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Suppressive Effects of Vermicomposted-Bamboo Powder on Cucumber Damping-Off

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Abstract

The suppressive effect of vermicomposted-bamboo powder (VB) derived from waste bamboo shoots on cucumber damping-off when VB is used as a nursery medium was characterized. A greenhouse inoculation experiment showed that the damping-off caused by each strain of *Pythium aphanidermatum*, *P. ultimum* var. *ultimum*, and *Rhizoctonia solani* AG1-IB was significantly reduced by VB compared to an autoclaved vermicomposted-bamboo powder (aVB) and a commercial-nursery medium (CNM). Due to the fact that the disease suppressiveness of VB was nulled by autoclaving, the soil biological factors were characterized with respect to those of the CNM by fluorescein diacetate (FDA) hydrolysis reactions, plate count techniques, and next-generation sequencing (NGS) analysis using prokaryotic universal primers. The FDA hydrolysis reaction and the plate count techniques showed that VB has a higher microbial activity and population density of fungi and bacteria than aVB and CNM. The NGS analysis revealed that the bacterial diversity was higher in VB than in aVB and CNM. In addition, the higher concentrations of NH_4^+ in VB may be related to the *Pythium* damping-off suppression. This is the first report on the suppressive effect of VB on the damping-off disease, which is at least partially due to its rich microbial activity and diversity.

Disciplines: Plant protection, Watershed and regional resources management
Additional key words: bacterial diversity, microbial activity, microbial population, waste bamboo

Introduction

Bamboos, especially moso bamboos (*Phyllostachys edulis* (Carrière) J. Houz.), grow vigorously in the vicinity of populated areas in Japan, causing substantial damage to agricultural production and rural ecosystems (Isagi et al. 1997, Imaji et al. 2013, Kajisa et al. 2011). Since moso bamboos are an abundant and sustainable resource throughout the world (FAO 2010), they have the potential to be an ideal feedstock for composting for agricultural use. Vermicomposting is an agricultural recycling process for biodegradable solid waste that is facilitated by the decomposition and digestion of earthworms and associated microorganisms (Elvira et al. 1998). The feedstocks that are commonly used for vermicomposting include animal manure and vegetable or fruit scraps from kitchens or farms (Atiyeh et al. 2000, Garg et al. 2006). Many kinds of vermicompost are known to be used as a component of nursery potting media (Scheuerell et al. 2005) owing to their ability to control plant pathogens such as *Pythium ultimum* Trow var. *ultimum*, *Rhizoctonia solani* Kuhn, and *Verticillium* sp. (Chaoui et al. 2002). Owing to their effective disease suppression, vermicomposts have become a promising alternative to chemical pesticides. However, vermicomposts produced from different feedstocks vary in disease suppressiveness. Szczech & Smolińska (2001), for example, showed that vermicomposts produced from

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cattle manure, sheep manure, or horse manure significantly suppressed *Phytophthora nicotianae* Breda de Haan var. *nicotianae*, but that produced from sewage sludge had no effect against the same pathogen. Considering that bamboo powder has relatively homogeneous qualities, it may generate a vermicompost superior to conventional vermicomposts for suppressing plant pathogens. However, vermicomposting of bamboo powder has never been examined, and consequently no information is available on its suppressive effects against plant pathogens.

The objectives of the present study were (1) to identify the suppressive effects of vermicompost produced from vermicomposted-bamboo powder (VB) against the damping-off of cucumber caused by *Pythium aphanidermatum*, *P. ultimum* var. *ultimum*, and *Rhizoctonia solani* AG1-IB under greenhouse conditions and (2) to understand the mechanism of the suppression, total microbial activity, population of microbes, and bacterial communities of VB.

Materials and methods

1. VB production

One-year-old shoots of moso bamboos were ground into a powder using a grinder and soaked in 10 times their volume of tap water for 24 h. Water-soluble phenolic compounds can be toxic to earthworms (Roberts & Dorough 1984, Abe et al. 2007), and they are considerably contained in bamboo shoots (Chongtham et al. 2011). Therefore, the powder was soaked in water before vermicomposting. 10 kg of the water-soaked bamboo powder was mixed with 100 g of pieces of kudzu vine (*Pueraria lobata* (Willd.) Ohwi, <10 cm in length) as a nitrogen source, along with 100 g of earthworms (*Eisenia fetida* Savigny, commercial name: "Kumataro-futomushi," Fishing Azumino Company, Azumino, Japan). Kudzu was chosen as the nitrogen source because it is abundant close to moso bamboo habitats (Kajisa et al. 2011). The earthworms were starved for at least 2 h to evacuate the intestinal residuals in advance. A small amount of a commercial horse manure/wheat straw compost (20 g of dry weight, Iris Ohyama Inc., Sendai, Japan) was added because it can provide suitable living conditions for earthworms (Huang et al. 2013). The mixture was placed in a plastic box covered with a lid, kept at 28 ± 2°C, and watered once a week to maintain moisture at approximately 80% (w/w). After incubation for two months, the earthworms and their eggs were removed manually and via a sieve (4 mm mesh), and the mixture was divided into two subsamples. One was used for chemical properties' analysis, and the other was used for the evaluation of cucumber damping-off suppressiveness as described later. Prior to the analysis of chemical properties, the samples were dried at 70°C for two days. NH^{4+}, NO_3^-, PO_4^{3-}, and K^+ were detected using an RQflex reflectometer (Reflectoquant ammonium test, nitrate test, phosphate test, and potassium test; Merck KGaA, Darmstadt, Germany). Ca^{2+} and pH were tested using handheld meters (LAQUAtwin series, model B-751 for Ca^{2+} and B-71X for pH; Horiba Ltd., Kyoto, Japan). Mg^{2+} was measured using portable photometers (HI96752; Hanna Instruments, Woonsocket, RI, USA). The autoclaved VB (aVB) (autoclaved at 121°C for 1 h and kept in the laboratory room for one day) and a commercial-nursery medium (CNM) (Aisai-1, Katakura Chikkarin Co., Ltd., Tsuchiura, Japan) were used for comparison.

2. Suppressive effects of VB against cucumber damping-off

The suppressive effects of VB on cucumber damping-off were evaluated under greenhouse conditions, using aVB and CNM as controls. *Pythium aphanidermatum* isolate MAFF245234, *P. ultimum* var. *ultimum* isolate MAFF240023, and *Rhizoctonia solani* AG1-IB isolate SLS1 (MAFF244980) were used. Each isolate was cultured on autoclaved bentgrass seeds at 25°C in the dark for one week as described by Tojo et al. (1993). 1 g of the culture of each isolate was suspended in 20 mL or 200 mL of 0.35% water agar and used as an inoculum. Seven-day-old cucumber (*Cucumis sativus* L., "Aonagakei-jibai") seedlings grown on CNM were transplanted into plastic pots each containing VB, aVB, and CNM. Each seedling received 1 mL of inoculum at the plant base (avoiding direct contact of the inoculum on the plants) and was irrigated daily with tap water via hand irrigation. Uninoculated (pathogen-free) treatments included uninoculated VB, uninoculated aVB, and uninoculated CNM. The number of plants showing damping-off was recorded 0, 5, 10, and 15 days after the inoculation. In all the experiments, the replicates of each treatment were arranged in a randomized block, with three replications for each treatment. Each treatment per replication consisted of 30 cucumber seedlings. The temperature of the greenhouse was maintained in the range of 23-33°C for the experimental period.

3. Microbial activity, populations, and diversity

The microbial activity in VB and CNM was estimated using the rate of hydrolysis of fluorescein diacetate (FDA) as described by Adam & Duncan (2001) with a slight modification. Briefly, 2 g of samples (fresh weight; $N = 4$) was put in 50 mL centrifuge tubes and mixed with 15 mL of potassium phosphate buffer (60 mM, pH = 7.6). After

20 min of incubation, 15 mL of chloroform/methanol (2 : 1, v/v) was added to terminate the reaction. The absorbance (490 nm) of the filtered solutions was determined spectrophotometrically. The number of bacteria and fungi in VB and CNM were determined using the plate count technique, employing selective media as described by Waksman & Fred (1922) and Martin (1950), respectively. The bacterial communities of VB and CNM were further examined using next-generation sequencing (NGS). VB and CNM were collected for three samples, with each sample corresponding to 1 L of organic matter. After the samples were homogenized by manual mixing, 4 g of the subsample was subjected to DNA extraction by MORA-EXTRACT (Kyokutoseiyaku Co., Ltd., Tokyo, Japan). The bacterial V3-V4 region of the 16S rDNA of each sample was amplified by a polymerase chain reaction (PCR) as described by Okano et al. (2016). The PCR products were sequenced using the Illumina MiSeq platform before the bacterial communities of each sample were identified by MagicSuite. In order to analyze the microbial diversity, operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Shannon's diversity index ($H' = -\sum p_i \ln[p_i]$, where p_i is the proportion of taxon i) was calculated (Shannon 1948). Simpson's diversity index ($D = 1 - \sum p_i^2$, where p_i is the proportion of taxon i) was calculated (Simpson 1949).

Results

1. Chemical properties

VB contained lower concentrations of NO_3^-, PO_4^{3-}, and Ca^{2+}, but similar Mg^{2+} levels, and higher NH_4^+ and K^+ levels than CNM. In comparison with aVB, VB contained lower concentrations of Mg^{2+} and Ca^{2+}, but similar levels of NO_3^-, PO_4^{3-}, and K^+, and higher levels of NH_4^+. Both VB and aVB had higher pH levels than CNM (Table 1). Because of the lower concentrations of total nitrogen and phosphorus in VB than in CNM, leaf yellowing and slightly poorer growth were observed in the seedlings grown in VB (Fig. 1B).

VB aVB CNM

Fig. 1. Suppressive effects of VB on damping-off of cucumber caused by *Pythium aphanidermatum* under greenhouse conditions.
(A) Damping-off appeared in VB five days after inoculation and transplanting. aVB and CNM (Aisai-1, Katakura Chikkarin Co., Ltd.) were used for comparison. (B) The cucumber seedlings' growth in the noninoculated controls including VB, aVB, and CNM in 10 days after transplanting.

Table 1. Comparison of chemical properties of vermicomposted-bamboo powder (VB), the autoclaved VB (aVB), and a commercial nursery medium (CNM)

Parameters	VB	aVB	CNM
NO_3^- (mg/g)	0.6±0.12 b	0.3±0.05 b	5.5± 0.45 a
NH_4^+ (mg/g)	0.7±0.08 a	0.2±0.00 b	0.2± 0.00 b
PO_4^{3-} (mg/g)	2.7±0.14 b	3.3±0.27 ab	4.6± 0.48 a
K^+ (mg/g)	34.0±2.66 a	36.8±0.27 a	0.8± 0.07 b
Mg^{2+} (mg/kg)	6.6±0.80 b	12.2±0.67 a	7.3± 0.29 b
Ca^{2+} (mg/kg)	111.0±6.06 c	420.0±4.47 a	282.0±11.88 b
pH	7.8±0.20 a	7.7±0.01 a	5.6± 0.03 b

Data are mean ± standard error (N = 3). Different letters indicate significant difference according to Tukey's HSD test ($P < 0.05$).

2. Suppressive effects of VB against cucumber damping-off

The damping-off caused by isolates of *P. aphanidermatum*, *P. ultimum* var. *ultimum*, and *R. solani* AG1-IB was significantly ($P < 0.05$) suppressed by VB as compared to CNM (Fig. 1A). After 15 days of inoculation, the damping-off severity of the three pathogens was reduced by 41%, 26%, and 47% in VB, as compared to CNM, respectively (Fig. 2). The disease suppressiveness appeared starting from five and ten days after the inoculation (Fig. 2). This effect was lost when VB was autoclaved. All pathogens were reisolated from the diseased seedlings by *Pythium*-selective NARM (nystatin + ampicillin + rifampicin + miconazole) medium (Morita & Tojo 2007) for the isolates of *P. aphanidermatum* and *P. ultimum* var. *ultimum* or 1.5% water agar for the *R. solani* AG1-IB isolate.

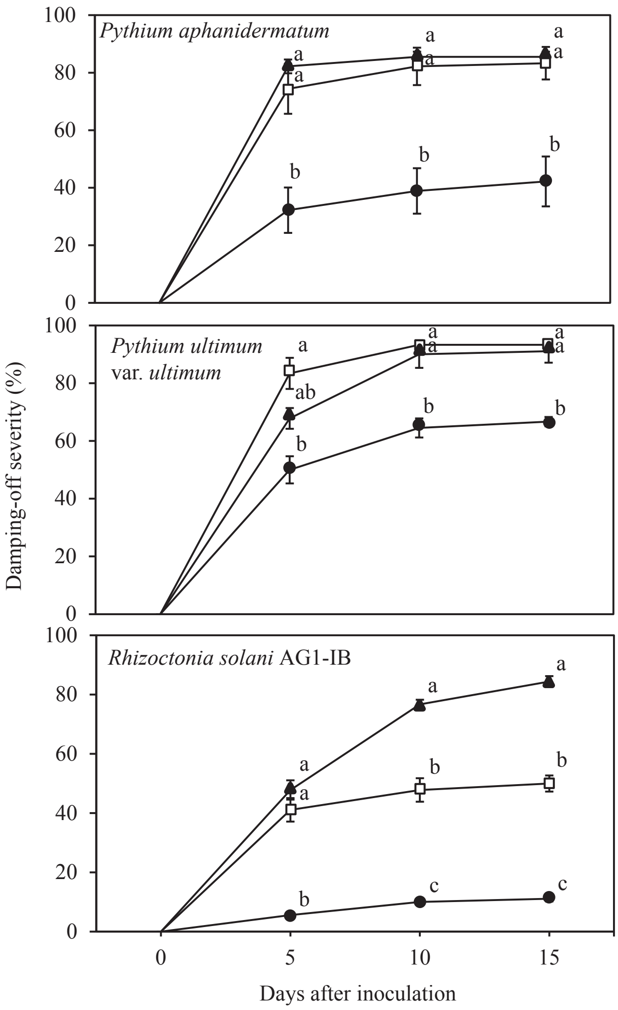

Fig. 2. The effect of VB on the damping-off severity of cucumber, caused by (A) *Pythium aphanidermatum*, (B) *P. ultimum* var. *ultimum*, and (C) *Rhizoctonia solani* AG1-IB under greenhouse conditions.
aVB and CNM (Aisai-1, Katakura Chikkarin Co., Ltd.) were used for comparison. VB (●), aVB (▲), and CNM (□). Bars indicate standard error ($N = 3$). Treatments with different letters indicate a significant difference at the 0.05 level, according to Tukey's HSD test ($P < 0.05$).

3. Microbial activity, populations, and diversity

The microbial activity and populations estimated by FDA hydrolysis and the soil plating technique were significantly ($P < 0.05$) higher in VB than in aVB and CNM (Table 2). The NGS analysis revealed that the bacterial communities in VB had significantly higher Simpson's diversity index than those in aVB and CNM did. Although no significant difference was observed in OTU richness and Shannon's diversity index among them, the average values of VB were higher than those of aVB and CNM (Table 3). The highest number and the most even distribution of bacterial genera were observed in VB (Fig. 3).

Discussion

In this study, we demonstrated that the vermicompost produced from moso bamboos had potential to suppress cucumber damping-off pathogens, including *P. aphanidermatum*, *P. ultimum* var. *ultimum*, and *R. solani* AG1-IB, under greenhouse conditions. The suppressiveness of VB on these pathogens is thought to be mainly due to living microorganisms, because the effect was nullified by the autoclave treatment. The highest ammonia concentration is probably also one of the factors of the suppression of VB to *Pythium*, because the suppression of *Pythium* damping-off is related to ammonia volatilization of composts (Scheuerell et al. 2005). As far

Table 2. Comparison of the activity and population of microbes among the vermicomposted-bamboo powder (VB), the autoclaved VB (aVB), and a commercial nursery medium (CNM)

	VB	aVB	CNM
FDA hydrolysis (µg/0.5 h)	8.2 a	4.9 b	1.7 c
Bacteria (cfu)	6.2×10^{10} a	—	1.5×10^{9} b
Fungi (cfu)	2.7×10^{6} a	—	4.0×10^{4} b

Means ($N = 4$) with different letters within a row indicate significant difference ($P < 0.05$) based on Tukey's HSD test for FDA hydrolysis and Student's *t*-test for the number of bacteria and fungi. The number of bacteria and fungi in VB and CNM were determined by a plate count technique using media described by Waksman & Fred (1922) and Martin (1950), respectively. Microbial activity was measured by FDA hydrolysis described by Adam & Duncan (2001). "—" no data.

Table 3. Comparison of the estimated operational taxonomic units (OTUs) richness, Shannon's and Simpson's diversity index of the 16S rRNA libraries for clustering at 97% identity, as obtained from the pyrosequencing analysis

	VB	aVB	CNM
OTUs richness	303±73 a	225±35 a	116±41 a
Shannon diversity index	6.2±0.3 a	5.3±0.7 a	4.3±0.2 a
Simpson's diversity index	0.96±0.01a	0.86±0.06 b	0.84±0.05 b

Data are mean ± standard error (N = 3). Different letters indicate significant difference according to Tukey's HSD test (P < 0.05).

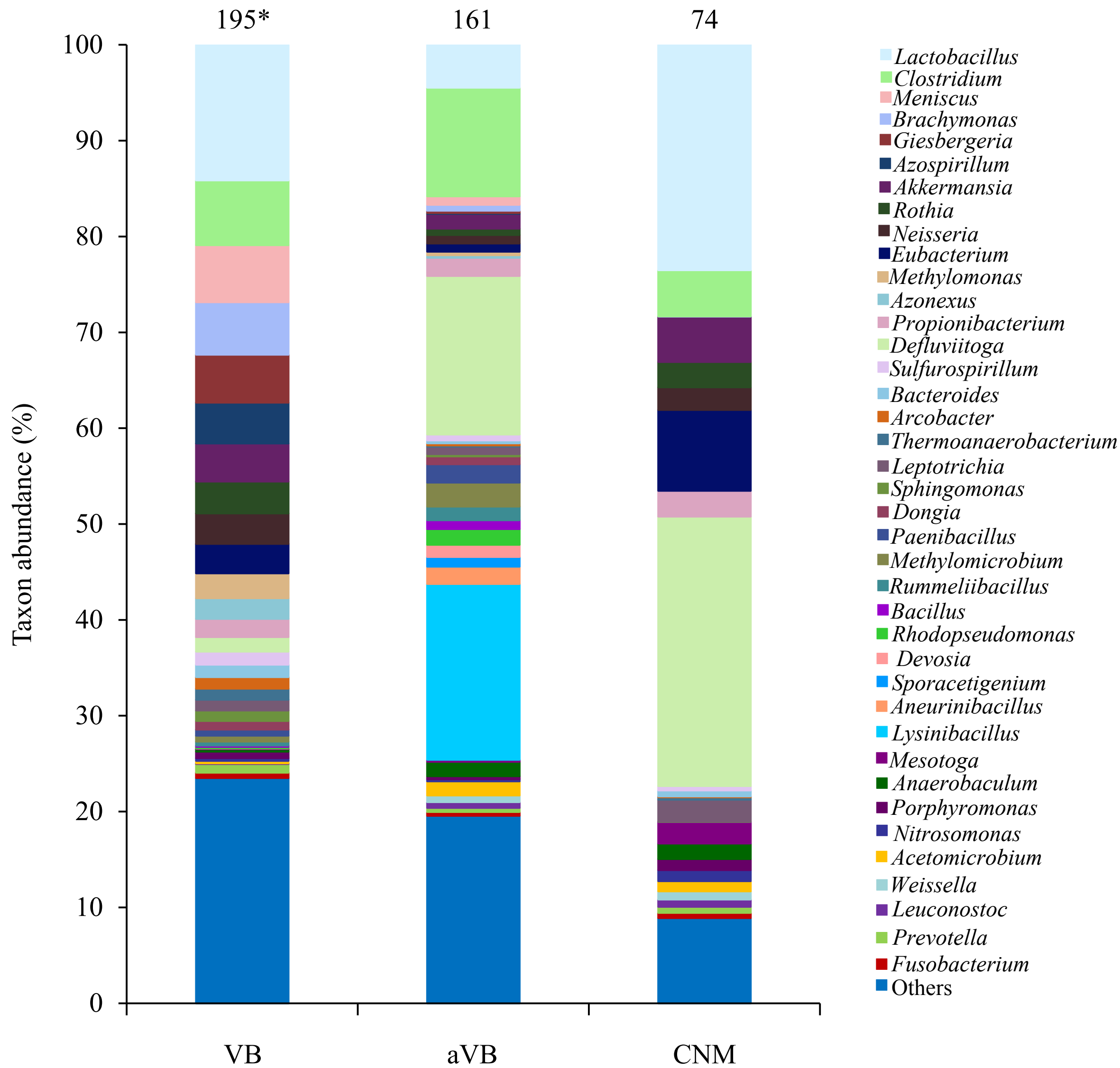

Fig. 3. The bacterial genera detected in VB, aVB, and CNM.

The others were *Methylocystis* spp. in VB, *Leptotrichia* spp. in aVB, and *Lautropia* spp. in CNM. aVB was prepared by autoclaving the VB at 121°C for 1 h and then kept in a laboratory room for one day. *Total number of the bacterial genera detected.

as we are concerned, this is the first report showing the suppressive effects of VB on plant diseases.

Microbial populations were more abundant, diverse, and even in VB than in aVB and CNM. Many soil-borne pathogens can grow saprophytically in the rhizosphere to reach and infect their host, and their success in the colonization of roots is influenced by competition with the native rhizosphere microbiome (Bakker et al. 2012, Chaparro et al. 2012). Thus, the suppressive effects of VB should be attributed to the higher activity, population, and diversity of the rhizosphere microbiome, which limits the growth and activity of soil-borne pathogens, and consequently plant diseases. This agrees with several previous studies demonstrating that the total microbial activity and diversity of soils or composts are highly positively correlated with *Pythium* spp. and *Rhizoctonia solani* suppression (Craft & Nelson 1996, Ghini & Morandi 2006). The suppressive effects of VB on *Rhizoctonia solani* AG1-IB were more significant than of the two *Pythium* species. This suggests that microbial populations in VB associated with the suppressive effects are different between *Rhizoctonia solani* AG1-IB and the *Pythium* species.

The present results also demonstrated that VB had more valuable characteristics with respect to equivalent or higher inorganic nutrients than CNM did. Leaf yellowing and slightly poorer growth were observed in the seedlings grown in VB, as compared with those grown in CNM. However, the amendments of organic fertilizers, such as oil cakes, resolved these problems without resulting in a loss of disease suppressiveness of VB (You unpublished). The pH of the VB was 7.8 ± 0.20, which is higher than that of CNM but is an acceptable range for cucumber growth (Old Farmer's Almanac 2016).

Bamboos grow vigorously in the vicinity of populated areas in Japan and cause substantial damage to agricultural production and rural ecosystems (Isagi et al. 1997, Imaji et al. 2013, Kajisa et al. 2011). The resent results suggested that the waste product moso bamboo can be recycled as an agricultural material through its powdering and vermicomposting.

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References

Abe, M. et al. (2007) Earthworm avoidance test for evaluation of environmental hazard of landfill leachate. *Environ. Sci.,* **20**, 503-510.

Adam, G. & Duncan, H. (2001) Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.*, **33**, 943-951.

Atiyeh, R. M. et al. (2000) Changes in biochemical properties of cow manure during processing by earthworms (*Eisenia andrei*, Bouché) and the effects on seedling growth. *Pedobiologia*, **44**, 709-724.

Bakker, M. G. et al. (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant Soil*, **360**, 1-13.

Chaoui, H. et al. (2002) Suppression of the plant diseases, *Pythium* (damping-off), *Rhizoctonia* (root rot) and *Verticillium* (wilt) by vermicomposts. *Brighton Crop Prot. Conf.−Pests Dis.*, *Proc.* **8**, 711-716.

Chaparro, J. M. et al. (2012) Manipulating the soil microbiome to increase soil health and plant fertility. *Biol. Fertil. Soils*, **48**, 489-499.

Chongtham, N. et al. (2011) Nutritional properties of bamboo shoots: potential and prospects for utilization as a health food. *Comp. Rev. Food Sci. Food Safety*, **10**, 153-168.

Craft, C. M. & Nelson E. B. (1996) Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. *Appl. Environ. Microbiol.,* **62**, 1550-1557.

Elvira, C. et al. (1998) Vermicomposting of sludges from paper mill and dairy industries with *Eisenia andrei*: A pilot-scale study. *Bioresour. Technol.,* **63**, 205-211.

FAO (2010) Global Forest Resource Assessment 2010. Rome, Italy: Food and Agriculture Organization of the United Nations. http://www.fao.org/docrep/013/i1757e/i1757e.pdf

Garg, P. et al. (2006) Vermicomposting of different types of waste using *Eisenia foetida*: A comparative study. *Bioresour. Technol.,* **97**, 391-395.

Ghini, R. & Morandi, M. A. B. (2006) Biotic and abiotic factors associated with soil suppressiveness to *Rhizoctonia solani*. *Sci. Agric.,* **63**, 153-160.

Huang et al. (2013) Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by *Eisenia foetida*. *Bioresour. Technol.*, **150**, 235-241.

Imaji, A. et al. (2013) Effects of bamboo colonization on water relations of Japanese cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*). *Nippon Shinrin-gakkaishi (J. Jpn. For. Soc.),* **95**, 145-146 [In Japanese with English Summary].

Isagi, Y. et al. (1997) Net production and carbon cycling in a bamboo *Phyllostachys pubescens* stand. *Plant Ecol.* **130**, 41-52.

Kajisa, T. et al. (2011) Situation of erosion, landslide, and limiting factors of vegetation recovery on abandoned clear-cut sites in Kyushu region. *Nippon Shinrin-gakkaishi (J. Jpn. For. Soc.),* **93**, 288-293 [In Japanese with English Summary].

Martin, J. P. (1950) Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.,* **69**, 215-232.

Morita, Y. & Tojo, M. (2007) Modifications of PARP medium using fluazinam, miconazole, and nystatin for detection of *Pythium* spp. in soil. *Plant Dis.,* **91**, 1591-1599.

Okano, R. et al. (2016) Microflora analysis of plug flow reaction of high temperature dry methane fermentation. *The 28th Annual Conference of Japan Society of Material Cycles and Waste Management*. B8-8P [In Japanese with English Summary].

Old Farmer's Almanac (2016) "Cucumbers: Planting, growing, and harvesting cucumbers." Yankee Publishing, Inc., Dublin, NH. http://homeguides.sfgate.com/soil-ph-cucumbers-73822.html

Roberts, B. L. & Dorough, H. W. (1984) Relative toxicities of chemicals to the earthworm *Eisenia foetida*. *Environ. Toxicol. Chem*., **3**, 67-78.

Scheuerell, S. J. et al. (2005) Suppression of seedling damping-off caused by *Pythium ultimum*, *P. irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of Pacific Northwest compost sources. *Phytopathology*, **95**, 306-315.

Shannon, C. (1948) A mathematical theory of communication. *Bell Syst. Tech. J.,* **27**, 379-423.

Simpson, E. H. (1949) Measurement of diversity. *Nature,* **163**, 688.

Szczech, M. & Smolińska, U. (2001) Comparison of suppressiveness of vermicomposts produced from animal manures and sewage sludge against *Phytophthora nicotianae* Breda de Haan var. *nicotianae*. *J. Phytopathol.* **149**, 77-82.

Tojo, M. (1993) Preparation of Pythium inocula using bentgrass seeds for glasshouse studies. *Proc. Kansai Pl. Prot.,* **35**, 1-5 [In Japanese with English Summary].

Waksman, S. A. & Fred, E. B. (1922) A tentative outline of the plate method for determining the number of microorganisms in the soil. *Soil Sci.,* **14**, 27-28.