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DIVERSITY OF SOIL MICROORGANISMS IN BANANA HABITATS WITH AND WITHOUT *FUSARIUM* WILT SYMPTOM

I Made Sudarma[1] and Dewa Ngurah Suprapta[2]

[1]Doctorate Program on Agricultural Science, School of Postgraduate Udayana University
Jl. PB. Sudirman Denpasar Bali, Indonesia
[2]Laboratory of Biopesticide, Faculty of Agriculture, Udayana University, Jl. PB. Sudirman,
Denpasar Bali, Indonesia. E-mail : biop@dps.centrin.net.id

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ABSTRACT

Fusarium oxysporum f.sp. *cubense* (Foc), the cause of the *Fusarium* wilt disease on banana plant, is one of the important soil borne pathogens which may lead to a significant loss of banana yield in Indonesia. This study was done in order to know the soil's microbial diversity in the banana habitat with and without *Fusarium* wilt symptom. The soil samples were collected from three regencies in Bali, *i.e.* Karangasem, Klungkung and Jembrana which are the main banana growing areas in Bali. Soil sampling was done in two sites in each regency representing the banana habitat with and without *Fusarium* wilt symptom, by collecting 100 grams of soil surrounding the banana plant at the depth of 20 cm. Soil microbes population density particularly for bacteria, fungi and actinomycetes were determined based on plate count technique, while the microbial diversity was determined based on the Diversity Index of Shannon-Wiener. Results of the present study showed that the density of soil microbes in the soil of banana habitat without *Fusarium* wilt symptom (HN) was 13.85 x 10^6 cfu per gram of soil, which was significantly higher than 1.02 cfu per g of soil of banana habitat with *Fusarium* wilt symptom (HF), while the density of Foc in the soil of HN is 0.003 x 10^6 cfu per g of soil, which is lower than 0.01 x 10^6 cfu per g of soil of HF. The diversity index of the soil microbes in the soil of HN was 2.03, which was higher than 1.91 of HF. The domination index of the soil microbes in the soil of HN was 0.81, which was higher than 0.78 of HF, wherein *Bacillus* spp. and *Pseudomonas* spp. were the dominant microbes. The density of soil microbes which are potentially antagonistic against Foc, such as *Bacillus* spp., *Streptomyces* spp., *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp. and *Gliocladium* sp. is higher in the soil of HN compared to the soil of HF. Among the antagonistic microbes, *Bacillus* sp. showed the highest inhibitory activity against Foc. These results suggest that the high microbial diversity index, the low population density of Foc, and the high population of antagonistic microbes in the soil of the banana habitat without *Fusarium* symptom were able to suppress the development of *Fusarium* wilt disease on banana.

Key words: Domination index, population density, antagonistic microbes

INTRODUCTION

Fusarium wilt disease caused by the fungus *Fusarium oxysporum* f.sp. *cubens*e (Foc)has led to a significant loss of banana fruit in Indonesia (Semangun, 2001). Almost all banana trees are highly susceptible to the disease (Ploetz, 2007). The disease has spread all over Indonesia and has also decreased banana yield by 63.33% (Semangun, 2001). In Bali, its serious widespread distribution in 1997, caused banana production to decrease from 134,000 tons to 54,000 annually (Sudana et al., 2000). To the Balinese people, banana fruit is not only for consumption but it has socio-religious values as it is used for religious ceremonies, accounting for 70% of the per capita total consumption.

About 70 cultivars of banana fruit are needed for the offerings offered in a temple festival (Subekti, 2008).

The percentage of the *Fusarium* wilt disease on a banana tree is determined by the diversity of soil microbes. The diversity and population of the microbes in the soil vary highly, depending on the soil fertility. Conklin (2002) stated that one gram of soil contains from 108 to 109 types of bacteria; from 107 to 108 types of actinomycetes, and from 105-106 types of fungal propagules. The soil microbial diversity is important in order to conserve it, as its existence plays an important role in the cycle of nutrients and the sustainable use of soil (Kalia and Gupta, 2005).

Soil microbes are important components of the soil habitat, as ecologically their role is to control the nutritional cycle to maintain the soil fertility, contributing to soil genesis and maintaining the soil structure (Clegg and Murray, 2002; Feeney et al., 2006). The microbial ecosystem includes biotic and abiotic components, such as the total number of microbes, microbial diversity, physical properties and chemical properties. In such an ecosystem, the microbial interactions such as neutralism, mutualism, commensalism, antagonism, competition and parasitism may occur (Whipps and Lumsden, 2001; Pelczar and Chan, 2005).

Suppressive soil is commonly dominated by the antagonistic microbes which produce a number of antibiotics, siderophores, fungicidal compounds, and competition with detrimental microbes, and induce plant resistance against pathogenic microbes (Arya, 2005; Alexander, 2006; Singh and Singh, 2008). Several examples of antagonistic microbes are *Trichoderma*, *Aspergillus, Penicillium* and *Actinomycetes* such as *Streptomyces*. The antibiotic produced by the antagonistic microbes may function as biostatic or biocide, which may affect the development of the soil borne pathogens (Higa and Parr, 1994; Haas and Defago, 2005). The greater the microbial population in the soil the more suppressed the soil borne pathogen. In the suppressive soil, the antagonistic microbes such as *Bacillus* sp., *Trichoderma*, *Pseudomonas* spp., *Actinomycetes* and non-pathogenic *F. oxysporum* effectively protect plants from soil borne pathogens such as *F. oxysporum*, *Phytophthora infestans*, *Rhizoctonia solani*, *Phytophthora cinnamon* and *Pythium* spp (Weller et al. 2002; Garbeva et al., 2004). The suppressive soil contains more bacteria and actinomycetes than the conducive soil (Peng et al., 1999).

The extent to which the antagonistic microbes suppress the pathogen depends on their microbiological activities in the soil. The greater the microbiological activities, the more carbon, nutrients and energy are used, thus, rendering the pathogen weak (Sullivan, 2004). The microbial diversity in the suppressive soil is usually higher than in the conducive soil; therefore, the suppressive soil produces more biomass and microbial activities that make the soil borne pathogen get suppressed. The microbes in the fertile soil tend to lower the intensity of the disease resulting from the soil borne pathogen (Garbeva et al., 2004). The more diverse the soil microbial population, the greater the possibility to find antagonistic microbes which potentially can be used to control the soil borne pathogens. This study was done from August 2009 to September 2010, in order to evaluate the soil microbial diversity, domination index and potential antagonistic microbes against *F. osyxporum* f.sp. *cubense* in the banana soil ecosystem, with and without *Fusarium* wilt symptom in Bali.

MATERIALS AND METHODS

Collection of soil samples

The soil samples were taken in August 2009 from three regencies in Bali, *i.e.* Karangasem Regency, Klungkung Regency and Jembrana Regency, which are the centers of banana cultivation in Bali. The soil samples were taken from two locations in each regency representing the banana habitat without *Fusarium* wilt symptom (HN) and banana habitat with *Fusarium* wilt symptom (HF). Three

samples were taken from each habitat. Four holes at a depth of 20 cm were made surrounding the banana plant, and 100 g of soil were taken from each hole, and combined together as one composite sample. The soil samples were then place in plastic bags and kept in an ice box. All samples were stored in a refrigerator for about 18 to 24 hours before they were used for microbial analysis. Other soil samples of 1 kg each were taken from each habitat for physical and chemical properties analysis.

Determination of the soil microbes population

One gram of soil sample was dissolved in distilled water and thoroughly mixed using vortex. This soil suspension was filled up to volume of 10 ml. A decimal serial dilution (10^{-2} to 10^{-7}) was made under sterile conditions. From the serially diluted solution, 1 ml was taken in every dilution which was then poured into the Petri dish together with the culture media. The nutrient agar (NA) medium which was made up of 3 grams of beef extract, 5 grams of peptone, 15 grams of bacteriological agar and distilled water to make 1000 ml, was used to isolate the bacteria. A 200 µl of 0.15% nystatin antibiotic (an antifungal antibiotic) was added to the solution. Five Petri dishes were prepared for each dilution. These cultures were incubated in the dark at room temperature ($27\pm 2^{o}C$) for 24 h, after which the bacterial colonies were counted as colony forming units (CFU). The single colonies were obtained by adopting the quadrant streak, which were then grown on slant medium for identification. The bacteria were identified based on physiological and biochemical tests with reference to the Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) and Soemarno (2000).

The Kenknight medium (1 gram of dextrose, 0.10 gram of KH_2PO_4, 0.10 gram of $NaNO_3$, 0.10 gram of KCl, 0.10 gram of $MgSO_4.7H_2O$, 15 gram of agar and 100 ml of distilled water (Rao, 1994) with 200 µl of 15% (w/v) nystatin antibiotic solution (an antifungal antibiotic) was used to isolate actinomycetes. The cultures were then incubated for three days in the dark at room temperature ($27 \pm 2^{0}C$), after which the colonies were counted as colony forming units (CFU). The single colonies were then transferred into a new Petri dish containing the KenKnight media before these were incubated at room temperature ($27 \pm 2^{0}C$). After 14 days, the isolates were identified macroscopically to determine colony colors, colony shape, the growth rate, and microscopically to observe the shape and the hypae branches and shape of the spores which were then matched with the Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) and the Actinomycetes Atlas (Miyadoh et al., 2002).

The potato dextrose agar (PDA) medium and 0.1% (w/v) livoplosaxin (an antibacterial antibiotic) was used to isolate the fungi. Five Petri dishes were prepared for each dilution. The cultures were incubated in the dark at room temperature ($27 \pm 2^{0}C$). A single colony was transfered into the Petri dish containing the PDA media and then incubated at room temperature. After 3 days, the isolate was macroscopically identified to determine the color of the colony and the growth rate, and microscopically to observe the presence of septa on the hyphae, the shape of spore/conidia and sporangiospore (Samson et al., 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; Indrawati et al., 1999).

Analysis of soil properties

Several soil properties, such as levels of organic C, total N, available P, available K, water content, pH and texture were analyzed according to the methods developed by Alef and Nannipieri (1995). The analysis was done in the Laboratory of Soil Science, Faculty of Agriculture, Udayana University, Bali.

Soil microbial diversity index

The Shannon-Wiener's index was applied to determine the soil microbial diversity index according to the following formula (Odum, 1998):

$$H' = - \sum_{i=1}^{s} Pi \ In \ Pi$$

where:

H' = Shannon-Winner's diversity index

S = the number of genera

Pi = ni/N as the proportion of type i (ni = the total number of individuals of microbe in total i type, N = the total number of all the individuals in total n)

The criteria adopted for interpreting the Shannon-Wiener's diversity (Ferianita-Fachrul et al., 2005) are as follows : H' <1 = low diversity ; H' 1-3 = fair diversity, and H' >3 = high diversity.

Domination index

The domination index is adopted for obtaining the information on the types of the dominating soil microbes at a community in every habitat. The following formula was applied to determine the Simpson index of soil microbes (Pirzan and Pong-Masak, 2008).

$$C = \sum_{i=1}^{s} Pi^2$$

where:

C = Simpson's index

S = the number of genera

Pi = ni/N, that is, the individual proportion of type i and all the individuals (ni = the total number of the individuals type i, N = the number of all the individuals in total n).

The Simpson index was used to determine the domination index (D), where D = 1 – C (Rad et al., 2009). The higher the domination index, the lower the Simpson index will be and vice versa. The criteria adopted for interpreting the domination index of the soil microbes were: the value of D is close to 0, meaning that no species which extremely dominates the others. The value of D is close to 1, meaning that one or several species of the soil microbes were dominant in a certain habitat (Pirzan and Phong-Masak, 2008).

Determination of antagonistic microbes

Determination of antagonistic microbes was done through dual-culture method on PDA in a Petri dish. A mycelial plug (4 mm in diameter) taken from the edge of three days old colony of *F. oxysporum* f.sp. *cubense* was grown side by side with a tested microbe with a 2-cm distance. The Foc alone, grown as single culture on a Petri dish, was prepared as control. The cultures were then incubated in the dark at room temperature (27 ± 2^0C) for five days.

The inhibitory activity of a tested microbe was determined using the following formula :

$$\text{Inhibitory activity (\%)} = \frac{A - B}{A} \times 100$$

A = Diameter of Foc colony in single culture (mm)

B = Diameter of Foc in dual culture (mm)

RESULTS AND DISCUSSION

The density of the soil microbial population

The number of the microbial colonies per gram of soil is significantly higher ($P<0.01$) in the banana plant habitat without the *Fusarium* wilt symptom (HN) than that in the banana plant habitat with the *Fusarium* wilt symptom (HF). The number of colonies in the soil of HN was 13.85 x 10^6 cfu per gram of soil, while 1.02 x 10^6 cfu per gram of soil was determined in HF (Table 1). The colonies of the bacteria, the colonies of actinomycetes and the colonies of fungi in HN were 7.8 x 10^6, 6.0 x 10^6, and 0.05 x 10^6 cfu gram^{-1} of soil, respectively, which were higher and highly significant ($P<0.01$) than those in the HF, with the total of 0.6 x 10^6 , 0.4 x 10^6 and 0.02 x 10^6 cfu per g of soil, respectively (Table 1). The population of Foc in the HN was 0.003 x 10^6 cfu per g of soil which was significantly lower than that of HF (0.01 x 10^6 cfu g $^{-1}$).

Table 1. Population of soil microbes in the soils of banana plant habitat without *Fusarium* wilt symptom (HN) and with *Fusarium* wilt symptom (HF).

Group of soil microbes	Number of colony in HN (x 10^6cfu per g of soil)	Number of colony in HF (x 10^6 cfu per g of soil)
Bacteria	7.8**	0.6
Actinomycetes	0.6**	0.4
Fungi	0.05**	0.02
Total microbes	13.85**	1.02
Foc	0.003**	0.01

** Number of colony in the soils of HN is significantly higher than those of the soils of HF according to the Chi-Square Test ($P<0.01$).

The population density of the bacteria available in the soil was higher than those of actinomycetes and fungi as shown in Table 1 and Table 2. These results are in agreement with Ogunmwonyl et al. (2008) that the average number of the soil bacteria at several different locations at the University of Obafemi Awolowo, Nigeria was $10^5 – 10^7$ cfu per g of soil, while the average number of fungus was $10^3 – 10^5$ cfu per g of soil. Widawati et al. (2005) stated that the density of the bacterial population was higher than the density of the actinomycetes population and the density of fungus at the Wamena Biological Park, Jayawijaya. Moore et al. (1995), likewise stated that the microbial population in the suppressive soil (the banana plant habitat without the *Fusarium* wilt symptom) could minimize the pathogen population so that the banana trees can grow without the *Fusarium* wilt symptom.

All the factors which contribute to the formation of soil also influence the soil microbes such as the climate, the nutrients and the soil texture (especially the clay), vegetation, topography and time. The chemical status and the physical condition of the soil may influence the soil microbial population and diversity (Granatsein and Bezdicek, 2003). The development of the *Fusarium* wilt symptom can be minimized by increasing the clay minerals of the suppressive soil into the conducive soil. The higher density of the microorganisms, resulting from such a treatment, increases the degree of its suppresiveness (Garbeva et al., 2004; Sikora and Pocasangre, 2006).

Several soil borne pathogens such as *F. oxysporum* (the causal agent of vascular wilt disease) and *Phytophthora cinnamon* (the causal agent of root rot disease of fruit trees) develop well and may cause serious diseases in the conducive soil; however, these develop poorly in the suppressive soil (Garbeva et al., 2004). A small amount of soil (1% of the total soil in the pot) taken from the suppressive soil with the *Fusarium* wilt symptom which was added to the pot in the green

house could effectively lower the damage caused by *Fusarium oxysporum* in carnation trees (McCain et al., 1980).

Table 2. Population density and proportion of various soil microbes in the soil of banana habitat without *Fusarium* wilt symptom (HN) and with *Fusarium* wilt symptom (HF).

Type of microbes	Population density (x 10^6 cfu per g of soil) and percent of the total population	
	HN	HF
Bacteria**		
Bacillus spp.	4.7 (33.9)	0.2 (19.6)
Micrococcus spp.	0.1 (0.7)	-
Pseudomonas spp.	3.0 (21.7)	0.4 (39.2)
Actinomycetes**		
Actinomyces sp.	0.1 (0.7)	0.01 (1.0)
Actinoplanes sp.	0.1 (0.7)	-
Agromyces spp.	0.1 (0.7)	0.01 (1.0)
Dactylosporangium sp.	0.4 (2.9)	0.03 (2.9)
Frankia sp.	0.2 (1.4)	0.02 (2.0)
Geodermatophilus sp.	-	0.04 (3.9)
Microbispora sp.	0.2 (1.4)	-
Micromonospora sp.	0.7 (5.1)	0.11 (10.8)
Nocardia sp.	2.0 (14.4)	0.07 (6.9)
Pilimelia sp.	0.1 (0.7)	-
Pseudonocardia sp.	0.1 (0.7)	-
Sacharomonospora sp.	0.5 (3.6)	0.10 (9.8)
Streptomyces spp.	0.9 (6.5)	-
Streptosporangium sp.	0.6 (4.3)	-
Fungi**		
Aspergillus nidulans	0.006 (0.04)	0.001 (0.1)
A. niger	0.006 (0.04)	-
A. terreus	-	0.001 (0.1)
Botrytis sp.	-	0.001 (0.1)
Chaetomium sp.	-	0.001 (0.1)
Fusarium oxysporum f.sp. *cubense*	0.003 (0.02)	0.01 (1.0)
Geotrichum sp.	0.001 (0.01)	0.001 (0.1)
Gliocladium sp.	0.001 (0.01)	-
Humicola sp.	0.002 (0.01)	-
Moniliella sp.	-	0.001 (0.1)
Mucor sp.	-	0.001 (0.1)
Paecilomyces sp.	0.003 (0.02)	0.001 (0.1)
Penicillium sp.	-	0.001 (0.1)
Penicillium digitatum	0.002 (0.01)	0.005 (0.5)
P. notatum	0.003 (0.02)	0.001 (0.1)
Rhizopus sp.	-	0.001 (0.1)
Trichoderma spp.	0.022 (0.01)	0.003 (0.3)

Type of microbes	Population density (x 10^6 cfu per g of soil) and percent of the total population	
	HN	**HF**
Vertilicium sp.	0.001 (0.01)	-
Total population	**13.85**	**1.02**
Number of types	**27**	**24**

** Highly significant according to the Chi-Square Test (P<0.01)

In the present study, the soil texture of HN and HF is relatively different, wherein the percentage of clay and dust in HN soil were relatively higher than those of HF, while the percentage of sand was lower in HN soil compared to HF soil (Table 3). Other soil properties such as pH, organic C, total N, available K and available P of both habitats were in the same category. The difference in the soil texture of HN and HF may be the influencing factor for the difference in the soil microbial population and Foc population in the HN and HF. The results of the present study showed that the density of the Foc in HF soil was 0.01 x 10^6 cfu per g of soil while in HN soil it was only 0.003 x 10^6 cfu per g of soil.

Table 3. Data for physicochemical properties of soil samples collected from banana habitat without *Fusarium* wilt symptom (HN) and with *Fusarium* wilt symptom (HF).

Soil properties	HN	HF
pH	7.2 ± 0.5 (N)*	7.1 ± 0.2 (N)
C-organic (%)	2.1 ± 0.8 (M)	2.8 ± 0.5 (M)
Available P (ppm)	97.6 ± 45.4 (VH)	77.5 ± 11.9 (VH)
Available K (ppm)	653.2 ± 15.1 (VH)	675.3 ± 260.3 (VH)
Water content :		
Air dried (%)	5.0 ± 4.1	5.0 ± 5.3
Field capacity (%)	24.4 ± 10.5	29.8 ± 10.6
Texture :		
Sand (%)	46.2 ± 21.7	57.6 ± 21.2
Dust (%)	34.2 ± 11.5	26.5 ± 14.4
Clay (%)	19.6 ± 10.7	16.0 ± 8.1

* N = neutral, M = medium, L = low, and VH = very high

The *Fusarium* wilt symptom was not observed on banana trees in the suppressive soil resulting from the high density of the actinomycetes and bacterial population. The pathogens were suppressed resulting from the interaction between the Foc and the antagonistic microbes such as *Pseudomonas fluorescens*, the non-pathogenic *F. oxysporum* and the *Trichoderma harzianum* (Perez-Vicente, 2004). Four antagonistic microbes in the soil around the banana trees, namely *Gliocladium* sp., the non-pathogenic *F. oxysporum*, the *Pseuodomonas fluorescens*, and the *Streptomyces* sp. effectively reduced the *Fusarium* wilt symptom when these were applied in the field (Subekti, 2008). Widodo and co-workers (2003) stated that the *Fusarium* wilt disease on the banana trees could be controlled by inoculating the bacteria, *Burkholderia ceparia* isolated from the rhizosphere of the banana trees in the suppressive soil. The results showed that the inoculation with *B. cepacita* which was done seven days before the inoculation with Foc could minimize the development of the *Fusarium* wilt symptom. The inoculation with the living cells of *B. cepacia* could increase the salicylic acid concentration in the banana root and stem; however, the correlation between the accumulation of the salicylic acid in extracts of root and stem of banana and the inhibitory activity against Foc *in vitro* has not yet been understood.

Diversity and domination indexes

The number of the soil microbial types in the soil of HN is greater than that HF, which was 27 and 24, respectively. The average diversity index (H') of the soil microbes in the soil of HN was 2.03±0.03, higher than 1.91±0.04 of HF (Table 4). The soil properties particularly the higher percentage of clay and dust and the low percentage of sand in the soil of HN compared to HF probably resulted in greater soil microbial diversity in the HN compared to HF.

The domination index of the soil microbes in HN soil was higher than that of HF, *i.e.* 0.81±0.03 and 0.78±0.01, respectively (Table 4).

Table 4. Domination and diversity indexes of microbes in the soil samples collected from banana habitat without *Fusarium* wilt symptom (HN) and with *Fusarium* wilt symptom (HF).

Type of Index	HN	HF
Diversity index	2.03 ± 0.03*	1.91 ± 0.04
Domination index	0.81 ± 0.03	0.78± 0.01

*Average of three replicates

Bacillus spp. and *Pseudomonas* spp. were dominant soil microbes in the soil of HN, accounting for 33.9% and 21.7%, respectively. *Bacillus* sp. was found to be a dominant antagonistic microbe against Foc in HN with an abundance of 0.2 x 10^6 cfu per g of soil, while the antagonistic *Bacillus* sp. was not found in HF (Table 5). Other potential antagonistic microbes against Foc were *Aspergillus* spp., *Gliocladium* sp., *Penicillium* spp., *Streptomyces* spp., and *Trichoderma* spp. (Table 5). The high abundance of the antagonistic *Bacillus* sp. in HN and the low population of Foc are probably the factors that suppressed the development of *Fusarium* wilt disease in HN.

Table 5. Population density of antagonistic microbes in the soils of banana habitat without *Fusarium* wilt symptom (HN) and with *Fusarium* wilt symptom (HF)

Type of microbes	HN (x 10^6 cfu per g of soil)	HF (x 10^6 cfu per g of soil)
Bacillus spp.	0.200	-
Aspergillus nidulans	0.013	0.004
A. niger	0.009	-
A. terreus	-	0.006
Gliocladium sp.	0.001	-
Penicillium digitatum	0.002	0.013
P. notatum	0.002	0.005
Streptomyces spp.	0.004	-
Trichoderma spp.	0.011	0.006
Population density**	0.242	0.034
Number of types	8	5

** Highly significant according to the Chi-Square Test (P<0,01)

Rao (1994) stated that the most common bacteria found in the soil were the genera of *Pseudomonas*, Arthrobacer, *Clostridium*, *Achromobacter*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Corynibacterium*, *Sarcina*, and *Mycobacterium*. Ogunmwonyl and co-workers (2008) showed that

the dominant bacteria at Obafemi Awolowo, Nigeria, was *Bacillus*, followed by *Pseudomonas*, while the dominating fungus was *Aspergillus*. *B. subtilis* and *P. fluorescens* play an important role in decomposing organic matter (Reid and Wong, 2005). *B. lichenoformis* and *B. amylolichefaciens* were good for controlling *Fusarium* sp. (Gheorghe et al., 2008). *Bacillus subtilis* as a biocontrol agent (BCA) forms a biofilm on the surface of the roots, include the surfactin secretion (a compound of lipopeptide which serves as the antimicrobial agent) (Bais et al., 2004). Many *Pseudomonas* spp. act as PGPR (plant growth promoting rhizobacteria) in suppressive soils, where these produce antibiotics and induce systemic resistance in the host plants (Haas and Defago, 2005).

Our present study found several potential antagonistic microbes against Foc namely *Bacillus* sp., *Aspergillus* spp., *Gliocladium* sp., *Penicillium* spp., *Streptomyces* spp., and *Trichoderma* spp. The abundance of antagonistic microbes such as *Bacillus* sp., *Aspergillus* spp., *Gliocladium* sp., *Streptomyces* spp., and *Trichoderma* spp. in HN was higher than that of HF. Among them, *Bacillus* sp. exhibited the highest inhibitory activity against Foc (Table 6).

Table 6. Inhibitory activity of the antagonistic microbes against *Fusarium oxysporum* f.sp. *cubense*

Antagonistic microorganisms	**Inhibitory activity (%)**
Bacillus sp.	89.8 $\pm$ 5.6*
Streptomyces sp.	79.6 $\pm$ 3.2
Aspergillus nidulans	83.3 $\pm$ 2.8
A. niger	84.7 $\pm$ 2.5
A. terreus	85.6 $\pm$ 1.1
Gliocladium sp.	77.8 $\pm$ 2.4
Penicillium digitatum	86.3 $\pm$ 0.9
P. notatum	81.5 $\pm$ 3.2
Trichoderma spp.	81.8 $\pm$ 3.2

*Average of five replications.

Further study is needed in order to evaluate the mechanism by which antagonistic microbes suppress the growth of Foc and affect the development of wilt disease in banana plant under field conditions. In addition, a study on the total DNA and DNA diversity in the soil of HN and HF is needed, as not all soil microbes can be cultured on synthetic medium.

CONCLUSIONS

The density and diversity of soil microbes in the banana habitat without *Fusarium* wilt symptom is significantly higher than in the banana habitat with *Fusarium* wilt symptom, while the density of *F. oxysporum* f.sp *cubense* in the soil of banana habitat without *Fusarium* symptom was lower than in the banana habitat with *Fusarium* symptom. The domination index of the soil microbes in the banana plant habitat without *Fusarium* wilt symptom was higher than that of banana habitat with *Fusarium* wilt symptom, wherein *Bacillus* spp. and *Pseudomonas* sp. were the dominant microbes in the banana plant habitat without *Fusarium* wilt symptom. The potential antagonistic microbes against *F. oxysporum* f.sp. *cubense* were *Bacillus* spp., *Streptomyces* spp., *Trichoderma* spp., *Aspergillus* spp., and *Gliocladium* sp. Among these, *Bacillus* sp. showed the highest inhibitory activity against Foc.

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