Universitas Esa Unggul

IC-BIOLIS
International Conference on Biotechnology and Life Sciences

IPSBI
IKATAN PROGRAM STUDI BIOTEKNOLOGI INDONESIA

Certificate

Presented to

Dr. rer. nat. Yunus Effendi

As Presenter of Call for Paper on

The 2019 International Conference on Biotechnology and Biodiversity

"Biotechnology in The Era of 4th Industrial Revolution"

8 - 9 October 2019, Universitas Esa Unggul - Jakarta, Indonesia

IC-BIOLIS
International Conference on Biotechnology and Life Sciences

Radisti Ayu Praptiwi, Ph.D
Chair of The 2019 International Conference on Biotechnology and Life Sciences

IPSBI
IKATAN PROGRAM STUDI BIOTEKNOLOGI INDONESIA

Dr. rer. nat. Maria Goretti M. Purwanto
Head of Indonesian Biotechnology Program Association

Potential of Soil Bacteria for Suppressive Soil Induction Against *Fusarium oxysporum* f.sp. *cubense*

Y. Effendi[1], S. A. Herdiansyah[2], Pancoro, A.[1]

[1]Biologi Dept. Universitas Al-Azhar Indonesia, Jl. Sisingamangaraja Jakarta, 12110
[2]School of Life Sciences & Technology, Institut Teknologi Bandung, Jl. Ganesa No. 10 Bandung, Indonesia 40132
Corresponding Author: pancoro@itb.ac.id

ABSTRACT

Banana (*Musa paradisiaca* L.) commodity in Indonesia has high market demands and intensively planted in large scale. A major problems in banana plantations is infection of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) which caused wilting of banana plants. *Foc* spores are able to persistent in soil for around 30 years, forcing abandonment of banana plantations. A type of soil known as natural suppressive soil can inhibit the infection of Foc spore through antagonistic interaction between microbes and *Foc*. Microbial composition in soils around healthy and *Foc* infected bananas were studied by comparative metagenomic analyses of bacterial *16S rRNA* in both soil conditions. Models of suppressive soils were generated by bioinformatics analysis for alpha diversity, beta diversity, and comparative abundances analysis with Qiime2 and R packages. Total 161.259 quality sequences from 4 samples (two infected and two healthy) acquired after sequences quality filtering. Alpha diversity analysis showed no significant differences in species diversity between soils with healthy and infected bananas. Beta diversity analysis showed a grouping between healthy soil samples but not in infected soils. Composition analysis on Phylum level revealed Actinobacteria, Proteobacteria, and Planctomyces abundances were higher on soils around healthy banana. Comparative analysis of abundance with deseq2 on family levels revealed *Gemmataceae*, *Hyphomicrobiaceae*, *Bradyrhizobiaceae, Chtoniobacteriaceae*, and *Nitrospiraceae* were more abundant in soils aroudn healthy plants. **Comparative abundance analysis with Gneiss and phyloseq shown several abundant genus in healthy soils, such as *Rhodoplanes*, *Gemmata*, *Pirellula*, *Nitrospira*, *Streptomyces*, *Reyranella*, *Kribbella*, and *Klaistobacter***. Members of family *Bradyrhizobiaceae* and *Nitrospiraceae* were dominantly functioning as a nitrogen fixating bacteria, promoting growth of plants. Some members of genus with higher abundant in healthy soils known to produces several active compounds. Based on the data, it is concluded that suppressive soils condition could be designed by increasing more abundances of bacteria with a role as a plant growth promoting bacteria (PGPR) and producing active metabolites compound such as siderophores.

Keywords : *Fusarium oxysporum*, Soil Microbiome, Metagenomic, 16S rRNA

INTRODUCTION

Banana (Musa spp.) is a food commodity that has high market needs on various countries in the world, especially in tropical country. In Indonesia, bananas are available with various varieties, which processed in many different ways according its varieties. Wide biogeographical distribution of banana in Indonesia, followed with domestication produced many varieties which place banana as one of the most important commercial crops (De Langhe, et al., 2009).Because of its importance, banana are found cultivated on small and large scale. In 2015, banana plantations production scale in Indonesia were in the range of 94 thousand hectares with a production rate of 77.64 tons/ha (Rohmah, 2016).

One of the frequent problems in banana production is the presence of Panamanian disease which causes wilting in banana plants. Banana wilt caused by infection of *Fusarium oxysporum* f. sp. *cubense* (Foc), blocking vascular systems in banana with its mycelial thus inhibits transportation of nutrition from soil (Dita, et al., 2018). Moreover, Foc spores are persistent in the plantations soils and easily spreads passively to infect another banana. *Foc* infested area found in Indonesia, with reports of findings in Sumatra, Java, Sulawesi, and Papua. Those frequent problems of banana plantation by *Foc* infection limits banana production in Indonesian (Molina & Fabregas, 2009).

Many efforts has been done to prevent Foc infection, one of the action is carried out by planting Foc resistant banana species, such as Cavendish banana. However, resistant outbreak can occur and made the solution ineffective. The emergence of *Foc* Tropical Race 4 (TR4) which infect Cavendish bananas that resistant to other type of Foc is one of the example of resistant outbreak ( ). Genetic engineering to produce more resistant banana can be done, but less favorable in society and threatened by the emergence of incoming resistant outbreak ( ).

The presence of natural soils with potential inducing of plant resistance to *Foc* can provide alternative solution to control *Foc.* Type of soils known as suppressive soils is rich of

microbe that prevents infection of *Foc* spore in soils to the plants. Beneficial interaction of microbes with associate plants is a form of coevolution to increase survivability of plants ( ). Microbes in soil can prevent infection by direct or indirect ways. Negative interaction of microbes with soil pathogen could be antagonistic, hyperparasitism, inhibition by secondary metabolites, or competition (Mehta, et al., 2014).

In order to understand the difference of microbiome composition on supressive soil compared to soil with low resistance to pathogenic infection called inducive soil, we conducted metagenomic analysis of either soil in banana plantation. This comparative study is aimed to reveals supressive soil models and finds potential biocontrol agents for *Foc* infection.

METHODS

Sampling procedure. Soils samples were taken from banana plantations on PTPN VIII Department in Parakansalak, Sukabumi, Indonesia. Soils were taken from bulk soils around rhizospere of *Musa acuminata* var. Maskirana (AAB ? genome) in July 2018. The soils sampled from farms which only consist of banana with no other co-cultivated plants. For each farms, two replicates plants rhizosphere is sampled for soils around healthy banana plants and *Foc* infected banana plants. For plants infested by *Fusarium* wilt, we choose the plants with physiological characteristics such as yellowing of old leaves and the presences of gray mucus in transverse slice of banana pseudostem (figure 1).

Total Soil DNA Isolation. DNA extraction from sampled soil were conducted with DNAeasy Powersoil Kit (Qiagene) based on protocol. Extraction process used 0,25 gram from each soil sample. Each DNA isolates were tested using 27F – 1492R for the presences of microbial DNA. DNA concentration, molarity, and library size were measured before sequencing. Each sample is encoded with abreviation indicating the condition of banana, H for healthy banana which mark the supressive soil, and I for infected banana which mark the condusive soil. Total 4 samples were used for metagenomic analysis (Two sample represents supressive soils, and the other two samples for conducive soil).

Illumina MiSeq Sequencing. Sequencing analysis targeting the hypervariable region V3 – V4 (±460 bp length) of 16s rRNA genes. Libraries were prepared and sequenced by paired-end approach using Illumina MiSeq platform (FirstBase, Singapore).

Raw Sequences Processing. Quality control for raw sequences were conducted using dada2 package in R (Callahan, et al., 2016). Considering the V3 – V4 region length, trimming is only done for small portion (280 bp onwards of forward sequences and 255 bp onwards of reverse sequences, although keep the sequences phred score higher than 15. Sequences were filtered with max error estimation (EE) value of 2,5. Samples were dereplicated and denoised to get rid of overrepresented sequences. Forward and reversed sequences then merged, and chimeric sequences is filtered by consensus methods. The processed sequences exported for analysis with Qiime2 2019.4 (Bolyen *et al.*, 2018) with package qiime2R (Bisanz, 2018). Taxonomy assignment conducted in Qiime2 with Greengenes database (DeSantis, et al., 2006).

Diversity Analysis. Microbial diversity were analyzed using Qiime2 and R package phyloseq (McMurdie & Holmes, 2013)with sampling depth value 38.500. Rarefaction curve on mentioned sampling depth were generated in qiime2. Alpha diversity analysis were conducted using qiime2 for evenness and Faith phylogenetic distance index, and phyloseq for Shannon and Chao1 diversity index. Beta diversity were analyzed with Bray-Curtis and Weighted Unifrac index in qiime2, displayed with principal coordinate analysis (PCoA).

Composition and Abundance Analysis. Abundance analysis on phylum level were done by generating taxa prevalence graph from R package Microbiome (Lahti *et al.*, 2017). Dominant phyla abundance were displayed by graph bar representing read counts per phylum for supressive and conducive samples. Taxa bar plot for order and family level were generated using Qiime2. Abundance analysis were done using Gneiss methods (Morton, et al., 2017) from qiime2 software, log scale abundance comparation methods using Deseq2 package (Love, et al., 2014), and comparation of dominan taxa based on read abundance using phyloseq and microbiome package. Correlation analysis …

RESULTS

Microbiome Diversity. Total quality sequences yield from processing of raw V3 – V4 region amplicon sequencing by Illumina Miseq were 161.259 from 4 sample,which is ranging from 38.534 – 42.977 persample. **Table S1** shown the number of sequences which reduced from each step of processing. Rarefaction analysis based on OTU observed on genetic dissimilarity level of 3% were shown in **figure S1.** Alpha diversity measured with various index were shown in **Figure 1**. Index measured shown the based on Chao1, Shannon, and Faith PD, the diversity only slightly higher on supressive soil for one of the healthy samples.

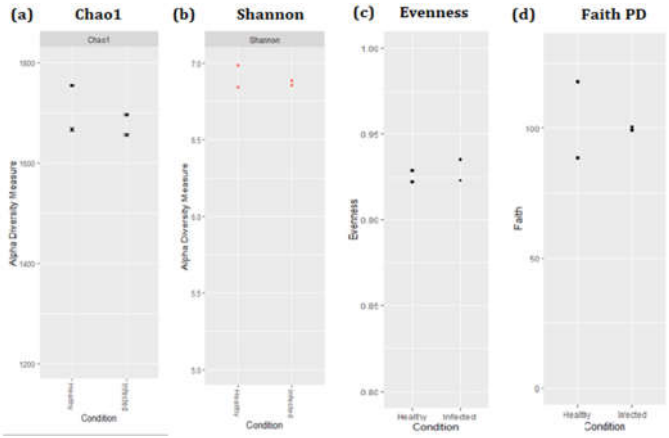


Figure 1. Alpha diversity on each sample, grouped by the soil type based on banana condition (healthy and infected bananas). Diversity calculated with (a) Chao1 index, (b) Shannon index (c) Evenness index, and (d) Faith phylogenetic distances index.

Beta diversity measured based on Bray – Curtis and Weighted Unifrac index shown in **Figure 2**. Bray Curtis index shown a grouping for healthy sample, indicating similar microbial composition based on discrete character. However, wighted unifrac index shown scattered distribution.

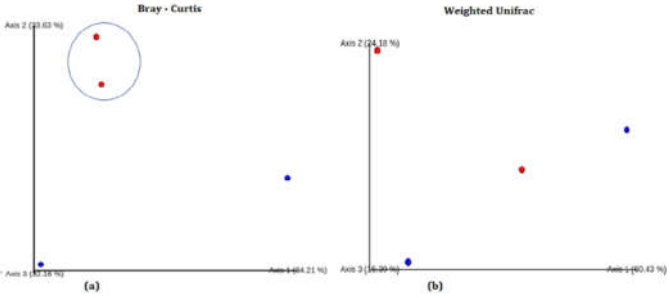


Figure 2. Principal Coordinate Analysis (PCoA) of beta diversity index : (a) Bray – Curtis index (b) Weighted Unifrac index from soil samples. Red colour indicate healthy samples, blue colour indicate infected samples.

Microbial Composition. Venn diagrams of OTUs compositions for the all samples were shown in **figure S2**. Total OTUs for all samples is 6311 OTUs. Respectively, unique OTUs found only samples H1, H2, I1, H2 is 1410, 1484, 1444, and 1699. All samples contained 1130 similar OTUs. Prevalence and Abundance analysis on Phylum levels shown in **figure S3**. Phylum with high abundance and prevalence were chosen for subsequent analysis. **Figure 3** shown the abundance comparation between healthy and infected phylum which chosen for high prevalence and abundances. Actinobacteria, Proteobacteria, and Planctomycetes were relatively more abundant on supressive soils. Acidobacteria and Verrucomicrobia abundance were higher on conducive soils. Microbial composition on Order and Family level were shown by taxa bar plot generated on **Figure s4**.

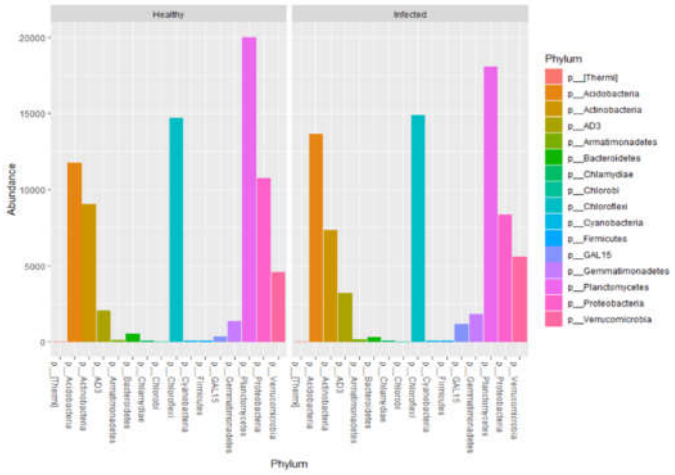


Figure 3. Phylum abundance comparation based on read abundance between *healthy* and *infected* sample.

Comparative Abundance Analysis. Gneiss methods was used to analyze microbial abundance comparation between supressive and conducive soils microhabitat. The methods used balance composition between taxa, to generate dendogram with nominator and denominator taxa. Heatmap dendogram and balance taxonomy were shown in **figure s5**. Proportion plot generated by Gneiss were shown in **figure 4**. We found that the abundances of genus Rhodoplanes, Gemmata, and unknown genus from Bradyrhizobiaceae were higher on supressive soils than infected soils.

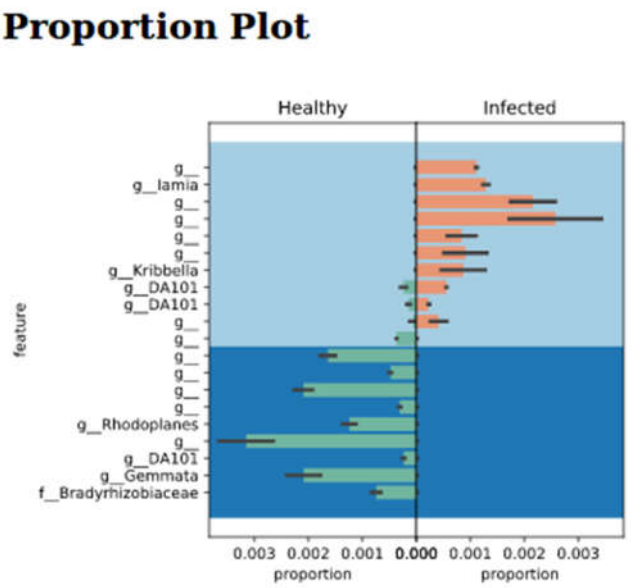


Figure 4. Proportion plot for genus with highest difference between healthy and infected samples.

Another approach we used to compare abundance were using log fold scale change on abundance with deseq2 methods. Abundance comparation by deseq2 for family level were shown in **Figure 5**. Figure shown the most differs abundance value by log scale between *infected* and *healthy* samples. We found that Lamiaceae and Koribacteraceae were higher on conducive soils. Hence, Nitrospiraceae, Chtoniobacteriaceae,

Bradyrhizobiacae, Hyphomicrobiaceae, and Gemmataceae found more abundant on supressive soils.

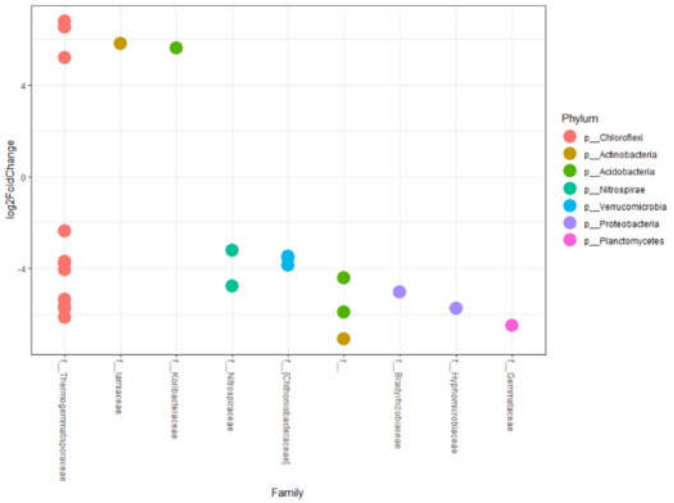


Figure 5. Comparative abundance analysis with Deseq2 package for infected vs healthy samples. Bacteria with higher abundance in infected soils has positive log2foldchange.

Comparative abundance analysis for dominant genus with highest abundance on our samples were done with package phyloseq in R. Boxplot for top Genus with highest abundance can be seen in **figure 6**. Top 5 dominant genus respectively were DA101 (Verrucomicrobia), Gemmata, Planctomyces Pirellula, and Rhodoplanes. Planctomyces and Pirellula were Genus with higher abundances on supressive soil. We also found genus Nitrospira, Streptomyces, Reyranella, Kribbella, and Klaistobacter were has higher abundance on supressive soils rather than conducive soils.

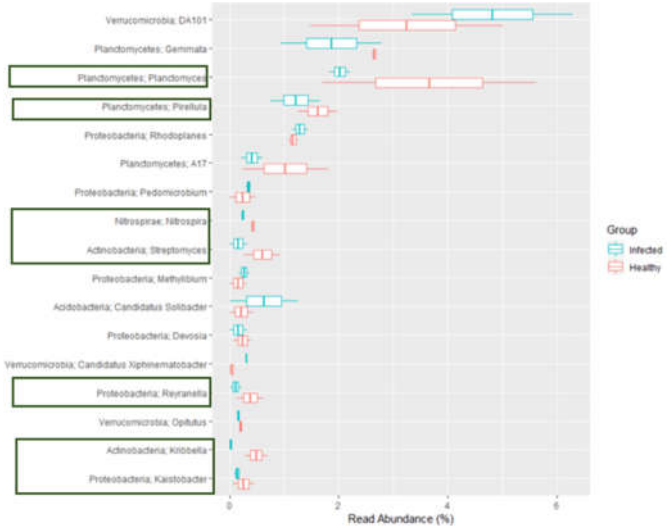


Figure 6. Abundance comparation form read abundance precentage for top 20 abundant genus.

Correlation analysis of abundance value for top dominant genus were done to study the co-occurence pattern of the members of bacteria population in studied samples. **Figure 7** shown the correlation patterns for top abundant genus in the samples. The highest positive correlation is between Nonomurea and Planctomyces (r = 0,9932), and strongest negative correlation found between Devosia and Verrucomicrobia DA.101 (r = - 0,9900).

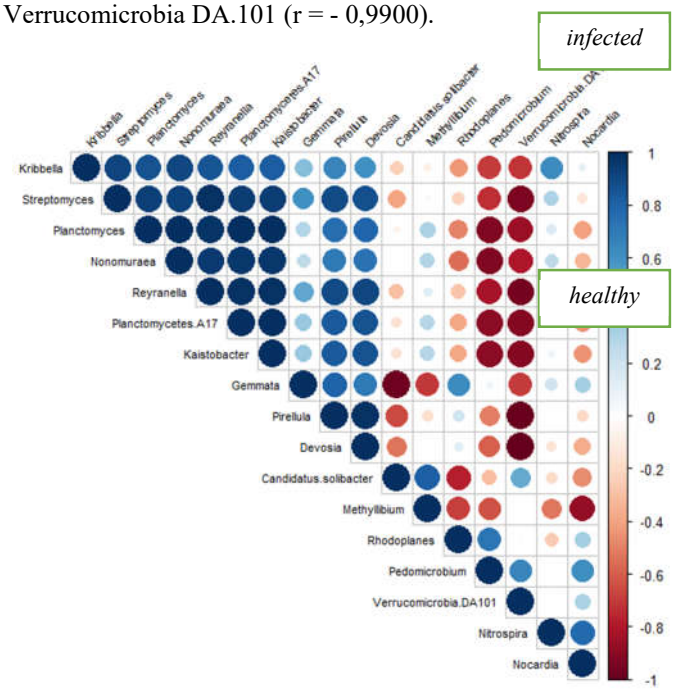


Figure 7. Correlation analysis of abundance between dominant genus on the sample. Blue colour indicates positive correlation and red colours indicates negative correlation.

DISCUSSIONS

In this study, we aim to describe the corresponding soil microbial composition with healthy and infected banana plants. Our study reveals a slight difference in microbial diversity from suppressive and conducive soils. There is a relation between soil microbial diversity and infection occurrences of bacterial pathogen to its host (van Elsas, et al., 2012). Soils with more complex microbial diversity tend to reduce invasion level of pathogen, thus its indirectly increase plant resistances to pathogen (Yang, et al., 2017). Soil bacterial communities with a clear niche overlap with pathogen and have stabilizing configurations tend to have lower diseased plants incident (Wei, et al., 2015) (Viaene, et al., 2016).

Bacteria	Role	Literature
Streptomyces	PGPR, Produce metabolites with antimicrobial activity → ex. VOCs with antifungal activity	(Viaene, et al., 2016), (Cordovez, et al., 2015)
Bradyrrhizobium	Nitrogen fixation, Siderophore production	(Omar & Abd-Alla, 1998)
Nocardia	Opportunistic pathogen, produce nargenycin, transvalesin, nocardiothicin with antimicrobial activity	(Sharma, et al., 2016)

Rhodoplanes	Potential for Nitrogen fixation (has gen Nifh)	(Buckley, et al., 2007)
Nitrospirae	Nitrification (Nitrites oxidation)	(Daims & Wahner, 2018)
Kribbella	Antibiotic production (Nocardioform)	

Soils microbial composition for healthy and infested were differs in each others. We found plant beneficials microbes ; *Bradyrhizobiaceae*, *Nitrospira*, *Nocardia*, *Rhodoplanes*, *Kribbella*, and *Streptomyces* founds more abundant in supressive soils. The role of the plant beneficial microbes can be seen on **Table 1**. Other study of natural supressive soils from banana rhizosphere found higher abundances of family Bradirhizobiaceae, Nitrospiraceae, Rhodospirillaceae, and Streptomycetaceae in supressive soils than conducive soils (Xue, et al., 2015). Increased abundances of nitrogen fixating bacteria *Nitrosomonas* and *Nitrosococcus* also increase disease supressiveness of banana in greenhouse conditions (Shen, et al., 2014). *Streptomyces* which can be found as banana endophyte were potential genus for biocontrol of Fusarium wilts, with siderophore producing ability (Cao, et al., 2005).

Cerita tentang bakteri bakteri yang berpotensi dalam supressive terhadap infeksi, atau sebaliknya.

Cerita perspektif biokontrol., potential health indicator. Menguatkan penelitian lain.

REFERENCES

Bisanz, J. E., 2018. Qiime2R : Importing Qiime2 artifacts and associated data into R sessions.. *[unpublished]*.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope E, Da Silva R, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley G, Janssen S, Jarmusch AK, Jiang L, Kaehler B, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MG, Lee J, Ley R, Liu Y, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton J, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson, II MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CH, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2018. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Preprints* 6:e27295v2. doi: https://doi.org/10.7287/peerj.preprints.27295v2

Buckley, D., Huangyutitham, V., Hsu, S. & Nelson, T., 2007. Stable isotope probing with 15N2 reveals novel noncultivated diazotrophs in soil. *Applied and Environmental Microbiology,* 73(10), pp. 3196-3204.

Callahan, B. et al., 2016. DADA2: High-resolution sample inference from Illumina amplicon data.. *Nature Methods,* Volume 13, pp. 581-583.

Cao, L. et al., 2005. Isolation and characterization of endophytic Streptomycete antagonist of Fusarium wilt pathogen from surface-sterilized banana roots. *FEMS Microbiology Letters,* Volume 247, pp. 147 - 152.

Cordovez, V. et al., 2015. Diversity and functions of volatile organic compounds produced by Streptomyces from a disease-supressive soil.. *Front. Microbiol,* 6(1081).

Daims, H. & Wahner, M., 2018. Nitrospira. *Trend in Microbiology.*

De Langhe, E. et al., 2009. Why Bananas Matter: An introduction to the history of banana domestication. *Ethnobotany Research and Application,* pp. 165-178.

DeSantis, T. et al., 2006. Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB.. *Applied and Environmental Microbiology,* 72(7), pp. 5096-5072.

Dita, M. et al., 2018. Fusarium Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. *Front Plant Sci.,* 9(1468), pp. 1-21.

Leo Lahti, Sudarshan Shetty et al. (2017). Tools for microbiome analysis in R. Version 1.9.1. URL: http://microbiome.github.com/microbiome

Love, M., W., H. & W, A., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology,* Volume 15, p. 550.

McMurdie, P. J. & Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE,* 8(4), p. e61217 https://doi.org/10.1371/journal.pone.0061217.

Mehta, C., Palni, U., Franke-Whittle, I. & Sharma, A., 2014. Compost: Its role, mechanism and impact on reducing soil-borne plant diseases. *Waste Management,* 34(3), pp. 607-622.

Molina, A. & Fabregas, E., 2009. Recent occurence of Fusarium oxysporum f.sp.cubense tropical race 4 in Asia. *Acta horticulturae,* pp. 109-116.

Morton, J. T. et al., 2017. Balance trees reveal microbial niche differentiation. *MSystems,* 2(1), pp. e100162-16.

Omar, S. & Abd-Alla, M., 1998. Biocontrol of fungal root rot diseases of crop plants by the use of rhizobia and bradyrhizobia. *Folia Microbiolofica,* 43(4), pp. 431 - 437.

Sharma, P., Kalita, M. C. & Thakur, D., 2016. Broad Spectrum Antimicrobial Activity of Forest-Derived Soil Actinomycete, Nocardia sp. PB-52. *Front. Microbiol.,* 7(347).

Shen, Z. et al., 2014. Deep 16S rRNA Pyrosequencing Reveals a Bacterial Community Associated with Banana Fusarium Wilt Disease Suppression Induced by Bio-Organic Fertilizer Application. *PLOS ONE,* 9(5), p. e98420.

van Elsas, J. D. et al., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *PNAS,* 10(4), pp. 1159-1164.

Viaene, T. et al., 2016. Streptomyces as a plant's best friend ?. *FEMS Microbiol. Ecol, ,* 92(8), p. Doi : 10.1038/srep11124.

Wei, Z. et al., 2015. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nature Communications,* 6(8143), p. DOI:10.1038/ncomms9413.

Xue, C. et al., 2015. Manipulating the banana rhizosphere microbiome for biological control of Panama disease. *Scientific Reports,* 5(11124), p. Doi : 10.1038/srep11124.

Yang, H. et al., 2017. An Integrated Insight into the Relationship between Soil Microbial Community and Tobacco Bacterial Wilt Disease. *Front. Microbiol. ,* 8(2179), pp. 1-11.

Table S1. Number of sequences in each raw processing steps.

Sample	Number of sequences						
	Raw sequences	Filter	Denoised Forward	Denoised Reverse	Merging	Non-chimeric	% Sequences
H1	269.700	181.330	150.926	151.919	53.031	38.721	14,36 %
H2	286.675	184.578	160.485	159.629	63.429	41.027	14,31 %
I1	252.689	172.929	148.099	147.108	56.384	38.534	15,25 %
I2	268.845	179.254	154.106	153.838	61.003	42.977	15,99 %

Figure S1.

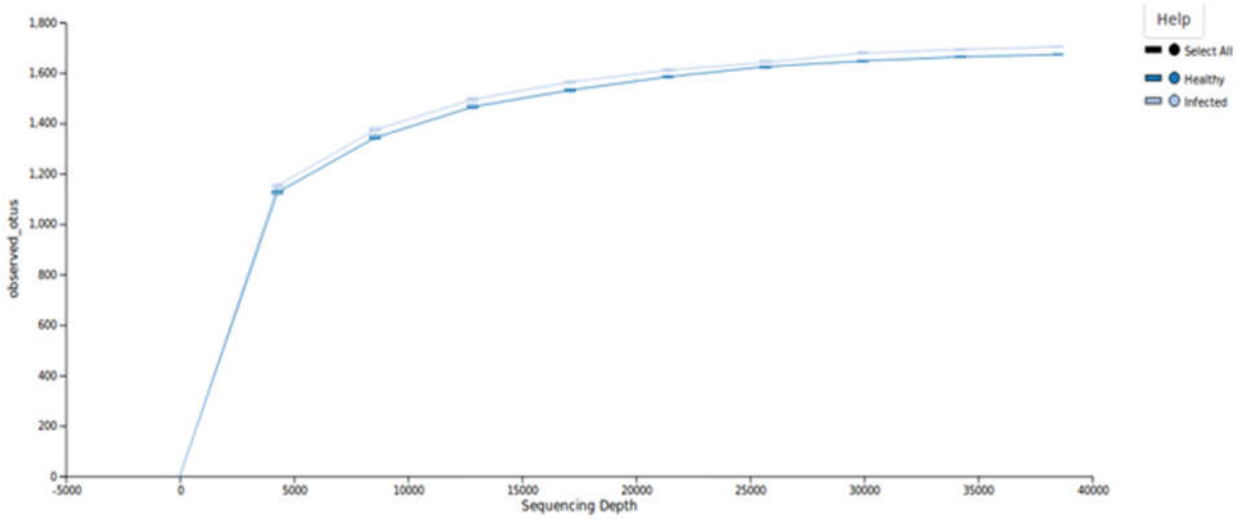


Figure S2.

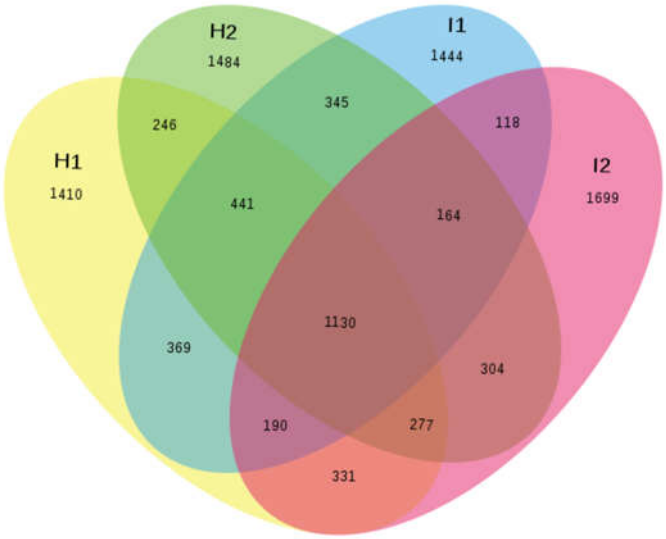
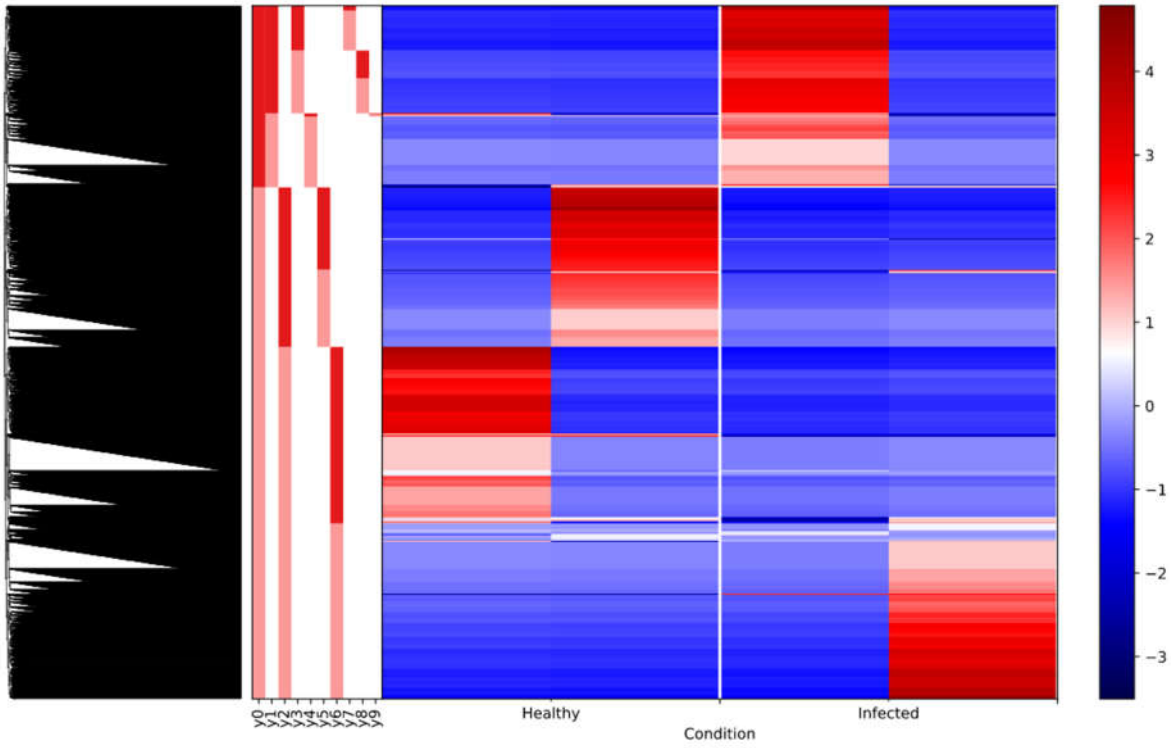


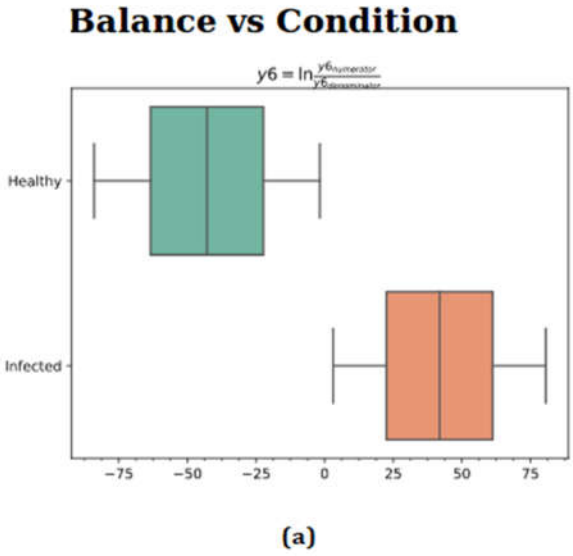
Figure s3.

Figure S4.

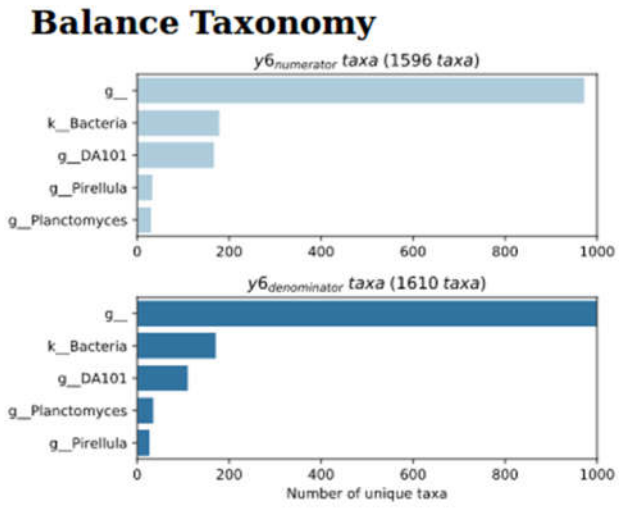


as PDF

Numerator Denominator



(a)



(b)