaphic factors between soil samples (suppressive/conducive and topsoil/subsoil), a barplot which is not included here, was constructed for each factor. The results show a significant difference in edaphic factors based on soil conduciveness, unlike the topsoil and subsoil samples. Lower soil pH also shows to induce Ganoderma conductivity in soil. Corley and Tinker (2016) also found the increased incidence of BSR at lower pH conditions [41]. This occurrence happens because the activity of xylase and cellulase is optimal at an acidic pH [41, 42].

Soil texture indicates that a higher sand percentage can suppress Ganoderma pathogens due to its low water retention capacity. Because of this, it is observed that soil of high sand percentage is suppressive. Likewise, soils with poor drainage, seen in soils of high clay percentage, are often associated with the increased incidence of BSR disease [41].

Other edaphic factors such as the content of carbon, nitrogen, phosphorus, calcium, magnesium and cation exchange capacity (CEC) were generally found to be high in suppressive soils. The physicochemical characteristics of nitrogen, calcium, magnesium, zinc and sodium have a positive correlation with the plant defense enzymes chitinase, glucanase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD). Research has observed the increased activity of these enzymes in soils with high nutrient content [41, 42].

The study observed higher nutrient content in suppressive soil samples compared to the conducive soil samples.

The relationship between edaphic factors and bacterial relative abundance was analyzed by Principal Component Analysis (PCA). PCA visualisation (Figure 10) showed that bacteria will be found in high abundance in edaphic factors most favourable. In Kalimantan, bacteria of the highest relative abundance is highly correlated to a high sand percentage. A soil with a sand-like texture retains water and provides space between particles, producing a hydrated microhabitat for bacteria. This condition encourages the coexistence of bacteria in close proximity without competing directly for nutrient sources [44].

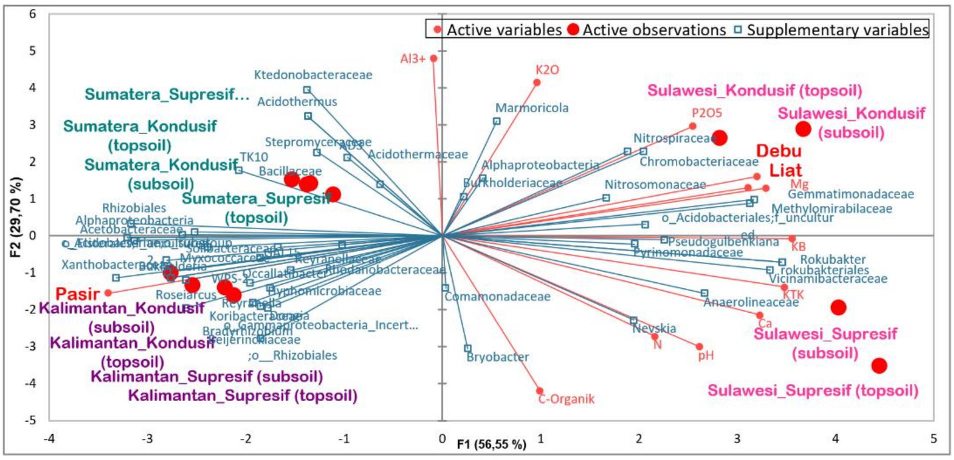

Figure 10 PCA plot of edaphic factor and bacteria in oil palm soil

4 Conclusion

Based on the results obtained from this study, it can be concluded that in suppressive soils, there is a high relative abundance and diversity of rhizosphere bacteria that have the potential to suppress Ganoderma infection in oil palms such as *Acidothermus*, *Burkholderia*, *Reyranella*, *Streptomycetaceae*, *Rokubacter*, *Bryobacter*, *Rhizobiales*, *Alphaproteobacteria* and *Bradyrhizobium*. The presence of these bacteria is supported by the predicted gene functions that show metabolic activity in the synthesis of antibiotics, antimicrobials, antifungals, enhancement of plant systemic defenses, and specific immune responses. In this study, a comparison of bacterial community structure based on soil depth was also carried out, namely topsoil (0-20 cm) and subsoil (20-40 cm), but the differences in the results were minute. Bacterial communities of oil palm soil were dependent on edaphic factors. High clay percentage and a low pH are associated with an increased incidence of BSR. Meanwhile, increasing soil nutrient content such as organic carbon, nitrogen, phosphorus, calcium, and magnesium can reduce the incidence of BSR.

References

[1] FAO, *Oil yields are measured as the amount of vegetable oil produced per hectare of land.*, 2017. http://www.fao.org/faostat/en/#data/QC (accessed Jun. 20, 2020).

[2] FAO, *Land use for palm oil production*, 2020. http://www.fao.org/faostat/en/?#data%5C (accessed Apr. 15, 2020).

[3] P. Turner, *Infection of oil palms by Ganoderma in Malaya*, Oléagineux, vol. 21, p. 73-76.

[4] V. Rao, C. Lim, C. Chia, and K. Theo, *Studies on Ganoderma spread and control*, Planter, vol. 79, pp. 367–368, 2003.

[5] R. Hustarian, N. Yusof, and S. Dutse, *Detection and control of Ganoderma boninense: strategies and perspectives*, Springerplus, vol. 2, p. 555, 2013, doi: 10.1186/2193-1801-2-555.

[6] R. Cook, *Plant Health Management: Patogen Suppressive Soils*, Encycl. Agric. Food Syst., pp. 441–455, 2014, doi: 10.1016/b978-0-444-52512-3.00182-0.

[7] M. K. Meghvansi and A. Varma, *Organic Amendments and Soil Suppressiveness in Plant Disease Management*. Cham: Springer International Publishing, 2015. doi: 10.1007/978-3-319-23075-7.

[8] S. Veresoglou, E. Barto, G. Menexes, and M. Rillig, *Fertilization affects the severity of disease caused by fungal plant patogen*, Plant Pathol, vol. 62, pp. 961–969, 2013, doi: 10.1111/ppa.12014.

[9] B. Tailliez, *The root system of the oil palm on the San Alberto plantation in Colombia*, Oléagineux, vol. 26, p. 435-448, 2017.

[10] N. Eisenhauer *et al.*, *Root biomass and exudates link plant diversity with soil bacterial and fungal biomass*, Sci. Rep., vol. 7, pp. 1–8, 2017, doi: 10.1038/srep44641.

[11] S. Andrew, *FastQC: a quality control tool for high throughput sequence data*, 2010.

[12] B. J. Callahan, P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes, *DADA2: High-resolution sample inference from Illumina amplicon data*, Nat. Methods, vol. 13, no. 7, pp. 581–583, 2016, doi: 10.1038/nmeth.3869.

[13] C. Quast *et al.*, *The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools*, Nucleic Acids Res., vol. 41, no. D1, pp. 590–596, 2013, doi: 10.1093/nar/gks1219.

[14] W. M. Tong and Y. Chan, *GenePiper, a Graphical User Interface Tool for Microbiome Sequence Data Mining*, Microbiol. Resour. Announc., vol. 9, no. 1, pp. 13–15, 2020, doi: 10.1128/mra.01195-19.

[15] G. M. Douglas *et al.*, *PICRUSt2 for prediction of metagenome functions*, Nat. Biotechnol., vol. 38, no. 6, pp. 685–688, 2020, doi: 10.1038/s41587-020-0548-6.

[16] N. P. Vidal, C. F. Manful, T. H. Pham, P. Stewart, D. Keough, and R. H. Thomas, *The use of XLSTAT in conducting principal component analysis (PCA) when evaluating the relationships between sensory and quality attributes in grilled foods*, MethodsX, vol. 7, p. 100835, 2020, doi: 10.1016/j.mex.2020.100835.

[17] N. Gotelli and R. Colwell, *Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness*, Ecol Lett, vol. 4, no. 4, pp. 379–391, 2001, doi: doi: 10.1046/j.1461-0248.2001.00230.x.

[18] L. Jost, *Entropy and diversity*, Oikos, vol. 113, no. 2, pp. 363–375, 2006, doi: 10.1111/j.2006.0030-1299.14714.x.

[19] R. G. Expósito, I. de Bruijn, J. Postma, and J. M. Raaijmakers, *Current insights into the role of Rhizosphere bacteria in disease suppressive soils*, Front. Microbiol., vol. 8, no. DEC, pp. 1–12, 2017, doi: 10.3389/fmicb.2017.02529.

[20] J. R. Bray, J. T. Curtis, and J. Roger, *This content downloaded from 147.8.31.43 on Mon*, Source Ecol. Monogr., vol. 27, no. 4, pp. 325–349, 1957.

[21] J. Chen *et al.*, *Associating microbiome composition with environmental covariates using generalized UniFrac distances*, Bioinformatics, vol. 28, no. 16, pp. 2106–2113, 2012, doi: 10.1093/bioinformatics/bts342.

[22] M. W. Friedrich, *Microbial Communities, Structure, and Function. In: Reitner J., Thiel V. (eds) Encyclopedia of Geobiology.*, in *Encyclopedia of Earth Sciences Series*, no. 9781402092114, Dordrecht: Springer, 2011, pp. 81–89. doi: 10.1007/978-1-4020-9212-1_16.

[23] A. Shade and J. Handelsman, *Beyond the Venn diagram: The hunt for a core microbiome*, Environ. Microbiol., vol. 14, no. 1, pp. 4–12, 2012, doi: 10.1111/j.1462-2920.2011.02585.x.

[24] T. Coenye and P. Vandamme, *Diversity and significance of Burkholderia species occupying diverse ecological niches*, Environ. Microbiol., vol. 5, no. 9, pp. 719–729, 2003, doi: 10.1046/j.1462-2920.2003.00471.x.

[25] P. Kämpfer, S. Glaeser, L. Parkes, G. Keulen, and P. Dyson, *The prokaryotes: Actinobacteria*, in *The Prokaryotes: Actinobacteria*, Berlin: Springer, 2014, pp. 889–1010. doi: 10.1007/978-3-642-30138-4.

[26] S. Gopalakrishnan, V. Srinivas, and S. L. Prasanna, *S. Gopalakrishnan, V. Srinivas, S.L. Prasanna*, Elsevier, pp. 55–71, 2020.

[27] Z. Wei *et al.*, *Initial soil microbiome composition and functioning predetermine future plant health*, Sci. Adv., vol. 5, no. 9, pp. 1–12, 2019, doi: 10.1126/sciadv.aaw0759.

[28] Y. K. Goh, M. Z. H. M. Zoqratt, Y. K. Goh, Q. Ayub, and A. S. Y. Ting,

Determining soil microbial communities and their influence on ganoderma disease incidences in oil palm (Elaeis guineensis) via high-throughput sequencing, Biology (Basel)., vol. 9, no. 12, pp. 1–21, 2020, doi: 10.3390/biology9120424.

[29] L. Xue, H. Ren, S. Li, X. Leng, and X. Yao, *Soil bacterial community structure and co-occurrence pattern during vegetation restoration in karst rocky desertification area*, Front. Microbiol., vol. 8, no. DEC, pp. 1–11, 2017, doi: 10.3389/fmicb.2017.02377.

[30] S. Omar and M. Abd-Alla, *Biokontrol of fungal root rot diseases of crop plants by the use of rhizobia and bradyrhizobia*, Folia Microbiolofica, vol. 43, no. 4, pp. 431 – 437, 1998.

[31] W. Y. Yu *et al.*, *Microbial community associated with ectomycorrhizal Russula symbiosis and dominated nature areas in southern China*, FEMS Microbiol. Lett., vol. 368, no. 6, pp. 1–11, 2021, doi: 10.1093/femsle/fnab028.

[32] J. Loh, E. Pierson, L. Pierson, G. Stacey, and A. Chatterjee, *Quorum sensing in plant-associated bacteria*, Curr Opin Plant Biol, vol. 5, no. 4, pp. 285–290, 2002, doi: 10.1016/s1369-5266(02)00274-1.

[33] K. Pal and B. Gardener, *Biological control of plant patogens*, Plant Heal. Instr., 2006, doi: 10.1094/PHI-A-2006-1117-02.

[34] P. Eckburg *et al.*, *Safety, Tolerability, Pharmacokinetics, and Drug Interaction Potential of SPR741, an Intravenous Potentiator, after Single and Multiple Ascending Doses and When Combined with β-Lactam Antibiotics in Healthy Subjects*, Antimicrob Agents Chemother, vol. 63, no. 9, 2019, doi: 10.1128/AAC.00892-19. PMID: 31262767.

[35] M. . Hashmi, V. Kumar, and A. Varma, *Xenobiotics in the Soil Environment: Monitoring: Toxicity and Management*. Springer International Publishing, 2017. doi: 10.1007/978-3-319-47744-2.

[36] N. Govender, M. Mahmood, I. Seman, and M.-Y. Wong, *The phenylpropanoid pathway and lignin in defense against Ganoderma boninense colonized root tissues in oil palm (Elaeis guineensis Jacq.)*, Front Plant Sci, vol. 8, 2017, doi: 10.3389/fpls.2017.01395.

[37] M. Reitz, P. Oger, and A. Meyer, *Importance of the O-antigen, core-region and lipid A of rhizobial lipopolysaccharides for the induction of systemic resistance in potato to Globodera pallida*, Nematology, vol. 4, pp. 73–79, 2002, doi: 10.1163/156854102760082221.

[38] S. Kordali, A. Cakir, A. Mavi, H. Kilic, and A. Yildirim, *Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish artemisia species*, J Agric Food Chem, vol. 53, no. 5, pp. 1408–16, 2005, doi: 10.1021/jf048429n. PMID: 15740015.

[39] T. Fouché, S. Claassens, and M. Maboeta, *Aflatoxins in the soil ecosystem: an overview of its occurrence, fate, effects and future perspectives*,

Mycotoxin Res, vol. 36, no. 3, pp. 303–309, 2020, doi: 10.1007/s12550-020-00393-w.

[40] N. Kondo and A. N. N. Azmi, *Temporal Changes Analysis of Soil Properties Associated with Ganoderma boninense Pat . Infection in Oil Palm Seedlings in a Controlled Environment*, 2021.

[41] R. H. V Corley and P. B. Tinker, *The Oil Palm*, 5th ed. UK: Blackwell Publishing, 2015.

[42] A. Surendran, Y. Siddiqui, H. Saud, and N. Ali, *Inhibition and kinetic studies of lignin degrading enzymes of Ganoderma boninense by naturally occurring phenolic compounds*, J Appl Microbiol, vol. 125, pp. 876–887, doi: 10.1111/jam.13922.

[43] A. Surendran, Y. Siddiqui, N. Ali, and S. Manickam, *Inhibition and kenetic studies of cellulose- and hemicellulose-degrading enzymes of Ganoderma boninense by naturally occurring phenolic compounds*, J Appl Microbiol, p. 124, doi: 10.1111/jam.13717.

[44] J. Ma, A. Ibekwe, C. Yang, and D. Crowley, *Bacterial diversity and composition in major fresh produce growing soils affected by physiochemical properties and geographic locations*, Sci Total Env., pp. 199–209, 2016, doi: 10.1016/j.scitotenv.2016.04.122.

Acknowledgement

The researchers would like to thank Dr. Adi Pancoro for his guidance and insight throughout this research. The researchers would also like to thank PT. Astra Agro Lestari, who have enrtusted us with the microbial data and edpahic data for this research.