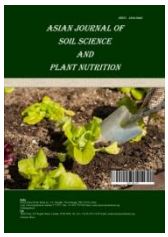


Asian Journal of Soil Science and Plant Nutrition

7(4): 63-75, 2021; Article no.AJSSPN.73886
ISSN: 2456-9682

Function and Characterization of Fungal Communities in Chestnut Soils (*Castanea crenata)* of Kansai Region, Japan

Hosne Ara Dilzahan[1*], Atsushi Okamura[2], Michelle Ann Calubaquib[3], Nolissa Delmo Organo[3], Masahide Kobayashi[4] and Andre Freire Cruz[1]

[1]Kyoto Prefectural University, Graduate School of Life and Environmental Sciences, Kyoto, Japan.
[2]Kyoto Prefectural University, Faculty of Agriculture and Life Sciences, Kyoto, Japan.
[3]University of the Philippines, Los Banos, Laguna, Philippines.
[4]Kyoto Prefectural Forestry Research Center, Kyotamba, Kyoto, Japan.

Authors' contributions

This work was carried out in collaboration among all authors. Author MK did the conceptualized. Data acquisition done by author HAD. Data analysis done by authors AO, MAC and NDO. Design of methodology done by authors AFC and HAD. Authors AFC and HAD writing and editing the paper. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJSSPN/2021/v8i130123
Editor(s):
(1) Dr. Pankaj Kumar, H.N.B.Garhwal Central University, India.
Reviewers:
(1) Cleide Barbieri de Souza, Lusíada University Center, Brasil.
(2) Suresh. G. Borkar,. Dr. Borkar's Diagnostic laboratory and Research Centre, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73886

Original Research Article

Received 08 July 2021
Accepted 18 September 2021
Published 23 September 2021

ABSTRACT

Chestnut (*Castanea crenata*) is an important fruit crop in Japan, grown under three cultivation systems in Kansai region, which succumb to fungal root disease pathogens. The fungal community in soils of chestnut in these cultivation systems were characterized along with the potential of soil bacterial species as biological control agent against these root-invading fungi. Bacteria from the chestnut soil rhizosphere were identified and their ability to suppress diseases *in vitro* was evaluated. Bacteria DAC17225011 and DAC17225014 showed 99% similarity to *Bacillus aryabhattai* and *Pseudomonas frederiksbergensis,* respectively, which could suppress the growth of *Armilaria mellea* and *Phytophtora cambivora,* respectively, *in vitro* conditions. The assay *in vivo* indicated the positive effect of these bacteria on the reduction of disease infection spots in chestnut roots; however, no visible symptoms were detected aboveground. For microbial community

**Corresponding author: Email: dilzahan@yahoo.com;*

analysis, chestnut soil was sampled from four locations (Wachi, Ayabe, Fukuchiyama and Sasayama) considering three management systems, conventional, organic and wild. The amplicon from the ITS region (The genomic library of the fungal detection in soils) was sequenced by Illumina MiSeq 250bp and used to analyze the fungal community in the sampled soil. *Nectriaceae*, which contains pathogenic fungi, was very common in all samples, but lower in wild areas. *Ceratobasidiacea* was also higher in conventional areas. For the symbiotic families, *Hypocraceae* and *Russulaceae* were typical in wild soils, whereas *Amanitaceae* was found in organic soils. The fungal community was clearly distinct in the wild system, differing from conventional and organic systems.

Keywords: Fungal diversity; rhizosphere soil; chestnut crop; biocontrol activity; amplicon sequencing; fingerprints.

1. INTRODUCTION

Chestnuts are native to Japan and the Korean peninsula, where they have been cultivated since the 11th century and used for sweet production. Chestnut is not only used for nut and wood production, but is also a good contributor to carbon sequestration [1,2]. The chestnut landscape is a good source of social welfare [3]. Ink disease of chestnut caused by the oomycete *P. cambivora* is a soil-borne pathogen with worldwide distribution. It causes the majority of disease problems in chestnuts and limits the yield in a large number of stands. This lethal disease may inhibit the establishment of new groves and threatens the survival of trees. Root rot of chestnut caused by *A. mellea* is found in a broad variety of host species, including chestnuts in natural forests [4]. This pathogen primarily attacks the root and lower trunk, while the top of the tree shows symptoms of wilting and, in severe cases, death. Several ectomycorrhizal fungi can exert a protective effect against root pathogens and thus can be used as biological control agents, as an alternative to or integrated with the application of fungicides or bacterial strains [5]. Some bacterial strains are already marketed for the biological control of plant pathogens [6].

In the modern world, agricultural productivity has increased due to improved fertilization and application of chemical pesticides, irrigation, soil management, and massive land conversions [7]. There is increasing concern that these agricultural practices lead to large-scale ecosystem degradation in the long term and loss of productivity. Effects of agricultural management on the soil microbiome are complex and diverse, and retrieving universally valid conclusions on organic and conventional farming systems is difficult (Nelson and Spaner, 2010). It has been stated that in low-input farming systems, the abundance and diversity of soil microorganisms can be greater [8]. Moreover, soil enzyme activities, nutrients, bacterial richness and diversity could be improved by organic management, as well as by enriching major bacterial lineages that contribute to nutrient (C, N, S, and P) cycling [9].

Novel high-throughput DNA sequencing technologies help us to explore the soil microbial community in details [10]. Analyses of soil fungal diversity could provide insight into soil development processes and microbial dynamics for better soil management, which in turn improves Chestnut production in the world as well as Japan. When exploring soil health status, it is important to know the microbial community of soil, so that it is possible to increase chestnut production to meet the present demand.

In this research, the objectives were to evaluate the antagonism of soil bacteria in two main diseases of chestnut, Ink disease and root rot. Moreover, the differences in the microbial community of chestnut soil according to the agricultural systems (organic, conventional and wild) from four locations of the Kansai region were verified.

2. MATERIALS AND METHODS

2.1 Use of Rhizosphere Bacteria for Biocontrol of Ink and Root Rot Pathogens

Collection of Soil Samples and Bacterial Isolation: Rhizospheric soil samples of chestnut were collected from Kyoto. The serial soil dilution method was used to isolate biocontrol bacteria from chestnut soil, in accordance with Somasegaran and Hoben [11]. The bacterial isolates were transferred, re-cultured and

multiplied in PDA medium from single colonies. The isolates were preserved with 20% Glycerol in a -80˚C freezer.

Collection of disease-causing agents of Chestnut: For this assay, *P. cambivora* (NBRC30471) and *A. mellea* (NBRC7037) were obtained from the NBRC (National Biological Resource Center) in Japan and then multiplied in PDA and Malt Extract Agar medium, respectively.

***In vitro* test:** The bacterial isolates were tested by the dual culture method in laboratory conditions. Two disks of pathogens (*P. cambivora* and *A. mellea)* of chestnut were put in the middle of the PDA plate. In the same plate, two paper discs soaked with biocontrol bacteria (6 mm) were put on the periphery. The evaluation of antagonism was done by visual diagnosis (fungal growth suppressed by bacteria), comparing with the controls.

Bacterial Identification Using 16S rDNA Gene Sequencing: The antagonistic bacterial activity against root disease pathogen under *in vitro* tests was identified molecularly. DNA was extracted by Isoplant kit (Nippongene Co., Japan). PCR was done to amplify the 16S rDNA region using universal primers (27f and 1492r) [12]. These amplified products were submitted to Sanger sequencing at Macrogen Inc., Kyoto, Japan. Useable sequences were compared with the existing sequence data in BLAST search. These sequences were submitted to the DNA Data Bank of Japan (https://www.ddbj.nig.ac.jp/index-e.html). Then the accession numbers were allocated.

2.2 *In-vivo* Test of the Biocontrol Bacteria Against Ink and Root Rot Pathogens

Pot experiment of chestnut: Chestnut seed was stored at 10 °C in peat moss soil bought from a supermarket. The seed of the chestnut cultivar named 'Sukuba' was sown in the tray. The seed was sown with the radical facing down. In some cases, the radical was not present, so it was planted with the flat side of the seed facing down. After 40 days, the seedlings were transplanted into the 12 L plastic pot containing peat, perlite and vermiculite (PPV) mixture 1:1:1 proportion. One seedling per pot was transplanted.

Inoculation of ink disease and root rot pathogens in chestnut: Rice Bran Medium (vermiculite and rice bran 10:3) was used to inoculate both *P. cambivora* and *A. mellea.* For inoculation, chestnut plants were taken out of the pot and their roots were cut about 30%. Then the rice bran medium with mycelia of the pathogen was mixed with the peat, perlite and vermiculite (PPV) mixture from the chestnut pot. The ratio of the mixture was 1: 10 (inoculated rice bran medium: PPV). After that, the chestnut plants were placed back.

Treatments for chestnut pot experiment: The biocontrol bacteria were tested along with AMF (Arbuscular Mycorrhizal Fungi). The treatments for ink disease were DAC17225011 (the most powerful bacteria found in an *in-vitro* test against *Phytopthora cambivora*); AMF; AMF+DAC17225011; Ink disease control. The treatments for Root Rot disease were DAC17225014 (the most powerful bacteria found inan *in-vitro* test against *Armillaria mellea*), AMF, AMF + DAC17225014, Root Rot disease control. One Healthy control was used as reference and all treatments were replicated 3 times. Chestnut roots were observed under a microscope to confirm the roots were infected or not with our inoculated pathogens. For this, the roots were stained by using a simple blue staining technique [13].

2.3 Evaluation of Microbial Community in Chestnut Orchard

Soil Sampling: Chestnut soil samples were collected from four chestnut fields located in Wachi, Ayabe, Fukuchiyama and Sasayama in Kansai region, Japan, where three types of systems were visited at each site (conventional, organic and wild). Four chestnut soil samples were taken from each site, composing 48 experimental units. Chestnut soil was taken from a depth of 10 cm. From the samples, some portion of the soil was air-dried and submitted to Tokachi Agricultural Union Federation, Nogyo Kyodo, Hokkaido, for chemical analysis.

DNA extraction and ITS (Internal transcribed spacer) library preparation: From the sampled soil, DNA was extracted by extraction buffer method [14] including purification by Promega PCR purification Kit (Promega Co., USA). The DNA concentration was measured with Nanodrop 2000 Spectrophotometer (Thermofisher Scientific Co. Japan). First, the amplicon PCR was performed to amplify the specific region of interest primers with overhang adapters attached. Our target was the ITS1 region. The forward primer sequence was ITS1

(5' TCCGTAGGTGAACCTGCGG) and the reverse was ITS2 (5' GCTGCGTTCTTCATCGATGC) [15] with the overhang adapter added (Illumina Co., USA). The amplicon PCR reaction was performed using 1ul DNA template, 10ul KOD FX Neo buffer (Toyobo, Co., Japan), 0.4ul KOD FX Neo (Toyobo, Co., Japan), 2ul of ITS1 (Final concentration of 0.5uM), 2ul of ITS2 (Final concentration of 0.5uM), 4ul dNTPs and 0.6ul DW. The thermal cycling program composed of 94˚C for 2 min followed by 30 cycles of 98˚C for 10 s, 55 ˚C for 30 s, 68 ˚C for 1 min. The amplicon PCR products were cleaned up using the Promega kit. The second one was index PCR. The PCR reaction was same as above with the Nextera XT Index Primers at final concentration of 0.5uM.

The library construction (Index PCR) was performed using the similar reagents of amplicon PCR and the Nextera XT Index Primers (Illumina. Co. USA). The reaction was performed under the following conditions: 94˚C for 2 min followed by 12 cycles of 98˚C for 10 s, 55˚C for 30 s and 68˚C for 1 min. The libraries were submitted to Genome Quebec, Canada for next generation sequencing using Illumina MiSeq 250bp. The analysis of the amplicon sequencing data was performed using the Qiime2-2019 pipeline [16]. From this, 2,458,248 sequences were obtained with an average of 47,774 sequences per sample, which were paired-end joined and filtered through a quality check and chimera check using DADA2 [17]. Sequences were deposited in the DDBJ (DNA Data Bank of Japan), registration number DRA012582. A taxonomic analysis was performed using the QIIME2 classifier from the UNITE Community database; the relative abundance (RA) of fungi at family level was calculated, and the most important ones (pathogenic and symbiotic) were selected for the analysis of variance. The Principal Coordinate Analysis (PCoA) was performed with the RA and used to plot the individuals and to verify the difference among the systems.

3. RESULTS

In *in vitro* test: In the paper disc method, the growth of *P. cambivora* was restricted by bacterial isolate DAC17225011 compared to control treatment (water) at 3 days of dual contact (Fig. 1a). A clear inhibition zone was found in PDA on the dish. The radial growth of *A. mellea* was significantly inhibited by bacterial isolate DAC17225014 compared to H_2O control treatment (Fig. 1b).

Identification of biocontrol bacteria from chestnut soil: About 40 bacteria were isolated from rhizospheric chestnut soil. Among these bacteria, the top five that showed biocontrol properties in dual culture method were identified molecularly. This *in vitro* assay was evaluated by the observation of the clear inhibition zone around the bacteria that indicated suppression of fungal growth. According to blast search, these bacteria were 99% similar to *Bacillus aryabhattai* and *Pseudomonas frederiksbergensis*, and received their appropriate codes DAC17225011 and DAC17225014, respectively (Table 2). The sequences were deposited in the DNA Databank of Japan (https://www.ddbj.nig.ac.jp/index-e.html).

Microscopic observation of chestnut root: We could not see any visible infection symptom in chestnut shoot, and neither of the treatments had a significant effect on plant biomass. However, infection spots were observed under the microscope in chestnut roots. We observed many more spores of *P. cambivora* and *A. mellea* in the root of the diseased control plant. Chestnut root was moderately infected with AMF in the AMF-treated plant and AMF + *Armillaria*-treated plant (Table 3).

Soil physicochemical properties: Soil chemical characteristics are listed in Table 4, and these results showed that the pH value, effective phosphoric acid and amount of humus were higher in the organic cultivation system.

Description of fungal community: In total, 2,458,248 ITS sequences were analyzed from all soil samples. PCoA graphs (Unweighted UniFrac) point out that the overall data had a significant separation between the OTUs (operational taxonomic unit) from the wild area and those from conventional and organic areas (Fig. 2). Most of the classifiable sequences were allied to 40 classes across the entire data set. A comparison of ITS rRNA profiles at the family level, considering those which contain pathogenic fungi, according to the literature, and symbiotic fungi, indicated that the composition of fungal communities was different between locations and systems. Only a few of these RA have shown significant effects ($p<0.05$; $p<0.01$) from location and systems (Table 1). The Chestnut soils mostly contained the *Nectriaceae* as a common family in all soils, with low

abundance in wild areas. Furthermore, the *Ceratobasidiaceae* was very typical in conventional areas and *Plectosphaerellaceae* in organic ones (Fig. 3A). The symbiotic group showed that *Hymenogastraceae* and *Russulaceae* are fingerprints for wild plots, whereas *Boletaceae* and *Amanitaceae* were strongly present in organic and conventional areas. The *Glomeraceae*, which contains the arbuscular mycorrhizal fungi, could be mostly found in organic soils.

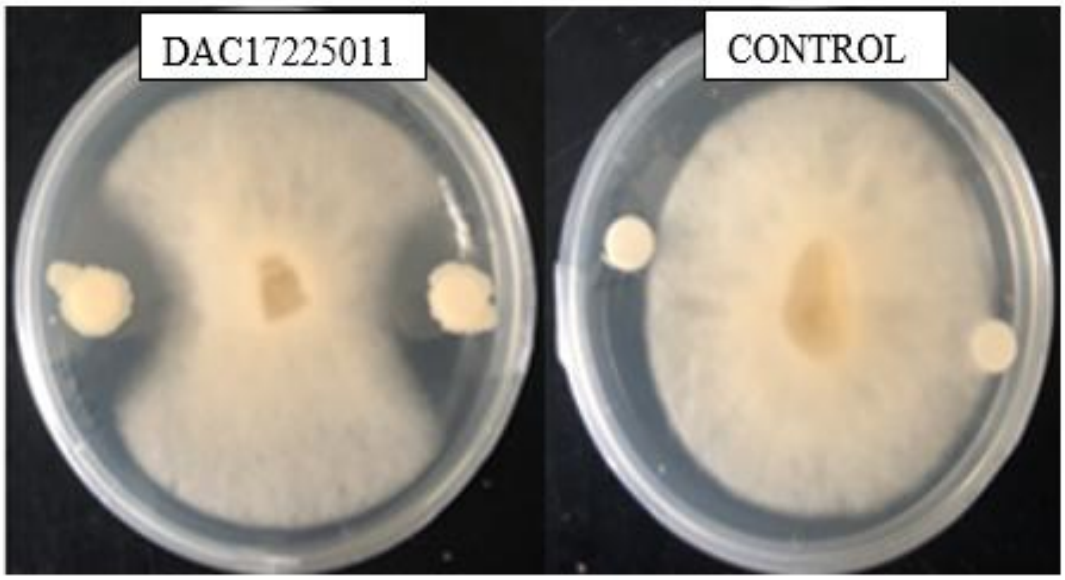


Fig. 1a. Effect of biocontrol bacteria on the inhibition of *Phytopthora cambivora* using paper disc method at 3 days after plating

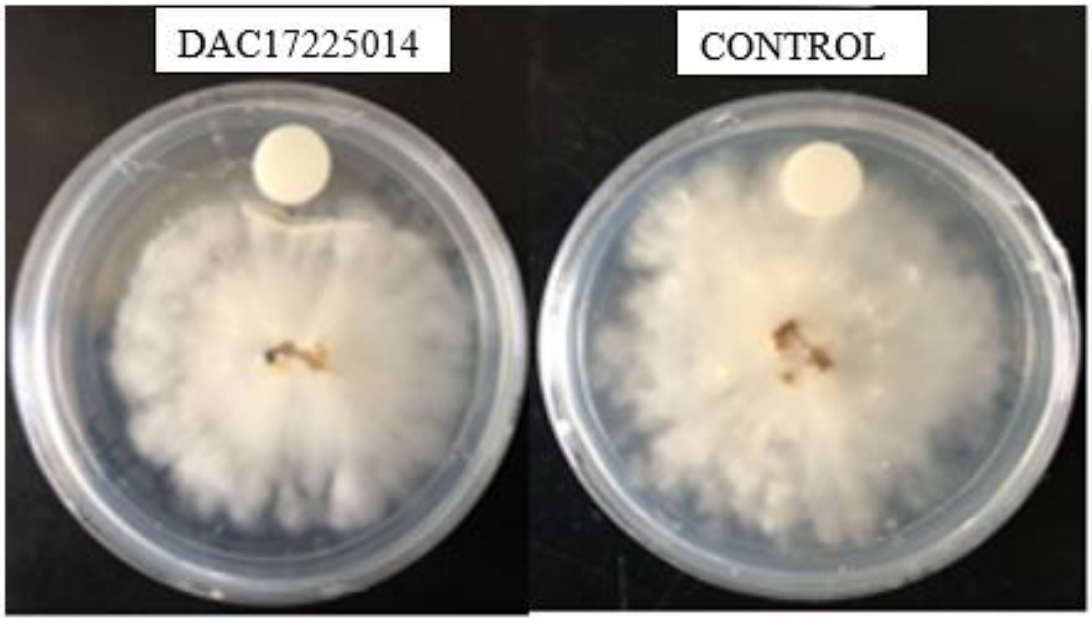


Fig. 1b. Effect of biocontrol bacteria on the inhibition of *Armillaria mellea* using paper disc method at 3 days after plating

Table 1. Analysis of variance regarding selected fungal families detected in chestnut soils

Group	Family	Location	System
Pathogenic	*Atheliaceae*	n.s.	n.s.
	Ceratobasidiaceae	n.s.	*
	Glomerellaceae	n.s.	n.s.
	Mucoraceae	n.s.	n.s.
	Nectriaceae	n.s.	**
	Physalacriaceae	n.s.	n.s.
	Plectosphaerellaceae	n.s.	**
	Sclerotiniaceae	**	*
	Trichocomaceae	n.s.	n.s.
	Xylariaceae	n.s.	n.s.
Symbiotic	*Acaulosporaceae*	n.s.	*
	Amanitaceae	n.s.	n.s.
	Bionectriaceae	*	**
	Boletaceae	n.s.	n.s.
	Claroideoglomeraceae	n.s.	n.s.
	Cortinariaceae	n.s.	n.s.
	Diversisporaceae	n.s.	n.s.
	Gigasporaceae	n.s.	n.s.
	Glomeraceae	*	n.s.
	Hymenogastraceae	n.s.	n.s.
	Hypocreaceae	n.s.	**
	Paraglomeraceae	n.s.	n.s.
	Russulaceae	n.s.	n.s.
	Sclerodermataceae	n.s.	n.s.

*n.s. – non-significant; * - Significant at 95%; ** - Significant at 99%*

Table 2. Molecular identification of the bacteria that functioned as a biocontrol of Chestnut pathogen

Code Locus	Species	Accession no	Similarity
DAC1722505	*Bacillus aryabhattai*	MH421842.1	99%
DAC1722507	*Bacillus aryabhattai*	KY855373.1	99%
DAC17225011	*Bacillus aryabhattai*	KY038668.1	99%
DAC17225014	*Pseudomonas frederiksbergensis*	KP407104.1	99%
DAC17225015	*Pseudomonas chlororaphis*	KJ831622.1	99%

Table 3. Infection level in chestnut root by pathogens and colonization level by arbuscular mycorrhizal fungi (AMF) according to their treatments

Treatments	AMF	*Phythopthora*	*Armillaria*
Control	N.A	N.A	N.A
AMF	++	N.A	N.A
AMF + Bact 1 (DAC17225014)	+	N.A	N.A
AMF + Bact 1 (DAC17225011)	+	N.A	N.A
Control + *Armillaria*	N.A.	N.A.	+++
AMF + *Armillaria*	+++	N.A.	+
Bact 1 (DAC17225014) + *Armillaria*	0	0	0
AMF + (DAC17225014) + *Armillaria*	++	N.A.	+
Control + *Phytopthora*	N.A.	++++	N.A.
AMF + *Phytopthora*	++	+	N.A.
Bact 1 (DAC17225011) + *Phytopthora*	N.A.	++	N.A.
AMF + Bact 1 (DAC17225011) + *Phytopthora*	++	+	0

0 – No infection/colonization; + - Slight infection/colonization (≥2 spots); ++ - Moderate infection/colonization (3-5 spots); +++ - High infection/colonization (6-8 spots); ++++ - Extremely high infection/colonization (≤8 spots); N.A. – Not applicable

Table 4. Results of chestnut soil chemical analysis

Manag.		Conventional				Organic				Wild			
Location		Ayabe	Fukuchiyama	Sasayama	Wachi	Ayabe	Fukuchiyama	Sasayama	Wachi	Ayabe	Fukuchiyama	Sasayama	Wachi
Soil pH	**-**	6.5	4.1	5.5	6.0	5.4	5.9	7	6.45	5.0	5.4	4.4	5.8
Bulk density	**g/cm3**	0.78	0.62	0.86	0.81	0.75	0.66	0.94	0.805	0.57	0.56	0.72	0.75
Electrical conductivity	**mS/cm**	0.47	0.20	0.29	0.22	0.14	0.16	0.14	0.225	0.18	0.18	0.07	0.25
Available phosphoric acid	**mg/100g**	211.6	89.3	66.7	117.6	50.2	106.7	125.8	274.1	5.9	112.7	6.6	48.2
Exchangable K	**mg/100g**	105.7	56.2	31.9	87.3	43.8	176.1	31.4	107.55	35.9	55.1	16.8	48.8
Exchangable MgO	**mg/100g**	106.3	14.6	23.7	44.6	63.5	127.4	51.4	56.05	56.6	39.6	9.3	51.1
Exchangable CaO	**mg/100g**	1053.6	65.0	381.3	510.3	455.2	1072.6	805.2	780.25	262.0	1028.2	47.8	448.8
MgO / K rate		2.3	0.6	1.7	1.2	3.4	1.7	3.8	1.3	3.7	1.7	1.3	2.4
CaO･MgO rate		7.1	3.2	11.5	8.2	5.2	6.1	11.3	10.75	3.3	18.6	3.7	6.3
MgO intensity	**%**	123.9	4.2	64.3	71.6	46.4	53.7	146.0	102.05	31.2	48.0	5.7	59.5
Base saturation	%	148.7	7.6	73.0	87.6	58.1	67.8	162.3	120.05	43.2	52.1	8.4	72.8
N-NO_3^-	%	7.86	7.88	10.57	7.92	4.85	5.08	2.89	6.475	0.36	6.90	0.81	3.64
N-NH_4^+	**mg/100g**	0.99	1.73	0.84	1.26	1.24	1.65	0.56	1.3	8.11	3.40	2.62	1.40
Total N	**%**	0.53	0.64	0.27	0.40	0.45	0.69	0.29	0.455	0.60	0.93	0.50	0.40
Total C	**%**	6.8	10.1	2.7	4.4	6.1	11.3	3.4	5.35	9.9	15.0	6.8	5.7
Humus amount	**%**	11.8	17.4	4.6	7.6	10.5	19.4	5.8	9.15	17.0	25.8	11.7	9.8
PO_4^- absorp. coef.	**-**	949	1592	1570	1640	1095	1646	1464	981	994	1696	911	1337
Base exch. capacity	**me/100g**	30.3	55.7	21.2	25.4	35.0	71.3	19.7	30.85	29.9	76.4	29.9	26.9

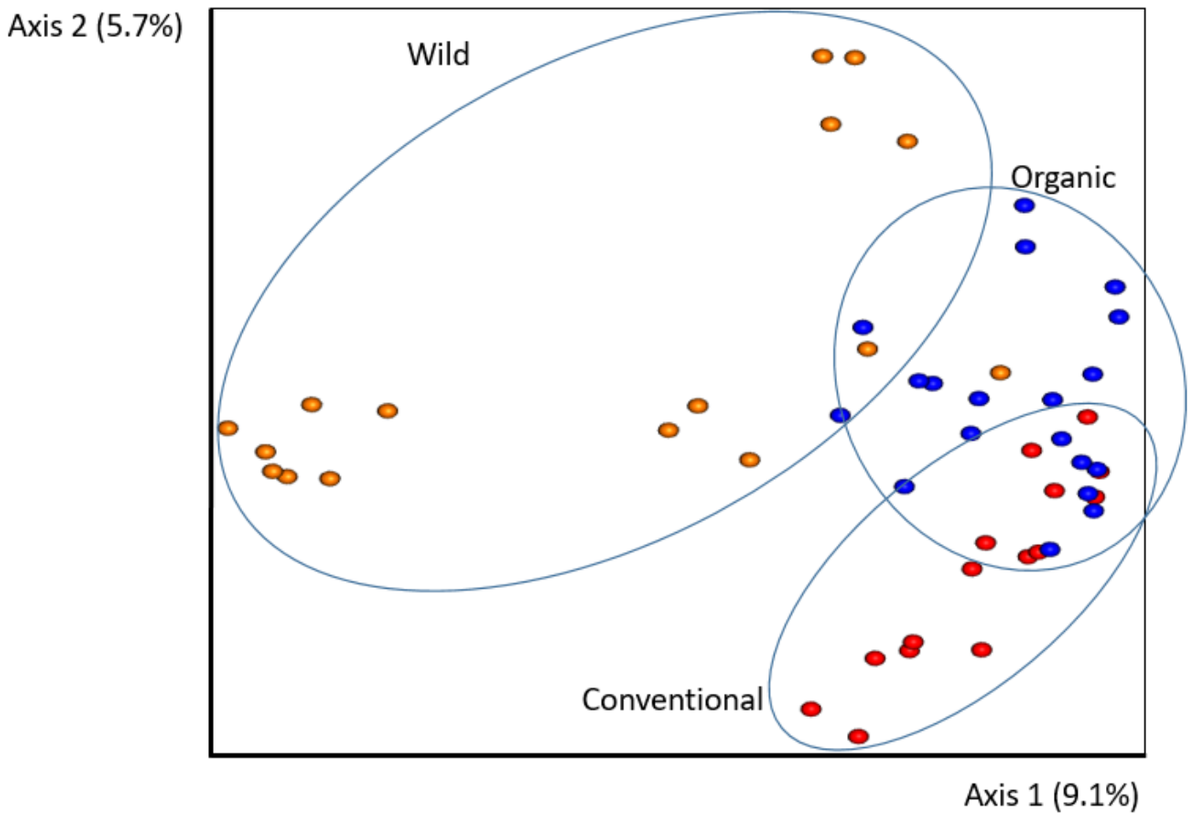
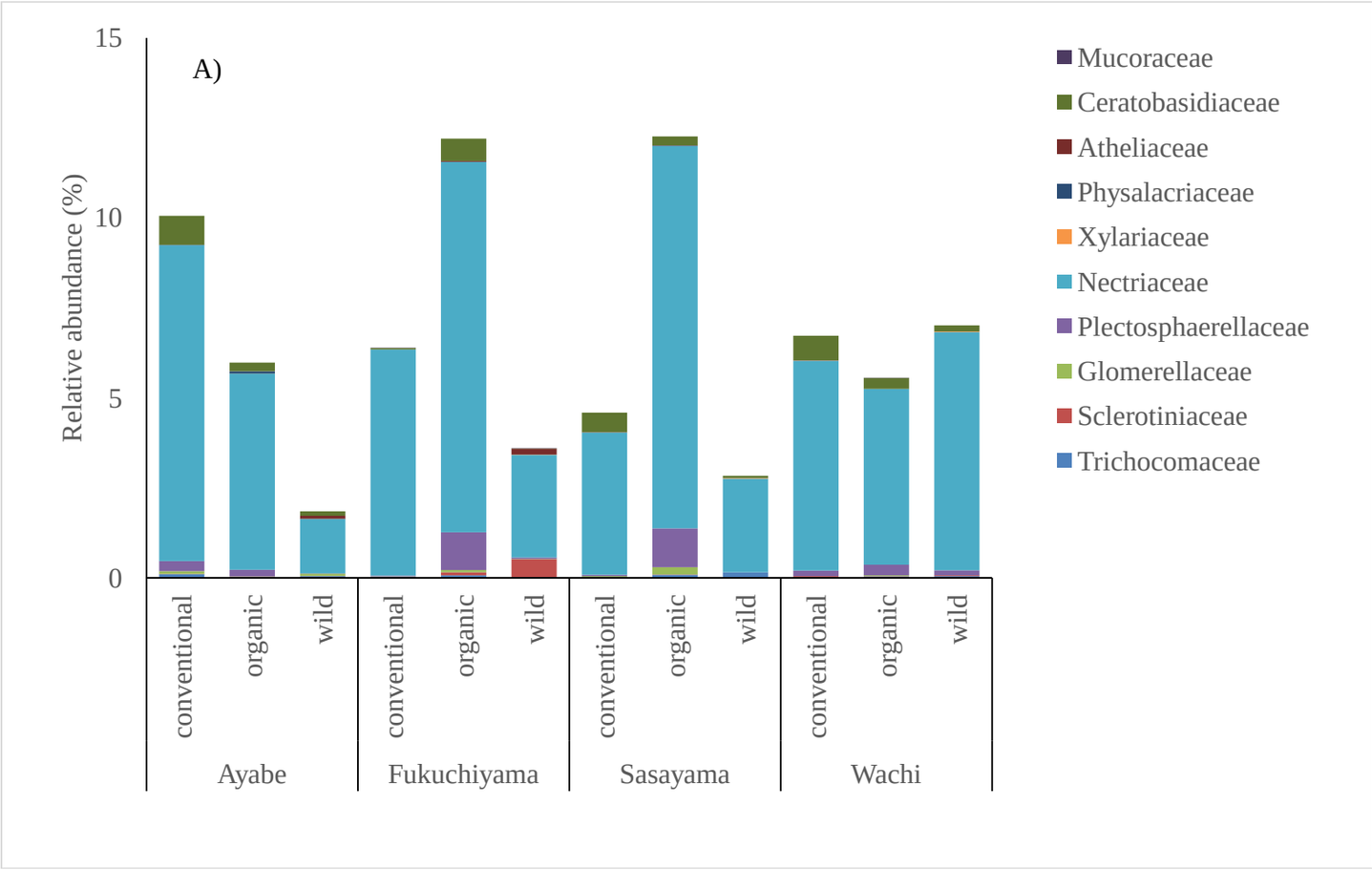


Fig. 2. Principal coordinate analysis (PCoA) of ITS rRNA genes in Chestnut orchards


A)
Relative abundance (%)
15
10
5
0
conventional
organic
wild
Ayabe
Fukuchiyama
Sasayama
Wachi
Mucoraceae
Ceratobasidiaceae
Atheliaceae
Physalacriaceae
Xylariaceae
Nectriaceae
Plectosphaerellaceae
Glomerellaceae
Sclerotiniaceae
Trichocomaceae

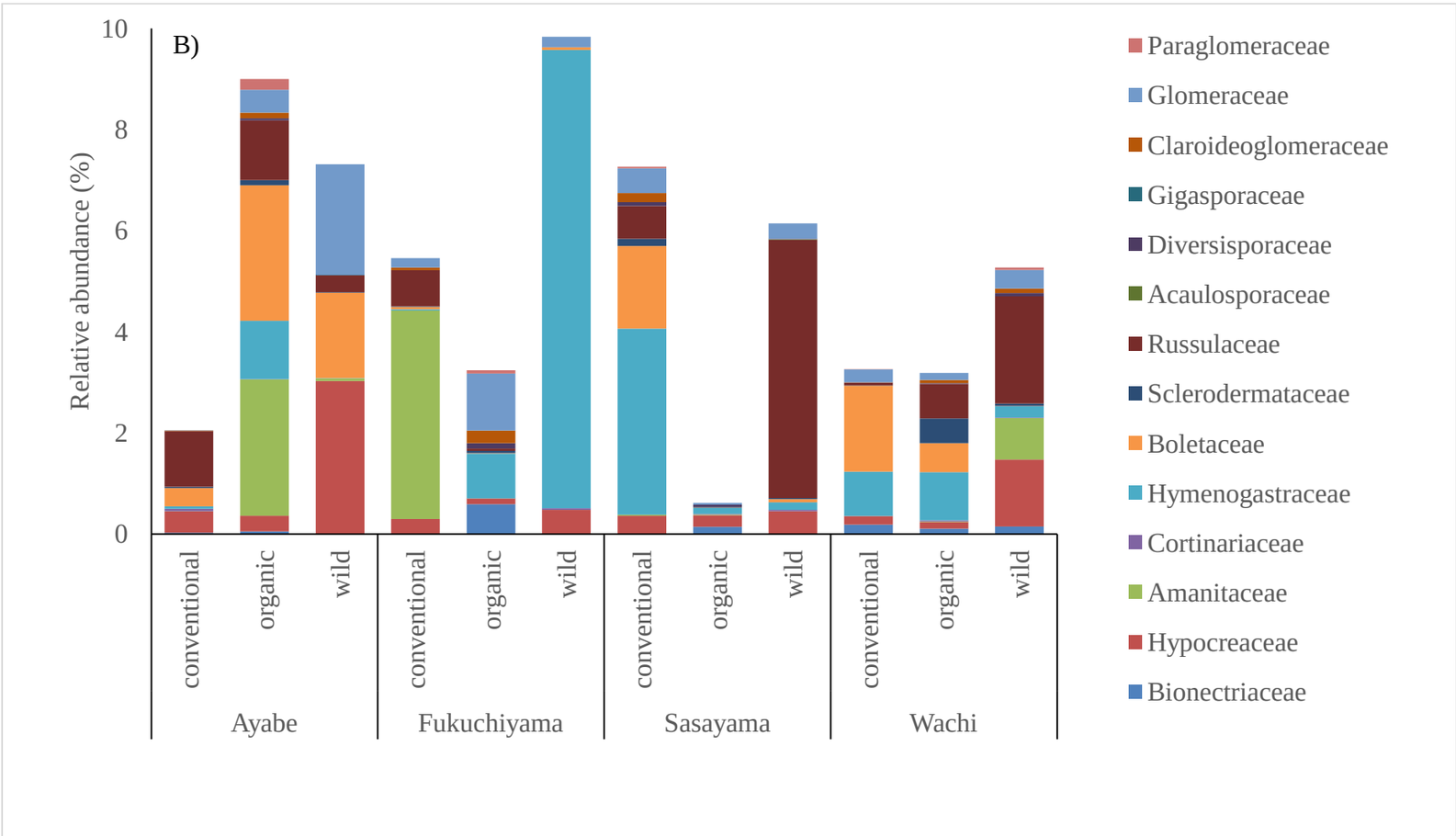


Fig. 3. Distribution of pathogenic (A) and symbiotic (B) fungal families among the Chestnut soils cultivated under conventional, organic and wild systems in four areas of Kansai region, Japan

4. DISCUSSION

The results of this study show that the isolated antagonistic bacterial strains DAC17225011 and DAC17225014 were able to suppress the growth of *P. cambivora* and *A. mellea* in *in vitro*. Earlier studies stated that endophytic and rhizospheric bacteria have significant antagonistic activity against the pathogenic fungus of chestnut [18]. The genus *Bacillus* has antagonistic properties that suppress various plant pathogenic bacteria [19]. The ability of these bacteria to inhibit the growth of pathogenic fungi suggest them as strong biocontrol agents. *Pseudomonas spp*. are ubiquitous bacteria in agricultural land, and can be suitable as biocontrol agents of soil-borne pathogens [20]. In the current research, molecular identification suggested that isolated bacterial strains DAC17225011 and DAC17225014 were 99% similar to *Bacillus* sp*.* and *Pseudomonas* sp*.* respectively, and demonstrated strong antagonism against Ink and root rot disease of chestnut.

In the *In vivo* experiment under greenhouse conditions, inoculated Chestnut seedlings did not show any visible symptom aboveground. However, spots (hyphae) observed in the stained roots were interpreted as signals of infection by *P. cambivora* and *A. mellea*. The number of infection spots in roots inoculated with these pathogens were higher than those co-inoculated with the antagonistic bacteria and/or arbuscular mycorrhizal fungi. These data suggest a similar effect of these antagonistic bacteria under *in vivo* conditions, but it might take time to see visible symptoms in the shoot, or the plants could develop resistance. The Japanese chestnut cultivars are more resistant to *Phytophtora* diseases as compared to the European ones [21], although they can carry the pathogen. This lack of symptoms aboveground might be another mechanism of this phenomenon. Furthermore, the *in vivo* performance of antagonistic bacteria may not always reproduce the same results seen *in vitro,* suggesting that other environmental factors (biotic and abiotic) might favor or suppress these isolates, causing infection until the plant is completely decayed. Sometimes, the opposite can occur, with no inhibition effect *in vitro*, but good effect *in vivo*, and thus it might be recommendable to carry out direct screening *in vivo* for some biocontrol agents [22].

The metagenome analysis showed a profile of the fungal communities in the soil of four locations under conventional, organic and wild systems. The microbial profile could be strongly modified by organic systems when compared to conventional ones [23]. Therefore, the evaluation of the soil microbial community leads us to observe the effect of soil management. The higher relative abundance of the family *Ceratobasidiaceae*, which contains many pathogenic species, in conventional areas, and of *Russulaceae* and *Boletaceae* in organic plots, suggests that management could favor some fungal groups and suppress others. In organic systems, in particular, the symbiotic fungi might be stimulated and the pathogens inactivated; however, this does not guarantee the plant root infection. Thus, the presence of some fungal groups could be used as indicators of these soil managements and wild areas. Corroborating this research, the cover crops [24] and organic management systems could affect the fungal community, where some species could increase to the detriment of others. Additionally, long-term organic management could increase fungal richness, especially when associated with animal manure, in parallel to decreasing potential pathogenic fungi [25]. However, despite the temporal stability, the sampling time and season had more influence on the fungal communities than the crop types and farming systems, perhaps due to climate and changes in the preceding crops [26]. Furthermore, the soil pH was higher in the organic cultivation system compared to the conventional and wild cultivation systems. In previous studies, the pH of soil was shown to drive differences in microbial communities [27]. Therefore, it could be one explanation for organic areas.

5. CONCLUSIONS

- Two diseases of chestnut, Ink disease and root rot, were suppressed by using biocontrol bacterial isolates.
- The infection of pathogens *in vivo* was observed only in the roots, without any visual symptoms in the shoot.
- Within the soil fungal community, conventional systems could be favorable to families that contain pathogenic species, and organic systems to symbiotic ones.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not

intend to use these products as an avenue for any litigation but for the advancement of knowledge. In addition, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

This research was partially supported by the Grant-in-aid for scientific research from the Ministry of education, sports and culture of Japan no. 16K07645. The authors are thankful to the chestnut growers in Kyoto and Hyogo prefectures – Japan for supplying the samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Beccaro G, Alma A, Bounous G, Gomes-Laranjo J. The chestnut handbook: Crop & forest management. CRC Press; 2019.
2. Ciesla, William M. Non-wood forest products from temperate broad-leaved trees. Food & Agriculture Organization. 2002;15.
3. Bounousa G. Perspectives and future of the chestnut industry in Europe and all over the world. Acta Horticulturae. 2014;1043:19–22.
4. Kile GA, McDonald GI, Byler JW. Ecology and disease in natural forests. In Armillaria root disease. Edited by C.G. Shaw III and G.A. Kile. U.S. Agriculture handbook 1991;691:102–121.
5. Hwang SF, Chakravarty P, and Chang KF. The effect of two ectomycorrhizal fungi, *Paxillus involutus* and *Suillus tomentosus*, and of *Bacillus subtilis* on *Fusarium* damping-off in jack pine seedlings. Phytoprotection. 1995;76:57–66.
6. Fravel DR. Commercialization and implementation of biocontrol. Annual Review of Phytopathology. 2005;43:337–359.
7. Tilman D, Cassman KG, Matson PA, Naylor R, aPolasky S. Agricultural sustainability and intensive production practices. Nature. 2002;418:671–677.
8. Postma-Blaauw MB, de Goede RG, Bloem J, Faber JH, and Brussaard L. Soil biota community structure and abundance under agricultural intensification and extensification. Ecology. 2010;91:460–473.
9. Wang W, Wang H, Feng Y, Wang L, Xia X, Xi Y, Luo X, Sun R, Ye X, Huang Y, Zhang Z, Cui Z. Consistent responses of the microbial community structure to organic farming along the middle and lower reaches of the Yangtze River, Scientific Reports. 2016;6:35046.
10. Taberlet P, Coissac E, Pompanon F, Brochmann C, and Willerslev E. Towards next generation biodiversity assessment using DNA metabarcoding. Molecular Ecology. 2012;21:2045–2050.
11. Somasegaran P, Hoben HJ. Handbook for rhizobia methods in legume rhizobium technology. New York, NY, Heidelberg: Springer; 1994.
12. Sambo F, Finotello F, Lavezzo E, Baruzzo G, Masi G, Peta E, Falda M, Toppo S, Barzon L, Camillo BD. Optimizing PCR primers targeting the bacterial 16S ribosomal RNA gene. BMC Bioinformatics. 2018;19:343
13. Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular arbuscular infection in roots, New Phytologist. 1980;84:489-500.
14. Kageyama K, Tsutomu K, Haruhisa S. Refined PCR protocol for detection of plant pathogens in soil, Journal of General Plant Pathology. 2003;69:153-160.
15. White T J, Bruns T, Lee S, and Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols. A guide to methods and applications. Innes, M. A., Gelfard, D. H., Sninsky, J. J., White, T. J. ed. San Diego, Academic Press; 1990.
16. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Caporaso JG. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. 2019;37:852-857
17. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13:581-583.
18. Krstin L, Katanic Z, Jezic M, Poljak I, Nuskern L, Matkovic I, Idzojtic M, Curkovic M. Biological control of chestnut blight in Croatia: An interaction between host sweet chestnut, its pathogen Cryphonectria parasitica and the biocontrol agent *Cryphonectria hypovirus* 1. Pest Management Science. 2017;73:582–589.

19. Revathi N, Kalaiselvi M, Gomathi D, Ravikumar G, Uma C. Antifungal activity of Bacillus species in bio-control of different plant pathogens. The Journal of Phytopharmacology. 2013;2(6):14–18.
20. David MW. Pseudomonas biocontrol agents of soilborne pathogens: Looking back over 30 Years, Phytopathology. 2007;97.
21. MSU (Michigan State University). Michigan chestnut management guide – Diseases. https://www.canr.msu.edu/chestnuts/pest_management/diseases; 2020. Accessed in January, 2021
22. Besset-Manzoni Y, Joly P, Brutel A, Gerin F, Soudière O, Langin, T, Pringent-Combaret C. Does *In vitro* selection of biocontrol agents guarantee success *in planta*? A study case of wheat protection against *Fusarium* seedling blight by soil bacteria. PLoS ONE. 2019;14(12):e0225655.
23. Chavarria DN, Perez-Brandan C, Serri DL, Meriles JM, Restovich SB, Andriulo AE, Jacquelin L, Vargas-Gil S. Response of soil microbial communities to agroecological versus conventional systems of extensive agriculture, Agriculture, Ecosystems & Environment. 2018;264:1–8.
24. Cloutier ML, Murrell E, Barbercheck M, Kaye J, Finney D, García-González I, Bruns MA. Fungal community shifts in soils with varied cover crop treatments and edaphic properties. Scientific Reports. 2020;10:6198.
25. Sun R, Dsouza M, Gilbert JA, Guo X, Wang D, Guo Z, Ni Y, Chu H. Fungal community composition in soils subjected to long-term chemical fertilization is most influenced by the type of organic matter. Environmental Microbiology. 2016;18(12):5137-5150.
26. Schneider S, Hartmann M, Enkerli J, Widmer F. Fungal community structure in soils of conventional and organic farming systems. Fungal Ecology. 2010;3(3):215-224
27. Dequiedt S, Saby NPA, Lelievre M, Jolivet C, Thioulouse J, Toutain B, Arrouays D, Bispo A, Lemanceau P, Ranjard L. Biogeographical patterns of soil molecular microbial biomass as influenced by soil characteristics and management, Global Ecology and Biogeography. 2011;20: 641.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/73886