InSME

The 1st Indonesian Symposium on Microbial Ecology 2018

YOGYAKARTA, INDONESIA

7th - 8th November 2018 University Club Hall Room, Universitas Gadjah Mada

DECLARATION OF ATTENDANCE

Yogyakarta, Indonesia, 7th - 8th November 2018

The First Indonesian Symposium on Microbial Ecology- InSME 2018 Organizing Committees hereby declare that the following delegate attended the InSME 2018 at University Club, Universitas Gadjah Mada in Yogyakarta, Indonesia from 7th - 8th November 2018:

Yunus Effendi

Universitas Al Azhar Indonesia

Sincerely yours,

Dr. Windi Muziasari
InSME 2018 - Co-Chairs Organizing Committee

Dr. M. Pramono Hadi
InSME 2018 - Co-Chairs Organizing Committee

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And contributed to a poster presentation with the title:

Metagenomic analysis of rhizosphere microbes from banana plantation Sukabumi-West Java

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CERTIFICATE
OF AWARD

This certificate is proudly presented to

Dr. Yunus Effendy

Poster title:

Metagenomic analysis of rhizosphere microbes from banana plantation Sukabumi-West Java

as The Best Poster Presentation

The First Indonesian Symposium on Microbial Ecology - InSME
7 - 8 November 2018
University Club, Universitas Gadjah Mada, Yogyakarta
INDONESIA

Dr. WINDI MUZIASARI
University of Helsinki
Co-Chair Organising Committee

Dr. M. PRAMONO HADI
Universitas Gadjah Mada
Co-Chair Organising Committee

InSME

First Indonesian Symposium on Microbial Ecology

Organized by

Center for Environmental Studies (PSLH), Universitas Gadjah Mada

Department of Microbiology, University of Helsinki

ABOUT

Indonesia, a home of 261.1 million people (2016), consists of five major islands (Sumatra, Java, Borneo, Celebes, and Papua) and other thousands of islands. Indonesia archipelago is located between two oceans, the Pacific and the Indian Ocean, and between two continents, Asia and Australia. Indonesia is also one of the world's richest countries in term of its biodiversity. All of these may influence the potential of microbial diversity in Indonesia, but the study on microbial ecology in Indonesia little is known. The presence of a society for microbial ecologists in Indonesia is important to facilitate all activities, promotions and communications related to microbial ecology.

Therefore, Pusat Studi Lingkungan Hidup, Universitas Gadjah Mada (PSLH-UGM) and Department of Microbiology, University of Helsinki are organising the First Indonesian Symposium on Microbial Ecology (InSME) which will be held on 7-8 November 2018 in Yogyakarta to fill the knowledge gaps regarding microbial ecology in Indonesia.

Specific topics that will be covered in InSME 2018:

(1) Exploring the opportunities and challenges of microbial ecology in Indonesia
(2) Founding The Society of Microbial Ecology in Indonesia
(3) The environmental dimension of antibiotic resistance in Indonesia

The symposium is targeted for researchers, policy makers and students in areas of microbiology, medicine, animal science, agriculture, environmental science, and other related areas.

The participants are welcome to submit research results on microbial ecology in Indonesia as consideration for Poster presentation.

The symposium is sponsored by PSLH-UGM, University of Helsinki, Academy of Finland and the International Society of Microbial Ecology (ISME) and supported by PT AmonRa - Science Communication, the Indonesian Society for Microbiology (PERMI) and the Indonesian Young Academy and Science (ALMI).

DAY 1

Day 1	Wednesday, 7th November 2018
08:30-09:30	**Registration and Morning Coffee**
09:30-10:15	**Opening Ceremony** Introduction to the building safety "Indonesia Raya" **Opening speeches:** Co-Chairs, *Dr. Pramono Hadi,* Center for Environmental Studies (PSLH) and *Dr. Windi Muziasari*, University of Helsinki Head of Center for Environmental Studies (PSLH), *Dr. Subaryono* Rector of Universitas Gadjah Mada, *Prof. Panut Mulyono*
10:15-11:00	**Session I: What is Microbial Ecology and Why is It Important** **Keynote Talk** What is Microbial Ecology and Why is It Important, *Prof. James M. Tiedje*, Michigan State University (USA)
	Invited Speakers: Microbial Ecology in Indonesia
11:00-11:30	Microbial diversity: The importance and the use of soil bacteria for cleaning up Polluted Environments, *Prof. Agnes Endang Sutariningsih Soetarto*, Universitas Gadjah Mada
11:30-12:00	Soil Microbial Ecology Plays Important Role in the Success of Bio(organic) Fertilizers, *Prof. Iswandi Anas*, Bogor Agriculture University
12:00-13:00	**Lunch break**
13:00-13:30	**Session 2: Founding the Indonesian Society for Microbial Ecology** **Opening Talk: Bacterial Community in Merapi Volcano** The role of microbes in soil formation: thinking from the early microbial ecosystems of Mt. Merapi volcanic deposits, *Prof. Hiroyuki Ohta*, Ibaraki University (Japan)
13:30-15:00	Panel discussions with the international and local experts on: Mapping the strength and challenges of microbial ecology What crucial issues that can be solved by microbial ecology Panelists: *Prof. James M. Tiedje*, Michigan State University (USA) *Prof. Agnes Endang Sutariningsih Soetarto*, Universitas Gadjah Mada *Prof. Iswandi Anas*, Bogor Agriculture University *Prof. Marko Virta,* University of Helsinki (Finland) *Prof. Satoru Suzuki,* Ehime University (Japan) *Prof. Hiroyuki Ohta,* Ibaraki University (Japan) Conclusion remarks by the International Society for Microbial Ecology (ISME) Ambassador for Indonesia, *Prof. Iswandi Anas* **"Founding of the Society of Microbial Ecology in Indonesia"**
15:00-17:00	**Poster Sessions and Coffee**
18:00-20:00	**Dinner and cultural nights at Jejamuran Restaurant**

DAY 2

Day 2	Thursday, 8th November 2018
08:30-09:00	**Morning Coffee + Snack**
09:00-09:30	**Session 3: Environmental Dimension of Antibiotic Resistance in Indonesia** **Scientific Talks: Antibiotic Resistance** Possible mechanisms of dissemination of antibiotic resistance genes in aquatic environment, *Prof. Satoru Suzuki,* Ehime University (Japan)
09:30-10:00	Risk assessment of antibiotic resistance and related genes in human impacted environments, *Prof. Marko Virta,* University of Helsinki (Finland)
10:00-10:30	Antibiotics and antibiotic resistance in Indonesia, *Prof. Iwan Dwiprahasto,* Universitas Gadjah Mada
10:30-10:45	**Coffee**
10:45-12:00	**Antibiotic Resistance in Indonesia Project Report:** "Study on antibiotic resistance genes and bacterial community in Indonesian River" *Dr. Pramono Hadi,* Center for Environmental Studies (PSLH) *Dr. Windi Muziasari,* University of Helsinki *Dr. Vanny Narita,* PT AmonRa - Science Communication *dr. Rd. Ludhang Pradipta Rizki, M.Biot, Sp.MK,* Universitas Gadjah Mada
12:00-12:30	**Closing remarks by *Prof. James M. Tiedje***

Moderators:

Dr. Vanny Narita
(PT AmonRa – Science Communication)

dr. Rd. Ludhang Pradipta Rizki M.Biot, Sp.MK
(Universitas Gadjah Mada)

Co-Chairs, Organizing Committees:

Dr. M. Pramono Hadi,
Center for Environmental Studies, (PSLH) Universitas Gadjah Mada,

Dr. Windi Muziasari,
Department of Microbiology, University of Helsinki, Finland.

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Poster Presentation

Poster No	Poster Presenter	Institution	Title
A-1	Aji Praba Baskara	Universitas Gadjah Mada	Antibacterial activity of cinnamon bark essential oil nanoemulsion as poultry feed additive candidate
A-2	Siti Rahmania Sari	Universitas Padjajaran	Antibiotic resistance activity of Pseudomonas stutzeri isolated from fire coral Millepora intricata in Morella Waters, Maluku, Indonesia
A-3	Akhirta Atikana	Indonesian Institute of Sciences (LIPI)	Antimicrobial screening and microbial diversity of marine sponges collected from Lembeh Strait, Indonesiat
A-4	Yuliana Retnowati	Universitas Gadjah Mada	Antibiotic-producing Actinomycetes isolated from mangrove rhizosphere on mangrove forest of Torosiaje, Gorontalo, Indonesia
A-5	Mohammad Ryan Alhakim	Universitas Gadjah Mada	Streptomyces and Bacillus volatile organic compounds adversely affect the ultra-structure and inhibit Ganoderma mycelial growth
A-6	Annisa Nur Lathifah	Tokyo University of Agriculture and Technologyt	Bacterial community composition of 2010 Mt. Merapi volcanic deposits assessed by pyrosequencing of 16S rRNA gene amplicons

Poster No	Poster Presenter	Institution	Title
A-7	Fitria Ningsih	Universitas Indonesia	16S rRNA gene sequences analysis of rare thermophilic Actinobacteria isolated from soil in Cisolok, West Java
A-8	Winona Wijaya	Nanyang Technological University	The monsoon season affects the microbial community structure of Singapore Coastal Waters
A-9	Anisa Lutfia	Universitas Sumatera Utara	Antagonistic fungal endophytes isolated from rhizomes of Elettaria sp.
A-10	Dewi Setyowati	Universitas Airlangga	Genotypes of Hepatitis A virus in outbreaks region, Lamongan and Bangkalan Districs, East Java, Indonesia in 2018
B-I-1 & 2	Yosmina Tapilatu	Indonesian Institute of Sciences (LIPI)	Exploration of sediment microbial community from Arafura Sea, Indonesia
B-I-3	Rully Adi Nugroho	Satya Wacana Christian University	Bacterial community of the Pine (Pinus merkusii) forest soils showed greater similarity across geographic distance than between soil layers
B-I-4	Yunus Effendi	Universitas Al Azhar Indonesia	Metagenomic analysis of rhizosphere microbes from banana plantation Sukabumi-West Java

Poster No	Poster Presenter	Institution	Title
B-I-5	Yoga Dwi Jatmiko	University of Brawijaya	Microbial composition of fermented Sumbawa Mere's milk using next generation sequencing
B-I-6	Muhd Danish Daniel Abdullah	Universiti Malaysia Terengganu	Metagenetic analysis of microbial communities associated with toxic and non-toxic Dinoflagellates
B-I-7	Isa Nuryana	Indonesian Institute of Sciences (LIPI)	The diversity of Arbuscular Mycorrhizal Fungi (AMF) associated with Lesser Yam (Dioscorea esculenta) plant's root
B-I-8	Keukeu Kaniawati Rosada	Padjadjaran University	Dynamics of bacterial communities of biofilm formation on metal surfaces in Saguling using DGGE techniques
B-I-9	Yurnaliza	Universitas Sumatera Utara	Presence and molecular identification of anti-ganoderma of Burkholderia from oil palm plantation in Medan, Indonesia
B-I-10	Diah Kusumawaty	Universitas Pendidikan Indonesia	Evaluation of identification techniques for fish pathogen, Aeromonas hydrophila, from Indonesia

Poster No	Poster Presenter	Institution	Title
B-I-11	Bodhi Dharma	Mulawarman University	Isolation and screening polysaccharide deacetylases (PDA) producer bacteria from shrimp ponds in East Kalimantan
B-II-1	Michael	Biogenesis Laboratory	Comparison of anti-biofilm activity from various microorganism in liquid and solid character
B-II-2	Evi Kurniati	University of Brawijaya	University of Brawijaya The role of Aspergillus flavus strain KRP1 in bioremediation of mercury contaminated soil
B-II-3	Fida Rachmadiarti	Universitas Negeri Surabaya	Ipomoea aquatic forsk, as phytoremediator of lead (Pb)
B-II-4	Ardhiani Kurnia Hidayanti	Bandung Institute of Technology	Characterization of silver and copper resistant bacteria from silver industrial wastewater Kotagede-Yogyakarta, Indonesia
B-II-5	Suharti Sastroredjoe	Universitas Pendidikan Indonesia	Exploring the genes encoded extremozymes from Domas Host Spring

Poster No	Poster Presenter	Institution	Title
B-II-6	Megga Ratnasari Pikoli	Universitas Islam Negeri (UIN) Syarif Hidayatullah	The interaction of bacteriain a consortium during their use of sulfur in coal
B-II-7	Mahanani Tri Asri	Universitas Negeri Surabaya	Histopatology of natural enemy tomcat (Paederus sp.) in soybean field aplied by biochemical and microbial biopesticides
B-II-8	Heli Siti Halimatul M	Universitas Pendidikan Indonesia	Characterization and photostability properties of chlorophyll and phycocyanin from Spirulina sp.
B-II-9	Achmad Gazali	University of Alghifari	Activities of noni leaf ethanol extracts (Morinda Citrifolia L.) against Staphylococcus aureus
B-II-10	Tien Turmuktini	Universitas Winayamukti	Application of straw compost and biochar as a soil, microbial carrier to increase pokcoy plants yield (Brassica lapa L.)
B-II-11	Shumpei Lehata	Universiti Malaysia Terengganu	Assessment of polyethylene degrading activity by bacteria from the guts of Namalicastic polychaete

Poster No	Poster Presenter	Institution	Title
B-II-12	Romaidi	Universitas Islam Negeri (UIN) Maulana Malik Ibrahim	Selenium recovery by selenite
B-II-13	Vincentia Irene Meitiniarti	Satya Wacana Christian University	What causes ethanol production is not optimal?
B-II-14	Evie Ratnasari	Universitas Negeri Surabaya	Effectiveness of fermented feed "Fermege Formula 3" made of water hyacinth (Eichornia crassipes), kale and tofu dreg in triggering of the goat growth
B-II-15	Afrizka Permana Sari	Universitas Islam Negeri (UIN) Walisongo	Antifungal activity of silver nanoparticles biosynthesized by Bacillus sp . (strain BAgBK-3)
B-II-16	Andi Kurniawan	University of Brawijaya	Analysisof the Cr(VI) biosorption characteristics of microbial biofilms
ISME17	Yuniar Devi Utami	Tokyo Instutite of Technology	Genome analysis of uncultured ZB3/TG2 bacteria ("Margulisbacteria ") attached to ectosymbiotic spirochetes of protist cells in the termite gut

METAGENOMIC ANALYSIS OF RHIZOSPHERE MICROBES FROM BANANA PLANTATION SUKABUMI-WEST JAVA

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[2]School of Life Science and Technology, Bandung Institute of Technology - Indonesia
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Abstract

Fusarium oxysporum is a soil borne pathogen fungus that has been known as caused Panama Disease in many horticulture plants, especially banana for Fusarium oxysporum cv Cubense TR4 (Foc). Currently, there is no effective method for controlling Panama Disease in banana has been applied. Utilization of novel Rhizosphere microbes as bio-agent for preventing the dispersal of microbial pathogens is a method that recently has been developing in agriculture system of many countries. In this study, metagenomic analysis of 16srRNA gene was utilized for analysing composition, richness, and abundance of soil microbes which is living in the rhizospheral area of banana plants. Comparison soil bacterial structure of healthy soils and Foc-infected soils was also performed for identifying key bacteria OTU which different in both soil conditions. Data showed more than 9000 OTU of bacteria was identified in both soil conditions. Of it, the Foc-infected soils showed a higher species abundance than healthy soil (ACE index 73,6 and 68,8 respectively). However, healthy soil has more taxa richness than infected-soil (Fisher index 447,7 and 343,4 respectively). Beta diversity analysis indicated infected-soils have lower bacterial diversity in comparison with health soils. No statistically difference between both soil conditions in phylum taxa. About 43 phyla have been identified and no significantly difference between both soil conditions. However, Acidobacteria (22%) and Verrucomicrobia (13%) were more abundance in the health soils in comparison in the infected soils 19% and 10% respectively, whereas Proteobacteria was found more abundance in the infected soil (11%) in comparison with the health soil (7%). High abundance of Xantomonadaceae, member of Proteobacteria in the infected soils might contribute on Fusarium development in soils

Keyword: Fusarium oxysporum, Banana, metagenomic analysis

Introduction

Banana is one of the most widely grown fruit along tropic and sub-tropics. Due to its high health benefit and nutrient contains, Banana is the most popular fruit which consumes many people and widely grown in the world (FAO 2018). Banana is also popular fruit and widely consumed in Indonesia. Mostly banana production is done by smallholders in almost area in Indonesia, although there are also present some Banana estates Sumatra and Java islands. Banana production in Indonesia are mostly found in Java (54%) and contributing to 68% of national banana production. However, large potential lands of banana plantation are available in Sumatera (over 1 million ha), Kalimantan, Sulawesi and Papua (over 3 million ha) (Dj har et al. 1999). Unfortunately national production of Banana showed decreasing since 1990s (Nurhadi et al,1994). Pests and diseases contributed mainly in production decreasing and a limiting factor of banana production worldwide

including in Indonesia (Getha et al. 2002; FAO,2015). From many banana diseases have been identified worldwide, Fusarium wilt of banana which is known as Panama disease, is the most devastating banana disease in the world. This disease is caused by pathogenic soil-borne fungi, Fusarium oxysporum, which is commonly colonizing in vascular tissues of banana and prevent transportation of water and nutrient in the pseudostem of infected banana. Thus, the plants are getting wilt which observed in yellowish leaves and later wilt totally on all leaves (Dita et al. 2010). Fusarium oxysporum f.sp cubense subgroup 'Tropical Race 4' (Foc TR4) is the most devastating race of Foc was recognized 1990s and had been identified as cause of serious losses of Cavendish banana in some areas of Southeast Asia (Ploetz and Churchill 2011). Currently, no effective methods have been applied for avoiding Foc spreading. Few methods have been reported applied in some areas, but mostly has less effective impact not only economically but also less environmentally safe (Lin et al. 2016).

During its life, plant develops important processes that necessary for its life. One of important processes is interaction with rhizosphere microbiome nearby plant root areas include bacteria, fungi, nematodes, protozoa, algae, and microarthropods (Raaijmakers et al. 2001). These microbiomes play important roles in ecological fitness of plants which interact with the microbiomes (Kent and Triplett 2002). Plant-microbe interactions may be considered beneficial, neutral, or harmful to the plant, depending on the specific microorganisms and plants involved and on the prevailing environmental conditions (Bais et al. 2006). In general, diversity of microbiomes in the soil is an important factor that has significant impacts on plant growth and development. However, to what level specificity of microbiomes especially bacteria, will contribute to plant-microbe interactions is remain unclear.

Most of bacteria in soils are unculturable (Nihorimbere et al. 2011) and using standard culturing techniques, less than 1% of bacterial diversity in most environmental samples was accounted (Amann et al. 1995). In other side, it well understands that knowing microbial diversity and their functional role in rhizosphere areas of plants is important information for understanding the role of bacteria and other microbes for plant growth.

Metagenomic is a molecular-ecology based technique which provides a high throughput method for analyzing collective genome of bacterial and other microbial from environmental samples without providing standard cultivation (Ravin et al. 2015). Metagenomic analysis is initially performed by extracting of total DNA from samples (soil, water, food, etc) and followed by constructing of genomic library. Using high throughput sequencing technique (Next Generation Sequencing/NGS), specific conserved genes or genomic fragments such as 16S rRNA, 18S rRNA or Internal Transcribed Spacer (ITS) were sequenced and analyzed for diversity, abundance, phylogenetic and functional analysis of the all microbes that identified in the samples (Riesenfeld et al. 2004; Ghosh et al. 2019). Since the technique analyzes the entire presented DNA from the sample, thus all microbes (cultured and uncultured microbes) are counted.

Experimental Design

Sampling

Soil samples were collected from Banana plantation of PTPN VIII in Parakan Salak Sukabumi, West Java, Indonesia with map coordinate 6°49'42.2"S 106°44'40.3"E. Four soils samples were collected from 2 different sites. Two samples were collected from Foc-uninfected soil, whereas two other samples were collected from Foc infected soils (Figure1). Each sample consisted of 500 g soil samples which collected from 3 different sites nearby rhizosphere areas of infected or uninfected banana plants. The soil mixes were then homogenized and took 100 g. The soil samples were kept in-4oC until used

DNA preparation

DNA extraction and molecular works Whole genomic DNA was extracted using PowerSoil DNA kit (MoBio). DNA extraction was done following manual procedure of the kit. A 0.25 gr soil of each sample were used as source of the genomic DNA. The DNA was used as template for PCR. A 2µL of DNA was added to 10µL PCR mix (GoTaq ® Green Master mix-PROMEGA) and 1 nmol of each forward and reverse 16srRNA primer. Reaction was performed 35 cycles which consisted of 30 sec at 94oC and continued with 57oC of annealing for 20 sec, followed with 2 min 72oC for elongation. A region V4 of 16srRNA gene was amplified with primer F515 (5'-GTGCCAGCMGCCGCGGTAA-'3) and 907R (5'-CCGTCAATTCMTTTRAGTTT-'3) (Lane 1991). The PCR products were purified and subjected for automated Illumina Miseq platform (1st BASE-Malaysia) after the PCR products were normalized in equimolar amounts.

Bioinformatic Anlaysis

Raw sequence data generated from Illumina Miseq platform were processed in QIIME Ver 6.0 (Caporaso et al. 2010). All sequences are shorter than 150 bp or longer than 600 bp are removed from downstream processing. Read were then aligned with 16srRNA SILVA database (www.arb-silva.de) and GRD database (metasystems.riken.jp/grd/), then followed inspected for chimeric errors. "Species-level" of OUT was used in analysis, thus reads then were clustered at 97% similarity into OTUs. In this step, rare OTUs with only 1 (singleton) or 2 reads (doubleton) are deleted from downstream processing. Taxonomic assignment was carried out with the RDP Classifier (Wang et al. 2007). Data analysis Alpha and Beta diversity analysis were performed using Explicet ver 2.10.5 software (Robertson et al. 2013). Heat map which showed relative abundances between samples in certain taxa level was generated with Explicet ver 2.10.5 software. Venn diagrams were made to visualize which OTUs were shared between infected and healthy soils using Explicet ver 2.10.5 software.

Result and Discussion

Structure and diversity of soil bacteria

A total of 37,909,152 reads was obtained using Illumina Miseq sequencing of 16srRNA gene. Of it total of 35,149,668 reads (87.5%) passed filter. After primer removal and length-and quality filtering, followed with removal of singleton as well as doubletons, about 20.000 reads for each sample were obtained. These reads have passed quality filtering control (mean of read length and GC%). "Species-level" of OUT was used in analysis, thus reads were clustered at 97% similarity into OTUs. About 9.000-11.000 OTUs were identified for each sample (Table 1).

Tabel 1. Number of Sequence and filtered OTU

Group	Screen < 150 bp and > 600 bp	Chimera, singleton, and doubletons removal	Number of OTUs
Health soil1	244147	118229	11364
Health soil2	213990	105205	9630
Infected soil1	205317	91449	10575
Infected soil2	247097	109523	11880

Alpha diversity analysis summarized that species diversity (richness) of health soils was higher than infected soils, which showed on higher Simpson diversity index (7.5-7.7) and Fisher alpha indexes (379.4-516.1). The richness index of the Chao1 estimator (Chao1) (Chao 1984) and the abundance based Coverage estimator (ACE) (Eckburg et al. 2005) was calculated to estimate the number of observed OTUs that were present in the sampling assemblage. The diversity within each individual sample was estimated using the nonparametric Shannon diversity index (Washington 1984).

Table 2. Alpha diversity index

Group	Chao1	ACE	Shannon	Simpson	Fisher
Infected soils1	9956	79.33211	48.0574	7.798442	504.8816
Infected soils2	8278	67.98454	42.94576	7.239521	181.9179
Health soils1	9300	63.20111	43.50184	7.773869	516.0863
Health soils2	9469	74.49042	46.99294	7.540323	379.3965

The ACE estimator indicated that species abundance was observed relative higher in the infected soils than in the health soils, even Chao1 index indicated only a slight difference of species abundance between these soils (Table 2). Metagenomic analysis showed that bacteria dominate the diversity of microbiome in the soil samples (99%) of both soil conditions. Archaea presented 0.04% and 0.012% in health soil and infected soil respectively. A total of 39 phyla were identified in the soil samples, however, the abundance of these phyla are statistically not different (Table 3).

Analysis of taxonomic abundance of species between the health and the infected soils showed varied diversity within samples. The abundance of major bacterial phylum was observed less different (Figure 1). From 11 major bacteria phylum which was compared, no significant difference between health soils and infected soils was found. However, some phyla tend to present more abundance in one of the soil conditions.

Table 3. The abundance of major phylum

Phylum	Health soil	Infected soil	*p-value*
Archaea			
Crenarchaeota	0.00033±0.00006	0.00017±0.00004	0.27
Euryarchaeota	0.00003±0.00002	0.00026±0.00020	0.07

Parvarchaeota.	0.00005±0.00005	0.00000±0.00000	0.26
Bacteria			
AD3	0.05517±0.02247	0.08320±0.01472	0.41
Acidobacteria	0.20938±0.01099	0.17915±0.00467	0.06
Actinobacteria	0.02296±0.00359	0.02922±0.00246	0.31
Armatimonadetes	0.00410±0.00054	0.00356±0.00041	0.42
BHI80.139	0.00010±0.00003	0.00009±0.00002	0.17
BRC1	0.00025±0.00025	0.00020±0.00020	0.56
Bacteroidetes	0.00789±0.00019	0.00232±0.00192	0.07
Chlamydiae	0.00094±0.00047	0.00102±0.00053	0.33
Chlorobi	0.00012±0.00001	0.00056±0.00044	0.11
Chloroflexi	0.21542±0.02283	0.20454±0.01260	0.46
Cyanobacteria	0.00129±0.00004	0.00194±0.00054	0.11
Elusimicrobia	0.00197±0.00028	0.00144±0.00011	0.25
FBP	0.00000±0.00000	0.00001±0.00001	0.21
FCPU426	0.00282±0.00067	0.00212±0.00015	0.51
Fibrobacteres	0.00014±0.00004	0.00042±0.00031	0.23
Firmicutes	0.00054±0.00032	0.00054±0.00012	0.24
GAL15	0.00716±0.00309	0.01918±0.00502	0.09
GN02	0.00001±0.00001	0.00001±0.00001	0.22
Gemmatimonadetes	0.00942±0.00192	0.00976±0.00069	0.31
Kazan.3B.28	0.00004±0.00004	0.00000 0.00000	0.34
NC10	0.00000±0.00000	0.00003±0.00003	0.44
NKB19	0.00124 ±0.00123	0.00058 ±0.00058	0.43
Nitrospirae	0.01218±0.00175	0.01494±0.00217	0.12
OD1	0.00004±0.00004	0.00000±0.00000	0.24
OP11	0.00000±0.00000	0.00003±0.00003	0.32
OP3	0.00124±0.00123	0.00058±0.00058	0.09
Planctomycetes	0.01218±0.00175	0.01494±0.00217	0.33
Proteobacteria	0.02411±0.00601	0.02245±0.00378	0.15
Synergistetes	0.00000±0.00000	0.00002±0.00002	0.23
TM6	0.00118±0.00092	0.00044±0.00025	0.15
TM7	0.16714±0.01058	0.00820±0.00639	0.37
Verrucomicrobia	0.06428±0.00510	0.10006±0.02639	0.31
WPS.2	0.00004±0.00004	0.00012±0.00009	0.25
WS3	0.00292±0.00269	0.00007±0.00002	0.24
Thermi	0.00294±0.00049	0.00820±0.00639	0.16

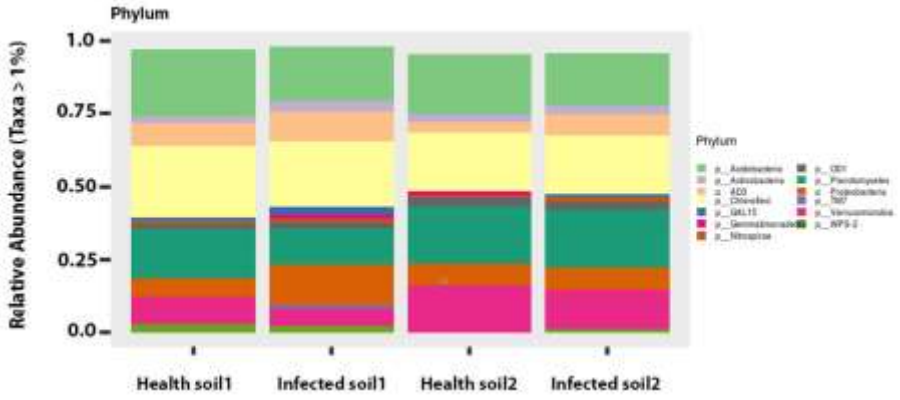

Figure 2. Relative abundance of highest phylum in healthy and infected soils

Relationship between soil conditions and microbial abundances

Acidobacteria (22%) (P<0.06) and Verrucomicrobia (13%) (P<0.31) were more abundant in the health soils in comparison in the infected soils 19% and 10% respectively, whereas Proteobacteria was found more in the infected soil (11%) (P<15) in comparison with the health soil (7%) (Figure 2). Nevertheless, there were no significant differences in the relative abundances of these dominant species in both soil conditions. Rarefaction curve confirmed the data that number of OTUs is varied abundance in range from 9360-11880 OTUs in the soil samples (Figure 2). Proteobacteria was found higher in infected soil than in health soils. The more abundance of Proteobacteria in infected soils in this study was suggested as one of important factors which composed a suitable environment for pathogenic microbes growing. Sanguin et al. (2009) reported Proteobacteria abundance was negatively correlated with disease suppression.

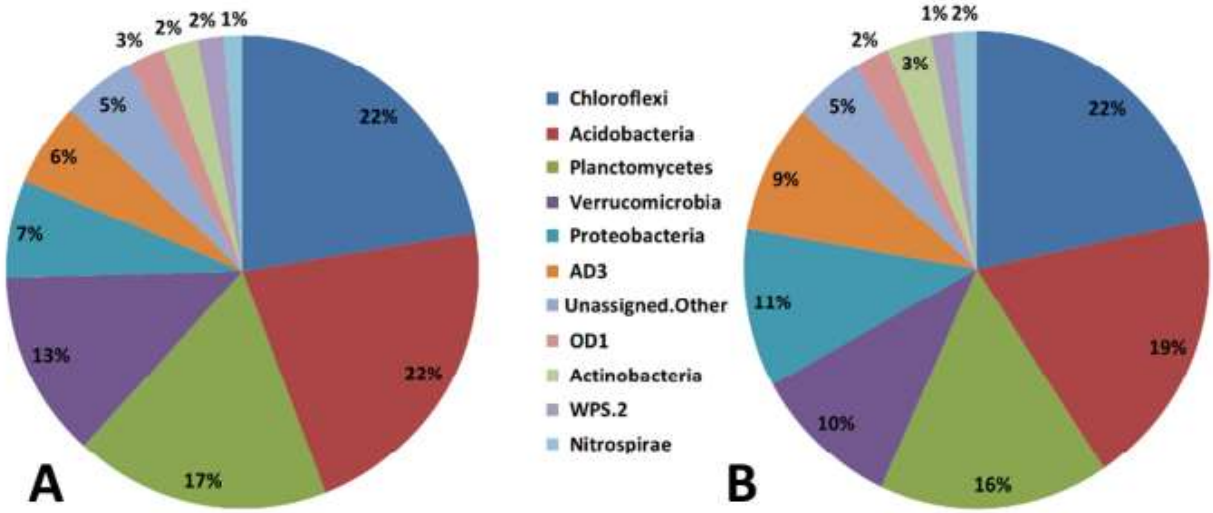

Figure 2. Comparison of phylum abundance of health and infected-soils. The most common bacterial phyla are Chloroflexi, Acidobacteria, Planctomycetes, and Verrucomicrobia (accounting ca. 67-74% of the total mappeed reads). From 11 tops phyla, Verrucomicrobia and Acidobacteria were found higher in health soils (A) than in infected soils (B). Moreover, Proteobacteria was found higher than in health soils (11% and 7% respectively). Abundance analysis of family taxa member of Protobacteria showed Xanthomonadaceae was found higher significantly in infected

soils than in health soils. Family of Xanthomonadaceae has been reported consisting of some pathogenic bacteria caused plant diseases.

The relatively high abundance of Acidobacteria in both soils in our study might correspond with acid soil condition (pH 4.9-5.1). In addition, the data showed that the Acidobacteria was found relative higher (22%) in the health soils than in the infected soil (19%). Whether it contributed to incidence of higher number of Foc in the soils or not, it remained a hypothesis. Soil pH strongly influences the composition of soil microbial community.

Soil acidity is linked to the decrease of available carbon for soil microbes (Wang et al. 2007), thus acidity of soil will contribute significantly diversity of soil microbes. Indeed, several study of a bio-organic fertilizer (BIO) application to various orchard with serious Fusarium wilt disease showed effectively enhancing suppression of Fusarium wilt disease by ameliorating structure of the microbial community (Cotxarrera et al. 2002; Kavino et al. 2010; Zhao et al.2011; Shen et al. 2013). Shen et al. (2014) showed that BIO-treated soil effectively decreased the number of soil Fusarium sp. and controlled the soil-borne disease. Correlation analysis indicated that there was a significant correlation between the abundance of Gemmatimonadetes ($r=-0.579$, $p=0.024$) and Bacteroidetes ($r=0.600$, $p=0.018$) phyla and Fusarium wilt disease incidence (Shen et al. 2014). Shen et al. result are more or less similar to our data. Our data showed infected soils have slightly lower abundance of Gemmatimodetes (0.947%) and higher abundance of Bacteroidetes (0.732%), whereas health soils have 0.972% and 0.289% respectively. Statistical analysis indicated Bacteroidetes phylum showed a slightly different in abundance (P-value > 0.07) (Tabel 2). In a high abundance, the Bacteroidetes has positive corresponding to initial and disease stage of Fusarium wilt disease incidence whereas decreasing of the Fusarium disease incidence was significantly shown when suppressiveness of this phyla was reached (Kyselková et al. 2009; Shen et al. 2014).

Moreover, significantly different in abundance of Sphingomonas genus which 3.4 time more frequently found in infected soils than in health soils is also consistent with Shen et al. (2014). On their report, Shen et al. (2014) described a strong negative correlation between Fusarium wilt disease incidence and Gemmatimonas ($r=-0.579$, $p=0.024$) and Sphingomonas ($r=-0.689$, $p=0.005$) abundance. Indeed, Gemmatimonas and Sphingomonas genus were found frequently in the Fusarium wilt disease infected soils than in the health soils (Shen et al. 2014). The composition of the soil microbial community and induced changes caused by its amendment, provide useful information on soil health and quality (Poulsen et al. 2013).

Maintaining biodiversity of soil microbes is crucial to soil health because a decrease in soil microbial diversity is responsible for the development of soil-borne diseases (Mazzola 2004). Determining the responses of soil bacterial communities to different organic amendments is particularly important because the bacterial community is one of the main components that determine soil health and is believed to be one of the main drivers in disease suppression (Garbeva et al. 2004). Naturally, suppressive soil condition on certain pathogenic microbes relies on at least two important factors, first a general mechanism of competition for nutrients caused by the whole soil microflora and the second a specific competition between pathogenic and nonpathogenic

microbes strains. Composition and diversity of microflora at the end will determine whether certain pathogenic microbes dominate the soil or not. The abundance of Proteobacteria members on healthiness banana plants has been reported in several studies (Shen 2014; Köberl et al. 2017). Comparative microbiome analyses between healthy and diseased Gros Michel plants on Fusarium Wilt-infested farms in Nicaragua and Costa Rica revealed significant shifts in the gammaproteobacterial microbiome (Köberl et al. 2017). The Author found diversity and community members of Gammaproteobacterial were identified as potential health indicators. Indeed, increasing of plant-beneficial Pseudomonas and Stenotrophomonas correlated positively with healthy plants (Köberl et al. 2017).

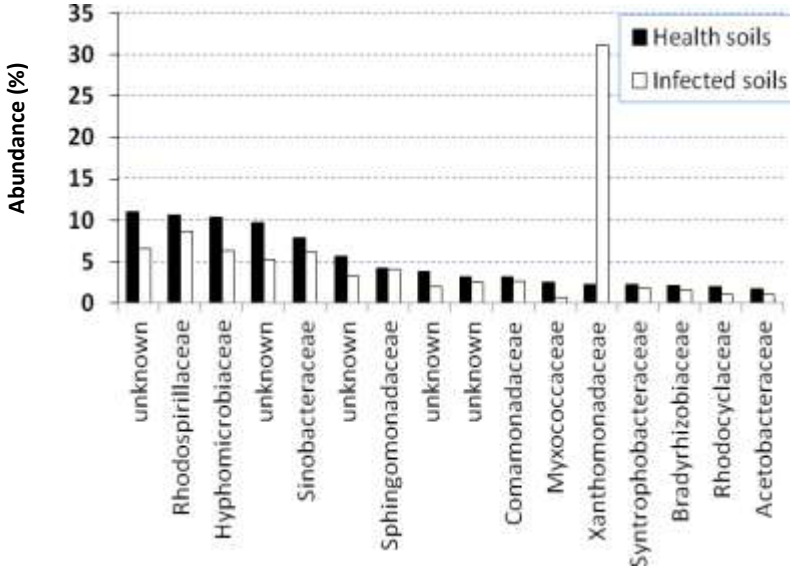

Figure 4. Top 16 families belong Proteobacteria which identified in healths and infected soils. Xanthomonadaceae showed more abundance in infected soils than in health soils (P-value > 0.07)

In contrast with our study, the abundance Xantomonadaceae family, one of Proteobacteria phyla member was found relatively higher in infected soils than in health soils (Figure 4). The family of Xantomonadaceae has been known well as one of the pathogenic family which caused Banana Xanthomonas Wilt (BXW) (Biruma et al. 2007). However, Köberl et al. (2017) reported that Xantomonadaceae presented in higher number in healthy plants. In other studies, Proteobacteria abundance was identified negatively correlated with Fusarium disease suppression thus confirming that the outbreak stage of wheat take-all disease is mainly attributed to the prevalence of Proteobacteria (Sanguin et al. 2009). It may be concluded Proteobacteria might present as positive or negative factors on the development of pathogenic bacteria in the soil is dependent on which specific bacterial taxa dominate in the soil (Biruma et al. 2007; Shen et al. 2015; Köberl et al. 2017).

In our data, the abundance of various genus members of Proteobacteria phyla which commonly are known as plant disease-caused bacteria (Peeters et al. 2013; Safni et al. 2018) was also found more in the infected soils than in healthy soils. *Ralstonia* genus present 5 times more frequently in infected-soils than in healthy soils. But we found some beneficial genus members of

Proteobacteria phyla were also higher abundance in infected soils than in health soils (Azospirillum-free-living aerobic nitrogen fixer, Rhodanobacter-denitrifying bacteria). Those indicated *Proteobacteria* was not a proper phylum for indicating healthiness of soils.

In conclusion, the result from the present study demonstrated that composition, diversity, and richness of microbiome in rhizospheral areas of banana plants in banana plantation Sukabumi might correspond with the incidence of *Fusarium* development in the rhizosphere soils. The more abundances of bacteria belong to *Acidobacteria* and *Verrucomicrobia* phyla might associate with the healthiness of the soils, whereas higher abundances of *Proteobacteria*, particularly *Xanthomonadaceae* family might contribute positively to *Fusarium* development in the soils.

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