

Analysis of microbial community in Japanese vineyard soils by culture-independent molecular approach

Keiko Fujita
Seiichi Furuya
Minako Kohno
Shunji Suzuki
Tsutomu Takayanagi

Institute of Enology and Viticulture,
University of Yamanashi, Kofu,
Yamanashi, Japan

Abstract: Soil microbes play an important role in the management of soil environment. They form various microbial communities in response to environmental factors, including soil texture and chemical components. Because of this, it is difficult to determine the microbial community structure of soil. In vineyard soils, the overall microbial community structure has yet to be unraveled. To understand the microbial community in vineyard soil, we surveyed comprehensively microbial communities in Japanese vineyard soils by using a culture-independent molecular approach. We identified 681 fungal clone sequences and 1076 bacterial clone sequences in soil samples collected from nine independent Japanese vineyards, and the results suggested that *Ascomycota* is the dominant group in the fungal community, whereas *Proteobacterium* and *Acidobacterium* are the dominant groups in the bacterial community. DNA was directly extracted from the soil samples, and the fungal internal transcribed spacer 1 (ITS 1) region or the bacterial 16S ribosomal DNA (rDNA) region was amplified by polymerase chain reaction. The recovered fungal clones were sorted into 225 operational taxonomic units and the majority of the clone sequences were assigned to *Ascomycota*. Meanwhile, the recovered bacterial clones were sorted into 17 phyla, and the abundant phyla were *Proteobacterium* and *Acidobacterium*. These results differed from the reported fungal and bacterial community structures in forest and agricultural soils. Moreover, we preliminarily generated a catalog of Japanese vineyard soils. The microbial community structures in the vineyard soils were extremely complex, suggesting that the microbial community structure in each vineyard soil has individual characteristics. Our study comprehensively showed for the first time fungal and bacterial community structures in Japanese vineyard soils, and is most likely to provide a clue to understand the nature of Japanese vineyard soils.

Keywords: Japanese vineyard, soil microbe, microbial community structure, 16S rDNA

Introduction

Soil microbes play an important role in the soil ecosystem, including formation of soil structure, decomposition of organic compounds, detoxification of toxins, and cycling of carbon, nitrogen, phosphorus, and sulfur.¹ Some soil microbes sustain and stimulate plant growth and suppress several diseases caused by soil-borne plant pathogens.¹ In addition, because soil microbial community structure is altered by environmental and anthropogenic factors,² its use as an indicator of soil quality has been proposed.³

Soil microbial communities have been assessed conventionally by culture-based methods.^{4,5} However, current culture techniques can culture less than 1% of environment-dwelling microbes from any experimental sources.⁶ Although culture-based methods allow for the isolation of microbial colonies, spores and/or hyphae, they

Correspondence: Shunji Suzuki
Institute of Enology and Viticulture,
University of Yamanashi, 1-13-1 Kitashin,
Kofu, Yamanashi, Japan
Tel +81 55 220 8394
Fax +81 55 220 8768
Email suzukis@yamanashi.ac.jp

present no clues to understanding exhaustively the microbial community structures in environmental sources. On the other hand, uncultured microbes have been investigated by molecular techniques, including polymerase chain reaction (PCR) and real-time polymerase chain reaction (RT-PCR), using DNA or RNA isolated from environmental sources.^{7,8} To understand the variation and/or composition of microbial community structures or microbial diversities, PCR products are subjected to fingerprinting techniques, including denaturing or temperature gradient gel electrophoresis, terminal restriction fragment length polymorphism, and automated ribosomal intergenic spacer analysis (ARISA).^{1,9} Using molecular techniques, microbial communities, and diversities in soils collected from grasslands, forests, alpine areas, and farms have been identified and the effects of chemical component,¹⁰ soil particle size,¹¹ cultivated plant,¹² seasonal condition,^{13,14} and agricultural management system^{15,16} on the diversity of soil microbes have been evaluated.

It is important for grape growers and winemakers to understand vineyard soil to satisfy the demand for high fruit yield, improved wine style, and superb wine quality. Books on soil for fine wines are available.^{17,18} However, there are few descriptions in those books about microbes (fungi and bacteria) in vineyard soils, although soil microbes play an important role in the management of soil environment. Microbial communities in vineyard soils may be unique compared with those in soils of other agroecosystems, because vineyards are generally subjected to less frequent tillage, less nitrogen fertilization, and less herbicide application.¹⁹ In addition, several farming practices may influence microbial communities in vineyard soils. For example, compost amendment induced the alteration and resiliencing of microbial community in vineyard soil.²⁰ In a pinot noir vineyard, microbial communities were also altered by soil morphology, soil depth, and grapevine root.¹⁹ However, in those two studies, an exhaustive evaluation of microbial diversities at the level of fungal species or phylogenetic bacterial taxa was not conducted because those studies were based on the evaluation by the phospholipid ester-linked fatty acid method. Currently, copper concentrations resulting from the use of fungicides and fertilizers are increasing in vineyard soil surface, and this trend is raising concern as regards its harmful and irreversible effects on the soil ecosystem.²¹ Thus, it is important to monitor the status of soil microbial population and diversity. Dell'Amico et al²¹ investigated ex-vineyard soil by the denaturing gradient gel electrophoresis method and identified copper-tolerant bacteria as the indicator of copper pollution in the soil. One study of the effects of copper contamination on the microbial community in vineyards

employed ARISA, and the findings suggested that bacterial genetic structure in nonamended soil varied significantly and *Actinobacteria* are the prevalent bacteria in copper-contaminated nonamended soil.²² In vineyard soils, however, the microbial community structure and the microbial species have not been comprehensively evaluated and are thus little understood. In Japan, no studies have been conducted on the microbial communities in vineyard soils. To improve Japanese viticulture and sustain vineyard soil environment, we tried to gain an overview of soil characteristics and microbial species in vineyard soils. For this purpose, soils were collected from some vineyards and the fungal internal transcribed spacer 1 (ITS 1) region or the bacterial 16S ribosomal DNA (rDNA) region was PCR-amplified to understand the microbial communities.

Materials and methods

Soil samples

To survey microbes in Japanese vineyard soils, we obtained 31 soil samples from nine vineyards. Four soil samples were obtained from pits located approximately 3–4 m apart in the experimental vineyard of The Institute of Enology and Viticulture, University of Yamanashi, Japan, in January 2010 (sample names K1–K4). Seven soil samples were obtained from seven commercial vineyards in Japan (A, B, C, D, E, F, and G), and 20 soil samples were obtained from two pits in the experimental vineyard of The Institute of Enology and Viticulture, University of Yamanashi, Japan, from May 2008 to December 2008 (BK3–BK12 and BC3–BC12). Soil pits located at a distance of 50 cm from each grapevine trunk were dug to a depth of 30 cm. Approximately 50 g of soil sample was taken from each pit. The properties of the soil samples are summarized in Table 1.

Soil texture analysis

Soil particle size was measured according to the Colorado State University Master Gardener manual.²³ Briefly, approximately 20 g of soil from which rocks and root tips were removed was strongly shaken in 40 mL of water for 15 minutes. Then, the soil particles were allowed to stand for one month. By measuring the thicknesses of sand, silt, and clay layers, the percentages of sand, silt, and clay were calculated. Soil texture was identified with a soil textural triangle.

pH and electrical conductivity of soil

Approximately 20 g of soil was strongly shaken in 100 mL of water for 10 minutes and the mixture was left to stand for 10 minutes. pH and electrical conductivity were measured

Table 1 Summary of sampling locations and physical and chemical properties of soils

Library name	Description	Site	Cultivated vine	Soil texture	pH	EC ($\mu\text{S/cm}$)
K1–K4 ^a	Conventional agriculture (Cover crops)	Kofu	CS ^c	Clay loam	6.8	14.3
A	Hardly use Bordeaux	Koshu	Koshu	Loamy sand	7.6	51
B	Conventional agriculture	Koshu	Koshu	Clay	6.9	86
C	Biodiversity	Koshu	CS	Sandy loam	7.0	60
D	Cover crops	Ibuki	CS	Sand	6.6	12
E	Sampling right after replant	Koshu	Koshu	Clay	7.4	25
F	Cover crops	Koshu	CS	Clay	7.1	43
G	Conventional agriculture	Ibuki	Koshu	Sandy loam	7.3	102
BK3–BK12 ^b	Conventional agriculture (Cover crops)	Kofu	Koshu	Sandy loam	6.2–7.7	39–93
BC3–BC12 ^b	Conventional agriculture (Cover crops)	Kofu	CS	Sandy loam	6.8–7.4	32–96

Notes: ^aFour clone libraries for soils sampled from four pits at vineyard K; ^bseasonal clone libraries for soil sampled from one vineyard monthly from March to December; ^cCabernet Sauvignon.

with a pH and electrical conductivity tester (Combol; HANNA Instruments, Padova, Italy).

DNA extraction from soil and preparation of clone library

Soils from each pit were mixed and large rocks and root tips were removed. Total DNA was directly extracted from 0.4–0.3 g of soil using a PowerMax™ Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. PCR amplification of fungal ITS 1 region or bacterial 16S rDNA region was performed using an ITS primer pair (5'-GTAACAAGGTTTCCGT-3' and 5'-CGTTCTTCATCGATG-3') or a 16S primer pair (5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-GTATTACCGCGGCTGCTG-3'), respectively. The PCR reaction mix consisted of 1 μL of 10 \times PCR buffer, 0.4 μL of 2.5 mM each dNTP, 0.25 μL of 20 mM each primer, 0.1 μL of Hot Start Taq polymerase (Takara, Shiga, Japan), 1 μL of DNA solution, and 7 μL of ddH₂O. PCR conditions were as follows: 95°C for three minutes (one cycle); 95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds (20, 22, or 24–27 cycles for fungal ITS 1 region, 14, 15, 18, or 28 cycles for bacterial 16S rDNA region); and 72°C for five minutes (one cycle). PCR cycles were controlled to prevent saturation of amplified PCR products. The PCR products were separated on 1.5% agarose gels. Agarose gels at 250–500 base pairs (bp) for fungal ITS or 450–800 bp for bacterial 16S rDNA were excised and the PCR products were extracted with a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. After extraction, the products were ligated to p-TAC-1 vector (Bio Dynamics Laboratory Inc., Tokyo, Japan) or pMD20-T vector (Takara, Shiga, Japan). By transforming the ligation product into *Escherichia coli* JM109 strain, fungal ITS clone,

and bacterial 16S rDNA clone libraries were constructed. DNA extractions from samples K1–K4 were used for the analysis of fungal community. K1–K4 clone libraries were generated from three replicates of one soil sample. Meanwhile, DNA extractions from samples A–G were used for the analysis of fungal and bacterial communities. The remaining samples, BK3–BK12 and BC3–BC12, were used for the analysis of bacterial community.

Sequence analysis

Sequencing of libraries was accomplished by DyeDeoxy terminator cycle sequencing with M13 forward or reverse primer. DNA sequences were subjected to a homology search using the BLAST search program.²⁴ Based on BLAST search results, fungal ITS clones were sorted into operational taxonomic units (OTUs). Fungal ITS clones in OTU have >98% sequence similarity. We confirmed the appropriate group using phylogenetic trees that were constructed with the neighbor-joining method of Molecular Evolutionary Genetics Analysis software,²⁵ although we do not show the phylogenetic trees. After BLAST search, bacterial 16S rDNA clone sequences were assembled into phylum groups using Classifier of Ribosomal Database Project (RDP-X).²⁶

Nucleotide sequence accession numbers

We have submitted all nucleotide sequence data to DDBJ database.

Results

Sequence analysis of fungal ITS clones

A total of 103 clones from K1, 86 clones from K2, 89 clones from K3, and 87 clones from K4 were recovered. Clones from plant ITS region sequences were included in the ITS libraries because the designed primer pair also amplified

the plant ITS region. Eventually, 81 K1 clones, 85 K2 clones, 62 K3 clones, and 73 K4 clones were examined for their similarity to a sequence from a known fungus in the database (Table 2). Clone sequences with BLAST scores exceeding 200 (100%–81% identity) were 70 K1 clones (86.4%), 71 K2 clones (83.5%), 32 K3 clones (51.6%), and 62 K4 clones (84.9%). K1–K4 clone libraries consisted of 49, 38, 33, and 40 OTUs, respectively (Table 2), and the obtained 303 clone sequences from K1–K4 were sorted into 109 different OTUs. These results suggested that this molecular method was able to detect many fungal species in vineyard soils. The fungal ITS clone libraries from samples K1–K4 had individual structures that consisted of unique and variously sized OTUs, although the clone libraries were generated from different pit soils collected from the same vineyard (Table 3, Supplementary Table 1). For example, the most abundant OTU (uncultured fungus) in K1 and K2 clone libraries was composed of 10 and 21 clones, respectively, while in K3 and K4 clone libraries, the OTU was represented by a single clone. OTU 83 (unidentified sequence) that included 11 clones was the largest OTU in K3 clone library while the clone sequences of the other libraries were not assigned to this OTU. The clone sequences of K1–K4 clone libraries were included in OTUs 3, 30, and 107. Moreover, 27.5% of K1–K4 OTUs were represented by the clone sequences from the above two clone libraries. These results suggest that fungi in vineyard soils do not have a uniformly distributed community structure.

To survey fungi in Japanese vineyard soils, we also examined fungi of seven soils sampled from other vineyards (vineyards A–G). A total of 516 clones were recovered and 380 clones (73.6%) resulted in successful fungal sequences. These clone sequences were of fungal origin and comprised

157 OTUs. Finally, we obtained and determined 681 clone sequences that were included in 225 OTUs in soils from eight vineyards, namely, samples A–G and K1–K4. The results of BLAST search of these clones are shown in Supplementary Table 1. The majority of the fungal ITS clone sequences were assigned to *Ascomycota* (30.8%) and unclassified fungi (38.2%, Figure 1A). The remaining clones were assigned to *Basidiomycota* (9.5%), *Zygomycota* (3.7%), *Glomeromycota* (2.5%), and *Oomycetes* (3.5%). Together, the results suggest that various kinds of *Ascomycota* fungi exist in the vineyard soils.

The fungal community structures were very complex in the seven vineyard soils as well as in the four pit soils taken from one vineyard, because common OTUs were rarely found in the seven vineyard clone libraries. By comparing the clone libraries of vineyards K, A, B, C, D, E, F, and G, we found original OTUs in the clone libraries of vineyards C and D. The clone library of vineyard C consisted of few OTUs included many clones, such as *Aspergillus* spp., *Aureobasidium pullulans*, *Phomopsis* sp., while the clone library of vineyard D had many unique OTUs (Table 2, Supplementary Table 1). Thus, soils of vineyards C and D may have original fungal community structures although we did not conduct further assessment because of the small sample size and the complete lack of replication at vineyards A–G.

Sequence analysis of bacterial 16S rDNA clones

To survey bacterial community structures in vineyard soils in a manner similar to the fungal community structures, 407 clones were recovered from the seven vineyards. To determine widespread bacterial community in vineyard, we sampled two pit soils from one vineyard monthly from May 2008 through December 2008, and 846 clones were recovered because of significant seasonal changes of the bacterial community, as reported by Lipson and Schmidt.¹⁴ Clones whose sequences were amplified by an unpaired primer were omitted from the clone library. Finally, we searched 1106 clone sequences in the National Center for Biotechnological Information (NCBI) database. Database sequences yielding the highest percentage identity to the clone sequences were chosen as the best match for the clones. The results that matched sequences in the NCBI database are shown in Supplementary Table 2, and 16S rDNA gene sequences with >97% similarity are considered to be the same species in phylogenetic position. However, as 30 clone sequences were not listed in the NCBI database and could not be sorted into “bacteria” by Classifier of RDP-X, those clones were omitted from the clone library

Table 2 Number of sequences and operational taxonomic units recovered from each fungal clone library

Library name	No. of sequences	No. of OTUs
K1	81	49
K2	85	38
K3	62	33
K4	73	40
A	56	21
B	57	25
C	53	10
D	56	21
E	46	27
F	54	28
G	58	32

Table 3 Fungal species from BLAST search with the highest sequence identity to some large OTUs and distribution of operational taxonomic units in K1–K4

OTU	Blast match	Accession no.	ITS size (bp)	Identity (%)	K1	K2	K3	K4	Total No. of clone
3	Uncultured mycorrhizal fungus	AB454411	237	100-99	4	2	1	3	10
23	Uncultured <i>Basidiomycota</i>	AY970109	240	99	1		1	7	9
25	Uncultured <i>Ceratobasidiaceae</i>	DQ182419	231	96-95		3	2	7	9
30	<i>Pythium sylvaticum</i>	DQ528741	346	100-99	4	5	2	2	13
40	Uncultured zygomycete	EU490044	213	85-84	4	8			12
54	Unidentified ^a	–	213	–	5		9	1	15
57	<i>Ceratobasidium</i> sp.	FJ435147	234	100-99				4	4
80	Uncultured fungus	FJ781421	245	97				5	5
83	Unidentified	–	426	–			11		11
85	Unidentified	–	249	–			4	1	5
96	Uncultured fungus	GQ866220	220	100-99	4	6			10
107 ^b	Uncultured fungus	GU370749	211	100-99	10	21	1	1	33

Notes: ^aClone sequences were not found in National Center for Biotechnological Information database; ^bFungal internal transcribed spacer clone sequences of operational taxonomic unit 107 had closest identity to GU370749, GQ509331, GQ509519, GQ510910, GQ511388, GQ515874, GQ523707, but most clone sequences were matched to GU370749.

and Supplementary Table 2. It was uncommon that more than three clone sequences were assigned to the same accession number, with the exception of “uncultured bacterium (FJ479014)” that was found in BK10, BK11, BK12, BC10, BC11, and BC12. BLAST search demonstrated that 80.6% of the 1076 clone sequences had high similarity to members of yet undescribed bacterial divisions. To sort the “unclassified” clones, we used Classifier of RDP-X (confidence threshold = 80%). This resulted in sorting into 18 groups that included “unclassified bacteria”. The most abundant phylum was *Proteobacteria*, which consisted of 23.1% of the total

clones (Figure 1B). The second most abundant group was “unclassified bacteria” (22.1%) and the third largest group was *Acidobacteria* (21.9%). Other relatively large groups were *Actinobacteria* (8.1%), *Bacteroidetes* (5.2%), *Chloroflexi* (4.0%), *Gemmatimonadetes* (3.2%), *Planctomycetes* (3.1%), and *Verrucomicrobia* (6.5%). The percentage abundance of these groups varied for each clone library, although the number of clones was not sufficient to compare with the percentage abundance of phylum within one clone library. However, bacterial clone libraries other than the clone libraries of soil collected from September 2008 to December 2008 constantly

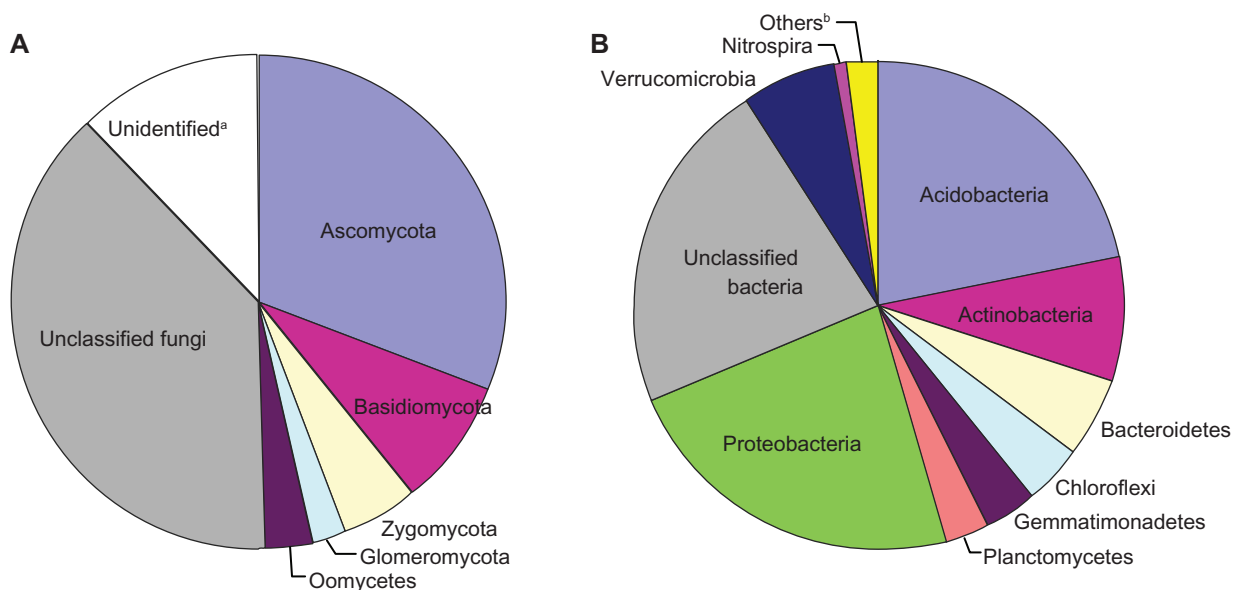


Figure 1 Frequencies of fungal ITS sequences belonging to each fungal phylum **A** and bacterial 16S rDNA sequences belonging to each bacterial phylum **B**. Fungal phyla were classified based on BLAST search results. Bacterial phyla were classified by using Classifier of RDP-X.

Notes: ^aClone sequences were not found in NCBI database; ^bothers include *Cyanobacteria*, *Firmicutes*, *Spirochaetes*, OD1, OP10, OP11, TM7, and WS3.

included *Proteobacteria*, *Acidobacteria*, and unclassified bacteria in abundance. The remaining phyla present within the clone libraries included *Nitrospira*, *Cyanobacteria*, *Firmicutes*, *Spirochaetes*, OD1, OP10, OP11, TM7, and WS3. Each consisted of less than 1% of the total clones.

Discussion

In this study, we determined fungal ITS and bacterial 16S rDNA clone sequences in vineyard soils and showed the fungal and bacterial community structures in the soils. Previous reports have demonstrated the diversity and community structure of mycorrhizal fungi in vineyards^{27,28} and the variation in vineyard soil microbial communities influenced by compost amendment and increasing depth.^{19,20} Whitelaw-Weckert et al²⁹ examined the effects of permanent swards and bare soil on soil microbial count in two Australian vineyards by culture methods. However, those studies did not determine the composition of fungi and bacteria at the species level in vineyard soils, and there are hardly any studies that deal with the comprehensive identification of clone sequences from vineyard soils. Thus, our study surveyed for the first time microbes present in vineyard soils. We recovered 109 OTUs from 303 fungal clone sequences of vineyard K clone libraries. As the rarefaction curves of the 303 fungal clones did not reach a plateau (data not shown), we were able to estimate only a small portion of fungi existing in the vineyard soils. We need to recover more clones to reveal the number of fungal species. Nevertheless, our results suggest that at least 109 fungal species exist in one vineyard and most of them belong to *Ascomycota*. These results have not been obtained in previous studies that used phospholipid ester-linked fatty acids or denaturing gradient gel electrophoresis to detect overall microbial community shifts and compare microbial community structures.³⁰ The main *Ascomycota* detected by prosequencing analyses of forest soils consist of lichen-forming fungi, litter/wood decomposers, plant parasites, endophytes, and saprotrophs.³¹ The *Ascomycota* in our study may include fungal groups causing plant disease, endophytes, wood decomposers, and saprotrophs, because such fungi as *Fusarium* spp,³² *Phomopsis* spp,³³ *Aspergillus* spp,^{34,35} *Pezizomycotina* spp,³⁶ and *Alternaria* spp abound in fungal clone libraries.

We noted that the OTU patterns in the clone libraries and the number of clones within each OTU are very diverse even among the clone libraries from the same vineyard, suggesting the complex distribution of the fungal community. Unfortunately, it was impossible to identify the dominant fungus at the species level in the vineyard soils due to the

limited sample size. However, we noticed that the species patterns of each clone library are interesting. Fungal clone libraries from vineyards C and D are the most unique among the eight vineyard clone libraries (Table 2, Supplementary Table 1). Microbial community structures are affected by environmental and anthropogenic factors.¹ Vineyard C is managed with a biodynamic agricultural system (Table 1). The soil characteristics of vineyard D differ from those of other vineyards: the soil has a sandy texture, the highest acidity, and the lowest electrical conductivity (Table 1). It was shown that the compositions of bacterial communities in soils of biodynamic, conventional, and unfertilized agricultural management systems were vastly different although the clone libraries revealed similar diversities.¹⁵ Mäder et al³⁷ found significantly higher microbial biomass and diversity in the soil of a biodynamic management system than in the soil of a conventional management system. Meanwhile, the effect of particle size on the microbial community structure was noted, in that small particles (silt and clay) yielded high microbial diversity compared with coarse particles (sand).¹¹ A strong correlation between bacterial diversity and soil pH was also shown by Fierer and Jackson.³⁸ Those reports agree with our results of fungal sequences and it seems that the characteristics of fungal community structures are reflected by soil environment and management. However, there was no obvious relationship between bacterial communities and environmental factors in vineyards C and D compared with fungal community structures in the same vineyards because each bacterial community was extremely complex and the variations in bacterial libraries might be restricted by the sample size. It is possible that the fungal community structure may be more sensitive to environmental factors than the bacterial community structure in vineyard soils. Further exhaustive studies are required to reveal the relationship between bacterial community structure and environmental factors.

Bates and Garcia-Pichel³⁹ indicated that bacterial diversity was noticeably and significantly higher than fungal diversity. One gram of soil contains one to four billion bacteria and less than one billion fungi.⁴⁰ Thus, to reveal the overall bacterial community structure in vineyard soil, it is necessary to conduct large-scale DNA sequence analysis. In this study, although we were not able to reveal the overall bacterial community structure, we were able to sort by phylum 1076 clone sequences recovered from samples collected monthly from one vineyard soil and from seven vineyard soils. We suggest that 17 of the 52 phyla determined exist in vineyard soils (see Figure 1B).⁴¹ Lejon et al²² showed that 30 clones derived from

350–450 bp bacterial-ARISA bands from soil collected from a copper-contaminated vineyard belonged to *Actinobacteria*, *Fibrobacteres*, *Gemmatimonadetes*, and *Proteobacteria*. Our results indicated that bacteria from numerous phyla exist in vineyard soil. The major phyla of vineyard soil bacteria are *Proteobacteria* and *Acidobacteria* (Figure 1B). In forest soil, *Proteobacteria* and *Bacteroidetes* are the dominant bacterial groups, followed by *Acidobacteria*.⁴² In agricultural soils where maize and sugarcane are grown, phylum diversity is poor and the predominant groups are *Actinobacteria*, *Bacteroidetes*, and *Fermicutes*.^{42,43} Therefore, our results suggest that the microbial community structure in vineyard soil may be unique, in support of the work of Steenwerth et al.¹⁹ Meanwhile, the dominant fungal group in forest soils is *Basidiomycota*,^{31,44} and this dissimilarity in the dominant fungi also supported our suggestion. However, we do not know why *Proteobacteria* and *Acidobacteria* are the dominant bacteria in vineyard soils. Moreover, it is interesting that the 31 clone sequences assigned to *Actinobacteria* were suddenly found in soils sampled from October 2008 to December 2008. Dell'Amico et al.²¹ described that a specific microbe in copper-contaminated soil may serve as an indicator of soil pollution status. Meanwhile, *Actinobacteria* are prevalent in copper-contaminated nonamended soil.²² Smit et al.⁴⁵ suggested that the ratio of the number of *Proteobacteria* to that of *Acidobacteria* may be an indicator of the nutritional status of soils. Therefore, the soils sampled from October 2008 to December 2008 might be organic-matter rich. However, because we assessed only the contents of copper and organic matter in vineyard soils, we could not determine whether the microbial community in the soils was a result of the effects of copper and organic matter. At the very least, we can say that there was no organic amendment before the appearance of *Actinobacteria* in any of the vineyard soils tested.

We were able to detect some species, including phytopathogenic and/or valuable fungi, and to observe the current status of vineyard soils. Although our sequence data are not sufficient to confirm the microbial community in Japanese vineyard soils, we preliminarily generated a microbial catalog that records the physical, chemical, and biologic properties of Japanese vineyard soils and their implications for viticulture (Supplementary Tables 1 and 2). This catalog may help further our understanding of the current state of vineyard soils and promote the management, maintenance, and improvement of vineyards. Although vineyard soil properties are complex, we may be able to elucidate gradually the characteristics of vineyard soils by using a PCR-culture-independent approach and generating a microbial catalog.

Conclusion

We demonstrated for the first time the community structures of fungi and bacteria in Japanese vineyard soils by a PCR-culture-independent approach and generated a microbial catalog. Our results suggest that many fungal and bacterial species exist in vineyard soils and the microbial community structure in each vineyard soil sample is extremely diverse. Moreover, the dominant fungal and bacterial groups are dissimilar to the dominant microbes in forest and agricultural soils. We were not able to determine the dominant microbes at the species level due to the limited sample size. By analyzing microbes on a large scale, vineyard soil characteristics will be revealed in detail and soil condition assessment using microbial community structures will be realized.

Acknowledgment

This work was supported by the Sasakawa Scientific Research Grant from The Japan Science Society.

Disclosure

The authors report no conflict of interest in this work.

References

- Garbeva P, van Veen JA, van Elsas JD. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu Rev Phytopathol.* 2004;42:243–270.
- Kennedy AC, Smith KL. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil.* 1995;170:75–86.
- Torsvik V, Øvreås L. Microbial diversity and function in soil: From genes to ecosystems. *Curr Opin Microbiol.* 2002;5:240–245.
- Garland JL. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C utilization. *Soil Biol Biochem.* 1996;28:213–221.
- Stieß J, Engelen B, Cypionka H, Sass H. Quantitative analysis of bacterial communities from Mediterranean sapropels based on cultivation-dependent methods. *FEMS Microbiol Ecol.* 2004;51:109–121.
- Hoshino YT, Hasebe R. DNA extraction from soil. *J Environ Biotechnol.* 2005;5:43–53.
- Anderson IC, Campbell CD, Prosser JI. Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. *Environ Microbiol.* 2003;5:36–47.
- Gelsomino A, Keijzer-Wolters AC, Cacco G, van Elsas JD. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. *J Microbiol Methods.* 1999;38:1–15.
- Anderson IC, Campbell CD. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular technique. *Environ Microbiol.* 2004;6:769–779.
- Anderson IC, Parkin PI, Campbell CD. DNA- and RNA-derived assessments of fungal community composition in soil amended with sewage sludge rich in cadmium, copper and zinc. *Soil Biol Biochem.* 2008;40:2358–2365.
- Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Appl Environ Microbiol.* 2001;67:4215–4224.

12. Marschner P, Yang CH, Lieberei R, Crowley DE. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol Biochem.* 2001;33:1437–1445.
13. Kennedy NM, Gleeson DE, Connolly J, Clipson NJW. Seasonal and management influences on bacterial community structure in an upland grassland soil. *FEMS Microbiol Ecol.* 2005;53:329–337.
14. Lipson DA, Schmidt SK. Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. *Appl Environ Microbiol.* 2004;70:2867–2879.
15. Hartmann M, Widmer F. Community structure analyses are more sensitive to difference in soil bacterial communities than anonymous diversity indices. *Appl Environ Microbiol.* 2006;72:7804–7812.
16. Upchurch R, Chiu CY, Everett K, Dyszynski G, Coleman DC, Whitman WB. Differences in the composition and diversity of bacterial communities from agricultural and forest soils. *Soil Biol Biochem.* 2008;40:1294–1305.
17. White RE. *Soil for Fine Wine.* New York, NY: Oxford University Press; 2003.
18. White RE. *Understanding Vineyard Soils.* New York, NY: Oxford University Press; 2009.
19. Steenwerth KL, Drenovsky RE, Lambert JJ, Kluepfel DA, Scow KM, Smart DR. Soil morphology, depth and grapevine root frequency influence microbial communities in a pinot noir vineyard. *Soil Biol Biochem.* 2008;40:1330–1340.
20. Saison C, Degrange V, Oliver R, et al. Alteration and resilience of the soil microbial community following compost amendment: Effects of compost level and compost-borne microbial community. *Environ Microbiol.* 2006;8:247–257.
21. Dell'Amico E, Mazzocchi M, Cavalca L, Allievi L, Andreoni V. Assessment of bacterial community structure in a long-term copper-polluted ex-vineyard soil. *Microbiol Res.* 2008;163:671–683.
22. Lejon DPH, Martins JMF, L  v  que J, et al. Copper dynamic and impact on microbial communities in soils of variable organic status. *Environ Sci Technol.* 2008;42:2819–2825.
23. CMG GardenNotes [Homepage on the Internet]. Colorado: Colorado State University Extension; c2007. Available from: <http://cmg.colostate.edu/gardennotes/214.pdf>. Accessed Jun 20, 2010.
24. Basic Local Alignment Search Tool [Homepage on the internet]. National Center for Biotechnology Information. Available from: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. Accessed Feb, 2010.
25. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007;24:1596–1599. Available from: www.megasoftware.net
26. Wang Q, Garrity GM, Tiedje JM, Cole JR. Na  ve Bayesian Classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol.* 2007;73:5261–5267.
27. Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytol.* 2005;165:273–283.
28. Schreiner RP, Mihara KL. The diversity of arbuscular mycorrhizal fungi amplified from grapevine roots (*Vitis vinifera* L.) in Oregon vineyards is seasonally stable and influenced by soil and vine age. *Mycologia.* 2009;101:599–611.
29. Whitelaw-Weckert MA, Rahman L, Hutton RJ, Coombes N. Permanent swards increase soil microbial counts in two Australian vineyards. *Appl Soil Ecol.* 2007;36:224–232.
30. O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. Fungal community analysis by large-scale sequencing of environmental samples. *Appl Environ Microbiol.* 2005;71:5544–5550.
31. Lim YW, Kim BK, Kim C, et al. Assessment of soil fungal communities using pyrosequencing. *J Microbiol.* 2010;48:284–289.
32. Michiels CB, Rep M. Pathogen profile update: *Fusarium oxysporum*. *Mol Plant Pathol.* 2009;10:311–324.
33. van Niekerk JM, Groenewald JZ, Farr DF, Fourie PH, Halleen F, Crous PW. Reassessment of *Phomopsis* species on grapevines. *Austral Plant Pathol.* 2005;34:27–39.
34. Leong SL, Hocking AD, Scott ES. *Aspergillus* species producing ochratoxin A: Isolation from vineyard soils and infection of Semillon bunches in Australia. *J Appl Microbiol.* 2007;102:124–133.
35. Perrone G, Susca A, Cozzi G, et al. Biodiversity of *Aspergillus* species in some important agricultural products. *Stud Mycol.* 2007;59:53–66.
36. Blanchette RA, Held BW, Arenz BE, et al. An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. *Microb Ecol.* In press 2010.
37. M  der P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U. Soil fertility and biodiversity in organic farming. *Science.* 2002;296:1694–1697.
38. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci U S A.* 2006;103: 626–631.
39. Bates ST, Garcia-Pichel F. A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: Their diversity and relative contribution to microbial biomass. *Environ Microbiol.* 2009;11:56–67.
40. White RE. The living soil. In: *Understanding Vineyard Soils.* New York, NY: Oxford University Press; 2009.
41. Rapp   MS, Giovannoni SJ. The uncultured microbial majority. *Annu Rev Microbiol.* 2003;57:369–394.
42. Roesch LF, Fulthorpe RR, Riva A, et al. Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J.* 2007;1:283–290.
43. Acosta-Martinez V, Dowd S, Sun Y, Allen V. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. *Soil Biol Biochem.* 2008;40:2762–2770.
44. Bu  e M, Reich M, Murat C, et al. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol.* 2009;184:449–456.
45. Smit E, Leeflang P, Gommans S, van den Broek J, van Mil S, Wernars K. Diversity and seasonal fluctuations of the dominant members of bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl Environ Microbiol.* 2001;67:2284–2291.

Supplementary tables

Table SI Catalog of fungi with the highest sequence similarity to clones by BLAST search and distribution of clones in each library

Phylum	Blast match/clone name	Accession number	ITS size (bp)	Identity (%)	Number of fungal ITS clones											
					K1	K2	K3	K4	A	B	C	D	E	F	G	
Ascomycota	<i>Alternaria tenuissima</i>	FJ766500	220	100							3					1
58 OTUs 210 clones	<i>Arthopyreniaceae</i> sp	FJ439584	220	98										1		
	<i>Ascochyta</i> sp	FJ032642	195	100	1		1									
	<i>Aspergillus japonicus</i>	GQ359413	232	100–99							6				1	
	<i>Aspergillus versicolor</i>	GU232767	211	100–99							7					
	<i>Aureobasidium pullulans</i>	GU062250	235	100–99	2			1			6					
	<i>Chaetomium</i> sp	EU750691	221	100												1
	<i>Cladophialophora chaetospora</i>	EU035406	247	100									1			
	<i>Cladorrhinum samala</i>	FM955447	224	90									2			3
	<i>Cladorrhinum samala</i>	FM955447	227	97						1						
	<i>Cordyceps robertsii</i>	AJ309335	224	95				1								
	<i>Corynascus verrucosus</i>	FJ537093	220	93									1			
	<i>Cylindrocarpon didymum</i>	GU067762	191	100												1
	<i>Cylindrocarpon liriodendri</i>	DQ178166	193	100									1			
	<i>Discosia</i> sp	AF405303	243	95						1						
	<i>Doratomyces nanus</i>	FJ914706	229	99												2
	<i>Exophiala pisciphila</i>	DQ826739	251	100		1										
	<i>Fusarium avenaceum</i>	GU454800	206	99					1							
	<i>Fusarium oxysporum</i>	GU445380	203	100–99							6					1
	<i>Fusarium solani</i> f. <i>radicicola</i>	AB513851	205	100–99			1	1								
	<i>Fusarium</i> sp	EU860071	207	88		1										
	<i>Fusarium</i> sp	GQ505759	203	99			1									
	<i>Fusarium</i> sp	GQ505761	204	100–99					1	1						1
	<i>Hirsutella minnesotensis</i>	DQ345572	230	99					1							
	<i>Lecythophora</i> sp	GU062252	228	97										1		
	<i>Leptodontidium orchidicola</i>	GQ302678	290	100–99				2								
	<i>Leptosphaerulina</i> sp	EF694653	269	100		1										
	<i>Microcypha ellisii</i>	U57493	216	98–96							1	24				2
	<i>Nectria mariannaeae</i>	FJ940904	208	99												1
	<i>Nectria</i> sp.	GU067752	208	99		1									1	
	<i>Onygenales</i> sp	GU212408	234	97										2		
	<i>Penicillium</i> sp	AB468053	228	100				1								
	<i>Penicillium</i> sp	GU270577	195	100					1							
	<i>Pezizomycotina</i> sp	GU212420	227	99–98					21				3			2
<i>Phialocephala fluminis</i>	AF486124	260	86		1											

(Continued)

Table S1 (Continued)

Phylum	Blast match/clone name	Accession number	ITS size (bp)	Identity (%)	Number of fungal ITS clones											
					K1	K2	K3	K4	A	B	C	D	E	F	G	
	<i>Phomopsis</i> sp	GU462150	230	100–99								10				
	<i>Phomopsis</i> sp	GU462149	232	98								10				
	<i>Pilidiella</i> sp	GU062317	261	98	1						1					
	<i>Plectosphaerella cucumerina</i>	GU062300	194	100	1		2									
	<i>Podospora didyma</i>	AY999127	210	89									6			
	<i>Pseudaleuria quinaultiana</i>	EU669387	252	86–85						2					1	
	<i>Sordariomycete</i> sp	EF694651	197	99						1						
	<i>Stilbella</i> sp	DQ993633	209	98												2
	<i>Trichoderma hamatum</i>	GQ331987	238	100–98				1					1	2		2
	Uncultured	EF619630	244	99						1						
	<i>Amphisphaeriaceae</i>															
	Uncultured	AM901745	248	100				1								
	<i>Ascomycete</i>															
	Uncultured	AM901946	202	99				1								
	<i>Ascomycete</i>															
	Uncultured	EU003078	228	98			3									
	<i>Ascomycete</i>															
	Uncultured	EU490130	229	100–99	1	3		3								
	<i>Ascomycete</i>															
	Uncultured	EU520620	202	98–97	1										2	
	<i>Ascomycota</i>															
	Uncultured	FJ440864	228	100									1			
	<i>Ascomycota</i>															
	Uncultured	GQ223459	244	98	1											
	<i>Geopyxis</i>															
	Uncultured	GU055674	199	93–92							5					
	<i>Lasiosphaeriaceae</i>															
	Uncultured	GU055705	216	85											1	
	<i>Tetracladium</i>															
	Uncultured	GU055705	216	100–99	1				3						9	
	<i>Tetracladium</i>															
	Uncultured	GU056018	198	99		1										
	<i>Xylariales</i>															
	<i>Verticillium balanoides</i>	AJ292413	231	98						1						
	<i>Wardomyces inflatus</i>	FJ946485	227	99												1
Basidiomycota	<i>Aqaricus romaquesii</i>	FJ478118	346	99	3											
15 OTUs	<i>Ceratobasidium</i> sp	FJ435147	234	100–99				4								
57 clones	<i>Conocybe rickenii</i>	AY194541	318	81	2											
	<i>Cryptococcus</i> sp	FJ210546	193	100–99	2											
	<i>Mallorcan basidiomycete</i>	AF438588	338	89									2			
	<i>Mycorrhiza</i> of <i>Neottia Nidus-avis</i> (<i>Sebacinaceae</i>) spp	AY052375	240	83				1								
	<i>Nematoctonus tyloporus</i>	EF409766	310	90		1										
	<i>Phlebia livida</i>	AB084618	238	89				1								
	<i>Phlebia</i> sp	AY787680	256	92		2										
	Uncultured	FJ554256	220	95												1
	<i>Agaricomycetes</i>															

(Continued)

Table S1 (Continued)

Phylum	Blast match/clone name	Accession number	ITS size (bp)	Identity (%)	Number of fungal ITS clones												
					K1	K2	K3	K4	A	B	C	D	E	F	G		
	Uncultured <i>Basidiomycete</i>	AM901821	162	100													1
	Uncultured <i>Basidiomycete</i>	AY970109	240	99	1		1	7									
	Uncultured <i>Ceratobasidiaceae</i>	DQ182419	231	96–95		3	2	7									
	Uncultured soil <i>Basidiomycete</i>	DQ672332	219	99–95		1		1									
	Uncultured <i>Tremellomycete</i>	EU030400	206	99						2					2		9
Zygomycota	<i>Endogone lactiflua</i>	AY997045	241	82				1									
6 OTUs	Uncultured <i>Mortierella</i>	FJ553286	202	100–99	2			1									
34 clones	Uncultured <i>Mortierellales</i>	FJ440914	203	88–87						4						7	2
	Uncultured <i>Zygomycete</i>	EF027378	248	95–94							2						
	Uncultured <i>Zygomycete</i>	EU490044	213	85–84	4	8											
	Uncultured <i>Zygomycete</i>	FN295478	196	99													3
Glomeromycota	<i>Glomus irregulare</i>	FJ009618	162	99	1	1		1									
11 OTUs	<i>Glomus</i> sp	AY174689	164	95				1									
16 clones	<i>Glomus</i> sp	FM865557	162	99		1											
	<i>Glomus versiforme</i>	AJ516931	158	98											1		
	Uncultured <i>Glomus</i>	AM494593	174	97				1									
	Uncultured <i>Glomus</i>	AM495189	170	97				1									
	Uncultured <i>Glomus</i>	AM992821	162	100		1											
	Uncultured <i>Glomus</i>	AY236284	167	95–94				1	1								
	Uncultured <i>Glomus</i>	AY236285	168	94				1	1								
	Uncultured <i>Glomus</i>	EU931248	162	93				1	1								
	Uncultured <i>Glomus</i>	FJ769323	162	98						1						2	
Oomycetes	<i>Pythium chamaeaphon</i>	AJ233440	297	97				1									
3 OTUs	<i>Pythium rostratifingens</i>	GQ160433	358	99													1
21 clones	<i>Pythium sylvaticum</i>	DQ528741	346	100–99	4	5	2	2								4	2
Unclassified	Environmental fungal	AM113724	204	95–94					2								1
96 OTUs	Fungal endophyte sp	FJ025263	227	97				1									
260 clones	Fungal sp	FJ612881	225	96											1		
	Fungal sp	FJ236011	226	99						1							
	Soil fungal sp	EU076954	218	95										3			
	Uncultured endophytic fungus	EF505576	225	100						1							
	Uncultured endophytic fungus	FJ524320	289	91	1												
	Uncultured endophytic fungus	FJ882010	206	95							3						
	Uncultured fungus	AY615930	264	99				1									
	Uncultured fungus	AY970243	172	100													1
	Uncultured fungus	DQ294949	206	89													1

(Continued)

Table S1 (Continued)

Phylum	Blast match/clone name	Accession number	ITS size (bp)	Identity (%)	Number of fungal ITS clones												
					K1	K2	K3	K4	A	B	C	D	E	F	G		
	Uncultured fungus	EF434103	217	94													1
	Uncultured fungus	EF521250	234	82	1											1	
	Uncultured fungus	EU096296	319	81		2											
	Uncultured fungus	EU096311	256	93													1
	Uncultured fungus	EU292672	234	82									2				
	Uncultured fungus	EU437428	201	100–99							17				1		
	Uncultured fungus	EU437434	205	99					1								
	Uncultured fungus	EU516926	398	98													1
	Uncultured fungus	FJ213503	246	99	1	1											
	Uncultured fungus	FJ386909	229	99									1				
	Uncultured fungus	FJ758408	208	100	1												
	Uncultured fungus	FJ761134	189	99			1										
	Uncultured fungus	FJ776046	192	96	2			2									
	Uncultured fungus	FJ776073	202	100					9							1	1
	Uncultured fungus	FJ776292	211	100			2			3							
	Uncultured fungus	FJ776415	216	99		1											
	Uncultured fungus	FJ776569	260	98–96			1	1									
	Uncultured fungus	FJ776813	209	99	1												
	Uncultured fungus	FJ777183	208	100													3
	Uncultured fungus	FJ777429	258	97	1												
	Uncultured fungus	FJ777950	247	99	1												
	Uncultured fungus	FJ778071	234	100					1								
	Uncultured fungus	FJ778090	218	98					1								
	Uncultured fungus	FJ778326	220	94	1												
	Uncultured fungus	FJ778637	261	86		3											
	Uncultured fungus	FJ779194	200	100												2	
	Uncultured fungus	FJ779858	215	100						1							
	Uncultured fungus	FJ780520	200	94							2						
	Uncultured fungus	FJ780554	246	93		1											
	Uncultured fungus	FJ780875	218	98												1	
	Uncultured fungus	FJ780914	217	98–95		1	1								1		
	Uncultured fungus	FJ781005	156	96–95				3							1		
	Uncultured fungus	FJ781013	224	93–91			1	1									
	Uncultured fungus	FJ781421	245	97				5									
	Uncultured fungus	FJ781525	249	87		1											
	Uncultured fungus	FJ782853	208	99–97	1		1										
	Uncultured fungus	FJ783024	189	97													1
	Uncultured fungus	FJ783279	222	100					1								
	Uncultured fungus	FJ857928	212	100–99	1		1	2									
	Uncultured fungus	FM999592	226	93												1	
	Uncultured fungus	FN397288	238	95							2						
	Uncultured fungus	FN397382	214	100	1												
	Uncultured fungus	FN397399	233	98						1							
	Uncultured fungus	FN397421	198	99				1									
	Uncultured fungus	GQ225117	244	100–99													5
	Uncultured fungus	GQ225149	205	99											1		
	Uncultured fungus	GQ225160	201	100–99						4						2	
	Uncultured fungus	GQ508521	211	100						1							
	Uncultured fungus	GQ509331	211	100		1											
	Uncultured fungus	GQ509370	211	100						1							
	Uncultured fungus	GQ509504	211	100						1							
	Uncultured fungus	GQ509519	211	100	1												
	Uncultured fungus	GQ510910	211	100	1												
	Uncultured fungus	GQ511189	198	100			1										
	Uncultured fungus	GQ511388	211	100		1											
	Uncultured fungus	GQ513703	262	90											1		
	Uncultured fungus	GQ515874	211	100		1											
	Uncultured fungus	GQ517307	220	100									1				

(Continued)

Table S1 (Continued)

Phylum	Blast match/clone name	Accession number	ITS size (bp)	Identity (%)	Number of fungal ITS clones												
					K1	K2	K3	K4	A	B	C	D	E	F	G		
	Uncultured fungus	GQ523707	211	100		1											
	Uncultured fungus	GQ866183	222	99							1						
	Uncultured fungus	GQ866184	193	98									1				
	Uncultured fungus	GQ866220	220	100–98	4	6				1	1					1	3
	Uncultured fungus	GU078626	217	100–99						1	1		1	1		6	1
	Uncultured fungus	GU078639	222	99		1											
	Uncultured fungus	GU256955	195	100						1							
	Uncultured fungus	GU366724	219	100	1	1										1	1
	Uncultured fungus	GU370749	211	100–99	8	17	1	1			4						
	Uncultured fungus	GU461346	198	100													1
	Uncultured fungus	GU461399	198	100													4
	Uncultured fungus	GU461434	216	100–98									3			1	
	Uncultured fungus	GU461440	234	100												1	
	Uncultured fungus	GU461574	201	100–99													2
	Uncultured mycorrhizal fungus	AB454411	237	100–99	4	2	1	3									
	Uncultured soil fungus	DQ420769	248	90–89	1		1									1	
	Uncultured soil fungus	DQ420800	216	97		1											
	Uncultured soil fungus	DQ420859	187	95	2												
	Uncultured soil fungus	DQ420918	214	100	1												
	Uncultured soil fungus	DQ421065	255	100													1
	Uncultured soil fungus	DQ421073	255	100						2							
	Uncultured soil fungus	DQ421079	259	100													1
	Uncultured soil fungus	DQ421237	228	97–94										1		2	
	Uncultured soil fungus	DQ421243	249	99													1
	Uncultured soil fungus	DQ980584	218	100									1				
	Uncultured soil fungus	EU675985	223	100–99	1		2										3
	Uncultured soil fungus	EU826920	257	100–99						1						3	1
Unidentified	OTU 87		357		1												
36 OTUs	OTU 34		210		1												
84 clones	OTU 14		216		2												
	OTU 39		234		1												
	OTU 66		270		1												
	OTU 33		301			1											
	OTU 20		233			1											
	OTU 13		220			2											
	OTU 26		352			1											
	OTU 104		333			1											
	OTU 44		429			5											
	OTU 105		207			1											
	OTU 60		239			2				1						3	2
	OTU 19		234				1										
	OUT 2		264				2										
	OTU 53		213				1										

(Continued)

Table S1 (Continued)

Phylum	Blast match/clone name	Accession number	ITS size (bp)	Identity (%)	Number of fungal ITS clones														
					K1	K2	K3	K4	A	B	C	D	E	F	G				
	OTU 54		213		5			9	1										
	OTU 56		223					1	2										
	OTU 83		426					11											
	OTU 85		249					4	1										
	OTU 45		320						1										
	OTU 55		235						1										
	OTU 72		249						1										
	OTU 95		352							2									
	OTU 86		250					1	2										
	OTU 108		208										1						
	OTU 109		259															1	
	OTU 110		271												2				
	OTU 111		173									1							
	OTU 112		263															1	
	OTU 113		244															1	
	OTU 114		196																1
	OTU 115		410															1	
	OTU 116		351															1	
	OTU 117		368															1	
	OTU 118		228																1

Abbreviations: ITS, internal transcribed spacer; OTU, operational taxonomic unit; bp, base pair.

Table S2 Catalog of bacteria with the highest sequence similarity to clones by BLAST search and bacterial phyla classified by using Classifier of RDP-X

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
Proteobacteria	BK5-17	<i>Aeromonas punctata</i>	GU205198	493	99
249 clones	BC8-1, BK10-25, F32	Agricultural soil bacterium	AJ252663	487	100-99
	BC5-16	Alpha proteobacterium	D84621	434	98
	D33	Beta proteobacterium	AY162061	485	99
	D27	<i>Burkholderia</i> spp	FJ478405	485	97
	BK11-3	Hyphomicrobium	AB543807	434	99
	A47, A48	<i>Janthinobacterium lividum</i>	AJ585218	487	100-99
	A53	<i>Nitrospira</i> sp	AY684260	485	99
	BK9-17	<i>Nordella</i> sp	AM411927	436	99
	BC9-15	<i>Pedomicrobium manganicum</i>	GU269549	434	98
	F35	<i>Pseudomonas</i> sp	AB461673	485	100
	A54	<i>Pseudomonas</i> sp.	EU681010	485	100
	G14	<i>Pseudomonas</i> sp	EU681013	485	99
	BK11-6	Rhizosphere soil bacterium	AJ252704	500	97
	BK3-35	<i>Sorangium cellulosum</i>	GU299041	500	99
	BK10-33	<i>Sphingomonadaceae</i> bacterium	AB269802	434	98
	BK8-44	Uncultured <i>Alcaligenaceae</i> bacterium	AM936094	485	99
	D41	Uncultured alpha proteobacterium	AY394616	432	100
	BC3-39	Uncultured alpha proteobacterium	AY921760	431	99
	BK12-39	Uncultured alpha proteobacterium	AY921974	421	99
	BC5-42	Uncultured alpha proteobacterium	FM252945	432	99
	BK9-36	Uncultured alpha proteobacterium	FN594676	434	98
	BK5-12	Uncultured bacterium	AB186825	444	95
	BK11-15	Uncultured bacterium	AF392673	483	100
	BC3-25, BC3-33	Uncultured bacterium	AJ576419	497	97
	BC8-38	Uncultured bacterium	AJ863370	434	99
	BK12-46	Uncultured bacterium	AM158328	485	98
	D49	Uncultured bacterium	AM158355	485	97
	BK5-39	Uncultured bacterium	AM909847	498	96

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK7-7	Uncultured bacterium	AM910073	482	97
	E38	Uncultured bacterium	AY425763	434	100
	BC8-17	Uncultured bacterium	AY917444	436	99
	BC3-37, BK7-29	Uncultured bacterium	DQ444030	504	98–97
	B9	Uncultured bacterium	DQ451508	440	97
	BC3-35	Uncultured bacterium	DQ815232	444	94
	D56	Uncultured bacterium	EF018952	436	99
	BC9-6	Uncultured bacterium	EF018996	432	97
	D13	Uncultured bacterium	EF019435	436	96
	BC8-37	Uncultured bacterium	EF019837	479	98
	BC7-34	Uncultured bacterium	EF392901	436	99
	BC7-28	Uncultured bacterium	EF474263	483	98
	B44	Uncultured bacterium	EF492895	485	98
	BK12-31	Uncultured bacterium	EF516458	501	95
	E19	Uncultured bacterium	EF516473	487	98
	G8	Uncultured bacterium	EF589979	444	100
	BK10-20	Uncultured bacterium	EF590047	434	99
	BK9-40	Uncultured bacterium	EF659421	489	96
	BK10-5, BK10-8	Uncultured bacterium	EU133360	432	99
	BK8-24	Uncultured bacterium	EU133467	432	99
	BC5-34	Uncultured bacterium	EU133479	432	98
	BK10-12	Uncultured bacterium	EU133780	484	98
	BK9-8	Uncultured bacterium	EU133792	487	99
	BK9-48	Uncultured bacterium	EU133860	486	99
	B24, B28, BK7-47	Uncultured bacterium	EU134376	504	98–97
	A36	Uncultured bacterium	EU134384	506	95
	BC7-26	Uncultured bacterium	EU134448	499	96
	G56	Uncultured bacterium	EU134453	504	96
	A56	Uncultured bacterium	EU134470	500	97
	B49	Uncultured bacterium	EU134472	499	97
	BC11-2	Uncultured bacterium	EU134486	500	96
	BK4-10	Uncultured bacterium	EU134524	501	93
	E41	Uncultured bacterium	EU134528	504	97
	A2	Uncultured bacterium	EU134706	486	95
	BC7-10	Uncultured bacterium	EU159984	485	98
	C1	Uncultured bacterium	EU160136	487	99
	BC7-9	Uncultured bacterium	EU160209	488	97
	BK9-15	Uncultured bacterium	EU160331	499	98
	BK7-23	Uncultured bacterium	EU280739	582	98
	A18	Uncultured bacterium	EU280747	400	96
	BK5-25	Uncultured bacterium	EU335214	506	98
	BK11-1	Uncultured bacterium	EU335283	506	93
	BC10-18	Uncultured bacterium	EU786147	502	97
	C20	Uncultured bacterium	EU869757	499	90
	BC3-44, BK12-3	Uncultured bacterium	EU881111	434	99
	BC4-12	Uncultured bacterium	EU881166	437	99
	E17	Uncultured bacterium	EU881168	436	99
	BC8-42	Uncultured bacterium	EU881171	436	100
	BC10-2	Uncultured bacterium	EU881183	498	97
	BK12-22	Uncultured bacterium	EU881205	485	99
	BC4-17	Uncultured bacterium	EU881293	499	95
	BK7-34	Uncultured bacterium	EU881332	499	98
	D50	Uncultured bacterium	FJ174981	487	98
	BC11-17	Uncultured bacterium	FJ175095	484	99
	BC12-44	Uncultured bacterium	FJ390543	530	100

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	E28	Uncultured bacterium	FJ409259	434	99
	B13	Uncultured bacterium	FJ409328	485	98
	BC9-9	Uncultured bacterium	FJ444700	485	99
	BK9-16	Uncultured bacterium	FJ478629	432	99
	BK9-47	Uncultured bacterium	FJ478723	504	99
	E46	Uncultured bacterium	FJ478734	434	99
	BK11-26, BK12-26, BK5-2, BK7-36, BC6-48	Uncultured bacterium	FJ478787	434	100-99
	BK9-18	Uncultured bacterium	FJ478818	487	99
	BK9-44	Uncultured bacterium	FJ478838	482	100
	BK11-13	Uncultured bacterium	FJ479038	482	99
	E20, E47	Uncultured bacterium	FJ479291	504	99
	BK6-31	Uncultured bacterium	FJ479372	506	100
	B55	Uncultured bacterium	FJ479441	484	98
	BK11-41	Uncultured bacterium	FJ479441	484	94
	BC9-25	Uncultured bacterium	FJ479505	484	98
	BK5-5	Uncultured bacterium	FJ479545	434	99
	A55	Uncultured bacterium	FJ479588	486	99
	BK10-13	Uncultured bacterium	FJ479597	503	98
	BC10-6	Uncultured bacterium	FJ532345	495	94
	BK11-4	Uncultured bacterium	FJ625364	446	98
	BC11-27	Uncultured bacterium	FJ719080	436	99
	D20, D51	Uncultured bacterium	FM956821	436	99
	BK11-28	Uncultured bacterium	FM956978	444	99
	BC12-7, BC8-7	Uncultured bacterium	FN401278	436	100-99
	B14, B6	Uncultured bacterium	GQ023358	436	99
	BC6-40	Uncultured bacterium	GQ094003	444	98
	D47	Uncultured bacterium	GQ127684	436	100
	BC10-3	Uncultured bacterium	GQ127692	495	97
	D22	Uncultured bacterium	GQ127702	483	99
	BC8-27	Uncultured bacterium	GQ128116	498	97
	D38	Uncultured bacterium	GQ128156	487	99
	BK7-44	Uncultured bacterium	GQ263248	504	97
	A35	Uncultured bacterium	GQ263698	491	100
	BK11-16	Uncultured bacterium	GQ264133	432	99
	BC7-47	Uncultured bacterium	GQ264141	489	88
	BC5-20	Uncultured bacterium	GQ264193	434	99
	A10, A36, BC7-36, BK12-10, BK7-12	Uncultured bacterium	GQ339232	485	99-98
	D55	Uncultured bacterium	GQ376803	451	98
	BK12-45	Uncultured bacterium	GQ376892	504	97
	BK8-9	Uncultured bacterium	GQ376899	436	99
	A13	Uncultured bacterium	GQ376913	487	100
	BC3-5	Uncultured bacterium	GQ376963	444	100
	BC3-48, BK3-51	Uncultured bacterium	GQ376972	434	96
	C27	Uncultured bacterium	GQ376996	501	96
	C45	Uncultured bacterium	GQ376996	501	92
	BK9-14	Uncultured bacterium	GQ461661	485	99
	BC9-23	Uncultured bacterium	GQ461662	503	97
	E51	Uncultured bacterium	GQ500733	484	100
	BC10-13, BC7-31	Uncultured bacterium	GQ500738	445	99-98
	B61	Uncultured bacterium	GQ860038	507	95
	BK5-11	Uncultured bacterium	GQ860122	444	100
	BC5-22	Uncultured bacterium	GQ860162	502	98
	BK3-19	Uncultured bacterium	GQ996436	459	97

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK8-39	Uncultured bacterium	GU256467	434	99
	BC5-24	Uncultured bacterium	GU256481	491	100
	BC8-4	Uncultured bacterium	GU272264	487	100
	BC5-25	Uncultured bacterium	GU444061	453	96
	BK9-7	Uncultured bacterium	GU444084	501	94
	BC11-39	Uncultured bacterium	GU444098	487	99
	D25	Uncultured beta proteobacterium	AB451788	485	99
	E30	Uncultured beta proteobacterium	AY921700	484	99
	G55	Uncultured beta proteobacterium	AY921788	483	95
	BC11-29	Uncultured beta proteobacterium	AY922034	485	98
	BC6-2	Uncultured beta proteobacterium	EU266802	484	99
	A4	Uncultured beta proteobacterium	EU979050	485	99
	BC5-7, BK5-21	Uncultured <i>Bradyrhizobium</i> sp	FJ495217	436	100
	BK9-21	Uncultured <i>Burkholderiales</i> bacterium	AM935842	487	100
	BC11-7	Uncultured <i>Caulobacterales</i> bacterium	EU665092	431	99
	F3	Uncultured <i>Cystobacteraceae</i> bacterium	EF019208	500	98
	G40	Uncultured delta proteobacterium	AM934955	506	99
	BK10-47	Uncultured delta proteobacterium	EF072538	488	96
	BC10-28	Uncultured delta proteobacterium	EF417732	504	94
	G26	Uncultured delta proteobacterium	EF417746	504	99
	C2, C5	Uncultured delta proteobacterium	EF651050	498	99
	F14	Uncultured delta proteobacterium	EF663249	504	99
	BK4-22	Uncultured delta proteobacterium	EU300451	504	98
	BK11-44	Uncultured delta proteobacterium	EU849397	499	92
	C58	Uncultured delta proteobacterium	EU979075	498	98
	BK8-33	Uncultured delta proteobacterium	FJ946562	491	89
	BC4-37, BK5-31	Uncultured <i>Desulfuromonadales</i> bacterium	AM934915	504	97
	BC7-24	Uncultured <i>Desulfuromonadales</i> bacterium	AM935776	504	99
	E49	Uncultured eubacterium	AJ292817	436	97
	C36	Uncultured <i>Ferribacterium</i> sp.	FM176903	487	98
	BC10-22	Uncultured <i>Flavimonas</i> sp	FM175401	485	98
	E11	Uncultured forest soil bacterium	AY913276	456	94
	BC6-26, BC6-30	Uncultured gamma proteobacterium	AM935211	502	100
	A15	Uncultured gamma proteobacterium	AY043794	489	94
	F1	Uncultured gamma proteobacterium	EF188451	485	99
	BC6-19	Uncultured gamma proteobacterium	EU979089	489	99
	BC9-12, E48	Uncultured gamma proteobacterium	EU979089	488	96
	G15, G17	Uncultured <i>Geothermobacter</i> sp	FM176316	505	99
	BC7-41	Uncultured <i>Hyphomicrobiaceae</i> bacterium	EU26680	432	100
	G50	Uncultured <i>Hyphomicrobiaceae</i> bacterium	FM956821	436	99
	BC5-6, E44	Uncultured <i>Nitrosomonadaceae</i> bacterium	EF019260	487	97–96
	A32	Uncultured <i>Oxalobacteraceae</i> bacterium	EF019185	483	99
	BC12-27	Uncultured proteobacterium	DQ828112	436	99
	BC9-48, F11	Uncultured proteobacterium	DQ828343	485	98
	BK3-15	Uncultured proteobacterium	DQ828413	522	91
	D19	Uncultured proteobacterium	DQ828444	487	97
	G7	Uncultured proteobacterium	DQ828498	487	99
	E27	Uncultured proteobacterium	DQ828518	504	99
	BC8-25	Uncultured proteobacterium	DQ829015	445	95
	BK3-33	Uncultured proteobacterium	DQ829067	434	99
	BC9-37	Uncultured proteobacterium	DQ829215	506	97
	BK4-1	Uncultured proteobacterium	DQ829395	506	95
	BC11-10	Uncultured proteobacterium	DQ829567	539	98
	D28, D52	Uncultured proteobacterium	EF019038	487	98
	B51	Uncultured proteobacterium	EF019739	482	98
	BK12-5	Uncultured proteobacterium	EU297804	510	98

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BC12-41	Uncultured proteobacterium	EU299489	498	98
	BC9-8	Uncultured proteobacterium	EU299841	502	97
	G41	Uncultured <i>Rhodobium</i> sp	GU556461	446	97
	BC10-15	Uncultured <i>Rhodocyclaceae</i> bacterium	EF019009	482	98
	C34	Uncultured soil bacterium	AF423222	485	99
	BK7-20	Uncultured soil bacterium	AM884623	485	99
	A20	Uncultured soil bacterium	AY242701	487	99
	BC10-37, BK8-42	Uncultured soil bacterium	AY242719	485	98–97
	BK7-46	Uncultured soil bacterium	AY493946	486	98
	B29, BC4-5	Uncultured soil bacterium	DQ128467	537	98–97
	BC11-18	Uncultured soil bacterium	DQ154524	487	99
	BC3-19	Uncultured soil bacterium	DQ154651	485	99
	BC10-45, BC4-25	Uncultured soil bacterium	DQ297965	504	98
	F26	Uncultured soil bacterium	DQ643718	491	100
	F34	Uncultured soil bacterium	DQ643724	491	100
	BK7-45	Uncultured soil bacterium	GQ918964	496	93
	A30	Uncultured soil bacterium	GU374985	485	98
	BC9-16, BK11-12	Uncultured soil bacterium	GU375476	504	99
	BC3-30	Uncultured <i>Sphingomonas</i> sp	AM936491	433	98
	BC3-10	Uncultured <i>Thermus</i> sp	DQ201611	482	96
Acidobacteria	BK8-48	Agricultural soil bacterium	AJ252612	502	96
236 clones	BC9-18	Bacterial species	Z95736	499	99
	BK5-51	Bacterial species	Z95734	501	99
	BK11-23	Uncultured <i>Acidobacteria</i> bacterium	GQ120660	501	99
	BC6-7	Uncultured <i>Acidobacteria</i> bacterium	GQ120636	501	98
	D26	Uncultured <i>Acidobacteria</i> bacterium	FM176437	502	99
	F16	Uncultured <i>Acidobacteria</i> bacterium	FJ478655	500	99
	BK7-8	Uncultured <i>Acidobacteria</i> bacterium	EU979105	498	99
	BK5-24	Uncultured <i>Acidobacteria</i> bacterium	EU426462	474	97
	BK12-13	Uncultured <i>Acidobacteria</i> bacterium	EU425874	500	97
	E29	Uncultured <i>Acidobacteria</i> bacterium	EU425837	499	98
	A17	Uncultured <i>Acidobacteria</i> bacterium	EU122945	502	97
	BK-18	Uncultured <i>Acidobacteria</i> bacterium	EU122922	501	99
	C51	Uncultured <i>Acidobacteria</i> bacterium	EU122900	501	99
	A45	Uncultured <i>Acidobacteria</i> bacterium	EU122894	501	97
	BC9-46	Uncultured <i>Acidobacteria</i> bacterium	EU122854	448	98
	BK8-29	Uncultured <i>Acidobacteria</i> bacterium	EU122809	500	98
	G28	Uncultured <i>Acidobacteria</i> bacterium	EU122663	499	99
	C40	Uncultured <i>Acidobacteria</i> bacterium	EU122654	501	99
	BC5-30	Uncultured <i>Acidobacteria</i> bacterium	EF664394	240	95
	B43	Uncultured <i>Acidobacteria</i> bacterium	EF457455	502	96
	BK4-27	Uncultured <i>Acidobacteria</i> bacterium	EF457425	499	98
	BC9-36	Uncultured <i>Acidobacteria</i> bacterium	EF457400	444	99
	C17	Uncultured <i>Acidobacteria</i> bacterium	EF457318	498	97
	D30	Uncultured <i>Acidobacteria</i> bacterium	EF447092	497	98
	BK10-3	Uncultured <i>Acidobacteria</i> bacterium	EF417701	481	99
	F44, F48	Uncultured <i>Acidobacteria</i> bacterium	EF019093	450	99
	D35	Uncultured <i>Acidobacteria</i> bacterium	DQ828831	501	99
	B10	Uncultured <i>Acidobacteria</i> bacterium	DQ828203	499	99
	BC6-24	Uncultured <i>Acidobacteria</i> bacterium	DQ828134	501	98
	BK4-18, BK4-19	Uncultured <i>Acidobacteria</i> bacterium	DQ648900	501	96
	BK11-21	Uncultured <i>Acidobacteria</i> bacterium	AY921811	518	95
	BK9-1	Uncultured <i>Acidobacteria</i> bacterium	AY214902	501	98
	A39	Uncultured <i>Acidobacteria</i> bacterium	AM936904	498	99
	G45	Uncultured <i>Acidobacteriaceae</i> bacterium	FM176935	471	98
	C26	Uncultured <i>Acidobacteriaceae</i> bacterium	FM176753	501	99

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	B46, C57	Uncultured <i>Acidobacteriaceae</i> bacterium	FM175766	506	98
	B56	Uncultured <i>Acidobacteriaceae</i> bacterium	EF018918	464	98
	BC10-7, BC7-46	Uncultured <i>Acidobacteriaceae</i> bacterium	AM936873	501	99
	BK4-17	Uncultured <i>Acidobacteriaceae</i> bacterium	AM936618	501	99
	F22	Uncultured <i>Acidobacteriaceae</i> bacterium	AM936458	501	99
	A1	Uncultured <i>Acidobacteriaceae</i> bacterium	AM936237	337	100
	BK5-33	Uncultured <i>Acidobacteriaceae</i> bacterium	AM935516	501	99
	BK9-46	Uncultured <i>Acidobacteriaceae</i> bacterium	AM935398	464	99
	BK5-9	Uncultured <i>Acidobacteriales</i> bacterium	EU276510	498	99
	BK4-28	Uncultured <i>Acidobacteriales</i> bacterium	EU202808	472	98
	BK8-11	Uncultured <i>Acidobacteriales</i> bacterium	AY150900	499	97
	BK3-9	Uncultured <i>Acidobacteriales</i> bacterium	AM935710	472	98
	BK5-19	Uncultured <i>Acidobacteriales</i> bacterium	AM934745	501	99
	BC12-36	Uncultured acidobacterium	GQ302590	481	97
	BC7-11	Uncultured acidobacterium	FM866294	501	98
	BC4-1	Uncultured acidobacterium	GQ302590	481	97
	D36	Uncultured bacterium	GU444075	499	98
	B2	Uncultured bacterium	GU366872	464	99
	BK7-40	Uncultured bacterium	GU359096	501	99
	BK10-27	Uncultured bacterium	GU359062	501	99
	BK12-1	Uncultured bacterium	GU326021	501	99
	BC9-38	Uncultured bacterium	GQ859685	501	99
	BC3-7	Uncultured bacterium	GQ376990	479	98
	D24	Uncultured bacterium	GQ376970	501	97
	G64	Uncultured bacterium	GQ376702	500	99
	D18	Uncultured bacterium	GQ376578	479	97
	C22	Uncultured bacterium	GQ264008	500	96
	C23	Uncultured bacterium	GQ128004	501	96
	A12	Uncultured bacterium	GQ127804	476	97
	BC7-30	Uncultured bacterium	GQ015431	465	99
	BC6-12	Uncultured bacterium	FN565564	463	96
	C12	Uncultured bacterium	FN565561	501	98
	BC6-27	Uncultured bacterium	FN434016	463	99
	BK7-33	Uncultured bacterium	FN401235	465	99
	BK8-37	Uncultured bacterium	FN401214	465	99
	BC3-14, BC3-29, BC8-13, BK6-12, BK8-47, BK9-35, G33	Uncultured bacterium	FM956949	501	99–97
	F33	Uncultured bacterium	FM956340	501	98
	C30	Uncultured bacterium	FJ891039	444	90
	BK10-42	Uncultured bacterium	FJ479576	501	98
	F28	Uncultured bacterium	FJ479553	502	99
	BC9-45, BK9-43, F38, G21	Uncultured bacterium	FJ479522	501	100–98
	BC4-7, BK6-42	Uncultured bacterium	FJ479518	500	99
	C44, C52	Uncultured bacterium	FJ479465	501	94
	F24	Uncultured bacterium	FJ479406	500	98
	BK9-38, F9	Uncultured bacterium	FJ479374	472	99–98
	BK4-42	Uncultured bacterium	FJ479328	481	98
	BC11-22	Uncultured bacterium	FJ479169	498	99
	B47	Uncultured bacterium	FJ479154	474	98
	BK9-11	Uncultured bacterium	FJ479073	498	99
	BC9-4	Uncultured bacterium	FJ479064	501	98
	BC9-41, E3	Uncultured bacterium	FJ479059	498	99
	BC7-44, BK10-15	Uncultured bacterium	FJ479043	501	99
	BC6-23	Uncultured bacterium	FJ478777	499	98

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	D58	Uncultured bacterium	FJ478732	501	97
	F21	Uncultured bacterium	FJ478677	498	98
	BC6-38, BK8-25, BK9-32, BK9-9	Uncultured bacterium	FJ478667	501	99
	BK10-39, C48	Uncultured bacterium	FJ478655	500	99–98
	BK8-30	Uncultured bacterium	FJ478638	501	100
	BK7-30	Uncultured bacterium	FJ478570	501	99
	BK6-36	Uncultured bacterium	FJ478551	499	98
	BK8-7	Uncultured bacterium	FJ444739	501	99
	BC6-14	Uncultured bacterium	FJ444736	500	98
	BC11-44, BK4-3	Uncultured bacterium	FJ444661	501	99
	BC8-36	Uncultured bacterium	FJ409404	466	100
	BC5-14	Uncultured bacterium	FJ409367	501	97
	BC7-17	Uncultured bacterium	FJ409345	499	98
	BC8-14	Uncultured bacterium	FJ175106	481	97
	BC4-18, BK3-38, BK7-13	Uncultured bacterium	EU881338	501	99–97
	BK7-22	Uncultured bacterium	EU881271	500	98
	A34	Uncultured bacterium	EU881240	447	98
	BC6-41, BK10-11	Uncultured bacterium	EU881121	474	99
	BC12-9, BK3-12	Uncultured bacterium	EU881115	474	98
	E23	Uncultured bacterium	EU881094	499	99
	B48	Uncultured bacterium	EU680444	478	100
	C42	Uncultured bacterium	EU680399	488	94
	BC6-10	Uncultured bacterium	EU669643	501	100
	F12	Uncultured bacterium	EU669608	501	98
	BK7-38	Uncultured bacterium	EU428874	501	93
	BC5-44, G30, G39	Uncultured bacterium	EU335268	500	99
	BK4-15	Uncultured bacterium	EU335199	466	98
	C60	Uncultured bacterium	EU335189	501	98
	BC6-21, G20	Uncultured bacterium	EU284522	501	98–96
	BK6-24	Uncultured bacterium	EU280609	503	94
	BC7-32	Uncultured bacterium	EU280600	501	99
	B11	Uncultured bacterium	EU280558	501	97
	BC7-2	Uncultured bacterium	EU234580	481	96
	A37	Uncultured bacterium	EU160390	466	99
	A11, B21	Uncultured bacterium	EU160114	499	99–98
	BC4-36	Uncultured bacterium	EU160017	502	99
	A16, E43, F19, F47	Uncultured bacterium	EU159978	501	99
	BK8-32	Uncultured bacterium	EU132830	507	95
	BC3-11	Uncultured bacterium	EU132407	496	95
	BC12-42	Uncultured bacterium	EU132236	481	95
	C56	Uncultured bacterium	EU132211	498	96
	BC5-39	Uncultured bacterium	EU132123	468	99
	BC6-22	Uncultured bacterium	EU132093	506	90
	BK11-48	Uncultured bacterium	EU132022	481	96
	BK10-9	Uncultured bacterium	EU132015	483	97
	BC8-3, BK12-12, F5	Uncultured bacterium	EU131964	501	100–98
	BC9-3, BK5-18	Uncultured bacterium	EU131918	500	99
	G10	Uncultured bacterium	EU044327	469	96
	G63	Uncultured bacterium	EF600574	448	99
	BC5-46, BC5-47	Uncultured bacterium	EF589982	466	99
	A33, B58	Uncultured bacterium	EF494375	501	97
	G5	Uncultured bacterium	EF494281	478	99
	B17, B41	Uncultured bacterium	EF492945	474	99
	BK4-13, D48	Uncultured bacterium	EF474272	487	97–96

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK6-33, C16	Uncultured bacterium	EF019724	461	99
	E33	Uncultured bacterium	EF018839	501	98
	D2	Uncultured bacterium	EF018668	501	97
	BC12-6	Uncultured bacterium	EF018599	501	98
	D42	Uncultured bacterium	EF018531	501	99
	G29	Uncultured bacterium	DQ833485	450	95
	G51	Uncultured bacterium	DQ463237	475	94
	BK7-32	Uncultured bacterium	DQ404682	501	99
	BK11-31	Uncultured bacterium	AY375053	506	94
	BC3-18	Uncultured bacterium	AY221067	487	95
	B40	Uncultured bacterium	AM909923	502	97
	BK4-31	Uncultured bacterium	AJ876730	497	98
	BK9-20	Uncultured bacterium	AJ863264	498	98
	G62	Uncultured bacterium	AJ863216	500	97
	E54	Uncultured bacterium	AB201600	501	98
	C11	Uncultured <i>Firmicutes</i> bacterium	EU300237	501	99
	G46, G49	Uncultured proteobacterium	EF662498	507	97
	F10, F31	Uncultured soil bacterium	GU375852	496	100
	BC10-30	Uncultured soil bacterium	GU375843	472	99
	F53	Uncultured soil bacterium	GU375570	500	100
	BC3-21	Uncultured soil bacterium	GU375568	479	96
	BC6-46	Uncultured soil bacterium	GU375540	501	97
	BC7-29	Uncultured soil bacterium	EU589261	481	97
	BC9-27, F13	Uncultured soil bacterium	EU554636	500	99–97
	BK3-27	Uncultured soil bacterium	EF688387	448	98
	BK6-28	Uncultured soil bacterium	EF494375	501	97
	BC10-14	Uncultured soil bacterium	EF455414	464	100
	BC7-37	Uncultured soil bacterium	EF455264	501	98
	D9	Uncultured soil bacterium	DQ154624	500	99
	B38, BK11-38	Uncultured soil bacterium	DQ154600	501	98–96
	BC4-9, F25	Uncultured soil bacterium	DQ154585	501	99
	C37	Uncultured soil bacterium	DQ154455	481	96
	B16, B7	Uncultured soil bacterium	DQ154434	498	99–98
	A5, G48	Uncultured soil bacterium	DQ154390	501	97
	BC10-25	Uncultured soil bacterium	DQ128777	461	99
	BK12-20, BK7-37	Uncultured soil bacterium	AY989076	503	98
	BK9-31	Uncultured soil bacterium	AY988661	501	97
	B30	Uncultured soil bacterium	AY493920	472	98
	BC9-42	Uncultured soil bacterium	AY326531	466	100
	D5	Unidentified bacterium	EF605742	503	99
	BC7-20, E12	Unidentified bacterium	EF605719	498	98
	BC7-21	Unidentified bacterium	EF221047	501	98
Bacteroidetes	E14	<i>Flavobacterium</i> sp	AJ319019	479	98
56 clones	A6, A41	<i>Flavobacterium</i> sp	AM934654	479	97
	BK12-8, BK5-15	Rhizosphere soil bacterium	AJ252690	484	97–96
	BK8-40	<i>Sphingoterra</i> bacterium	AB267719	483	95
	E2	Uncultured alpha proteobacterium	EU979053	489	99
	D14	Uncultured bacterium	AM158409	481	97
	G53	Uncultured bacterium	DQ444033	489	100
	BC8-48	Uncultured bacterium	EF020137	485	96
	BK5-6	Uncultured bacterium	EF378163	479	99
	BK5-16	Uncultured bacterium	EF378437	485	99
	BC8-31	Uncultured bacterium	EF393429	492	98
	BK8-31	Uncultured bacterium	EF516306	487	96
	BK7-18	Uncultured bacterium	EU133670	491	99
	BC12-33	Uncultured bacterium	EU133713	484	96

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK9-5	Uncultured bacterium	EU133731	485	95
	BK9-42	Uncultured bacterium	EU160351	482	98
	BC5-2	Uncultured bacterium	EU218971	484	95
	BK9-13	Uncultured bacterium	EU280678	486	97
	BK8-3	Uncultured bacterium	EU431776	489	98
	BC5-31	Uncultured bacterium	EU715822	491	100
	BC9-19, BC9-33, BK4-29	Uncultured bacterium	EU881186	484	95
	BC11-25	Uncultured bacterium	FJ175022	484	96
	BK9-37	Uncultured bacterium	FJ440512	484	96
	BC10-16, BC3-46	Uncultured bacterium	FJ478792	491	97-95
	BC4-14	Uncultured bacterium	FJ478792	491	97
	BC10-8	Uncultured bacterium	FM201103	485	96
	BK9-23	Uncultured bacterium	GQ202640	491	98
	D23	Uncultured bacterium	GQ376555	491	94
	BC9-17	Uncultured bacterium	GU256508	483	97
	BC12-13, BC9-47, BK3-13	Uncultured bacterium	GU256510	483	99
	BK10-43	Uncultured <i>Bacteroidetes</i> bacterium	AM935033	484	93
	BC3-38, BK3-49, BK7-14	Uncultured <i>Bacteroidetes</i> bacterium	AY921902	489	97
	BC10-42	Uncultured <i>Bacteroidetes</i> bacterium	AY921967	491	99
	BK9-3	Uncultured <i>Bacteroidetes</i> bacterium	EF018676	485	98
	BC4-35	Uncultured <i>Flavobacterium</i> spp	DQ628948	479	95
	BC4-43	Uncultured soil bacterium	AY037572	479	98
	BK9-45	Uncultured soil bacterium	AY037613	484	97
	BK7-27	Uncultured soil bacterium	AY493953	484	92
	BK6-35	Uncultured soil bacterium	AY988731	491	95
	BK4-37	Uncultured soil bacterium	DQ643677	483	100
	BC7-42, G4	Uncultured soil bacterium	EF455100	484	98-97
	BK6-15	Uncultured soil bacterium	GU375722	484	98
	A25	Uncultured <i>Sphingobacteriales</i> bacterium	FJ536888	487	98
	BC6-35	Uncultured unclassified bacterium	CU921805	486	94
	BK12-9, BK8-26	Uncultured yard-trimming-compost bacterium	AY095411	483	96
Verrucomicrobia 70 clones	BC12-21	<i>Rhizosphere</i> soil bacterium	AJ252700	491	97
	BK4-14	Uncultured bacterium	AB179534	463	98
	D32	Uncultured bacterium	AJ863246	507	92
	BK4-26	Uncultured bacterium	AM490658	507	99
	BC8-8	Uncultured bacterium	AY218557	509	95
	BK9-22	Uncultured bacterium	DQ521482	507	96
	G59	Uncultured bacterium	EF019315	507	95
	F23	Uncultured bacterium	EF492973	493	99
	BK6-44	Uncultured bacterium	EF516676	507	99
	A14	Uncultured bacterium	EF600576	501	98
	BC5-19	Uncultured bacterium	EU135416	502	95
	BC9-11	Uncultured bacterium	EU135417	491	95
	BC5-5	Uncultured bacterium	EU135425	507	99
	BK5-26	Uncultured bacterium	EU135432	507	98
	B5, BC10-10, BC9-31, BK12-19	Uncultured bacterium	EU135436	507	99-97
	BK8-6	Uncultured bacterium	EU135478	507	98
	BK7-43	Uncultured bacterium	EU135494	509	99
	B54	Uncultured bacterium	EU135506	508	91
	BK4-21, BK6-1, BK6-10, BK7-48	Uncultured bacterium	EU284207	507	97-96

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	B45, G37, G42	Uncultured bacterium	EU335150	509	96–95
	BC7-48, BK9-12	Uncultured bacterium	EU335182	507	99–98
	BK12-14, BK12-43	Uncultured bacterium	EU746731	507	97
	BK7-39	Uncultured bacterium	EU881208	502	99
	BK9-2	Uncultured bacterium	FJ409284	524	95
	BC5-43, BC5-48	Uncultured bacterium	FJ409381	498	93
	BK9-39	Uncultured bacterium	FJ478519	507	99
	BC4-8, BK5-46, F42	Uncultured bacterium	FJ478711	507	100–98
	BK3-2	Uncultured bacterium	FJ478711	507	95
	BC6-42	Uncultured bacterium	FJ479069	507	98
	BC12-15	Uncultured bacterium	FJ479252	506	99
	BC7-33	Uncultured bacterium	FJ479285	507	97
	A24	Uncultured bacterium	GQ127764	502	96
	BC6-34	Uncultured bacterium	GQ128057	500	91
	BC5-12, BK9-27	Uncultured bacterium	GQ264267	485	98
	BK10-18	Uncultured bacterium	GQ472352	507	96
	BK11-34	Uncultured soil bacterium	DQ128695	502	99
	BK3-16	Uncultured soil bacterium	DQ128804	502	99
	BK10-44, BK6-30	Uncultured soil bacterium	DQ154416	507	95–94
	A43	Uncultured soil bacterium	DQ154590	507	96
	BK12-32	Uncultured soil bacterium	GU375614	507	99
	D34	Uncultured <i>Verrucomicrobia</i> bacterium	AY874111	507	97
	BK9-29	Uncultured <i>Verrucomicrobia</i> bacterium	DQ828013	509	92
	BK4-48, BK9-28	Uncultured <i>Verrucomicrobia</i> bacterium	DQ828099	507	98
	BC8-11, BK6-9	Uncultured <i>Verrucomicrobia</i> bacterium	DQ828726	507	95
	BC10-47	Uncultured <i>Verrucomicrobia</i> bacterium	DQ829116	502	100
	BC7-15, BK5-38	Uncultured <i>Verrucomicrobia</i> bacterium	DQ829188	507	97–96
	A7	Uncultured <i>Verrucomicrobia</i> bacterium	DQ829377	501	98
	BK6-2	Uncultured <i>Verrucomicrobia</i> bacterium	EF188508	507	94
	BK4-11	Uncultured <i>Verrucomicrobia</i> bacterium	EF417730	491	97
	BC6-47	Uncultured <i>Verrucomicrobia</i> bacterium	EF662304	507	95
	BK6-23	Uncultured <i>Verrucomicrobium</i> sp	FM866301	506	99
	BC5-18	Unidentified eubacterium	AF010081	507	98
Actinobacteria	A50	<i>Arthrobacter</i> sp.	AY561601	467	99
87 clones	A28, A52	Bacterium Ellin 6023	AY234675	469	98
	BC8-20	<i>Dactylosporangium salmoneum</i>	FJ973607	466	96
	BC9-28, BC9-43	<i>Kibdelosporangium albatum</i>	NR_025570	463	97
	BC4-11	<i>Micromonospora</i> sp	EU437793	461	95
	C53	<i>Nocardioides oleivorans</i>	AB365060	465	98
	BC9-21	<i>Nonomuraea maheshkhaliensis</i>	EU741190	460	99
	BK5-36	<i>Rhodococcus kunmingensis</i>	DQ997045	463	100
	BK3-47	<i>Streptomyces argenteolus</i>	FJ486449	442	100
	BC5-45	<i>Streptomyces</i> sp	FJ857945	463	98
	BK10-38	Uncultured <i>Acidimicrobiales</i> bacterium	FMI75217	469	99
	BC11-33	Uncultured <i>Actinobacteria</i> bacterium	AY922063	466	95
	BC5-9, BK6-11	Uncultured actinobacterium	DQ827792	457	99
	BC3-43, BK3-50	Uncultured actinobacterium	DQ827848	471	97
	BK11-47	Uncultured actinobacterium	DQ827876	497	99
	F4	Uncultured actinobacterium	DQ827895	494	99
	BC4-3	Uncultured actinobacterium	DQ828615	493	99
	BC5-3	Uncultured actinobacterium	DQ828987	427	99
	E42	Uncultured actinobacterium	DQ829467	468	99
	BK7-21	Uncultured actinobacterium	DQ829571	465	100
	C24	Uncultured actinobacterium	GQ115620	470	99
	BK10-41	Uncultured bacterium	DQ125885	467	100
	G68	Uncultured bacterium	DQ125915	469	100

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK11-24	Uncultured bacterium	DQ136135	461	99
	BK8-12	Uncultured bacterium	EF507914	462	95
	BK8-38	Uncultured bacterium	EF590054	464	99
	BK10-23	Uncultured bacterium	EU132491	477	100
	BC11-41	Uncultured bacterium	EU132926	470	98
	BK7-11	Uncultured bacterium	EU133125	471	98
	BK6-8	Uncultured bacterium	EU133143	463	95
	BC3-31	Uncultured bacterium	EU335288	474	99
	BC10-29	Uncultured bacterium	EU335412	494	98
	BK8-27	Uncultured bacterium	FJ409341	470	99
	BC6-9	Uncultured bacterium	FJ409423	494	98
	BK5-37, BK5-48	Uncultured bacterium	FJ478477	495	97
	E37	Uncultured bacterium	FJ478608	463	99
	BC10-26, BC10-35, BC10-5,	Uncultured bacterium	FJ479014	477	99–98
	BC11-28, BC11-32, BC11-35,				
	BC11-45, BC12-19, BC12-25,				
	BC12-26, BC12-28, BC12-3,				
	BC12-35, BC12-4, BC12-43,				
	BC12-45, BC12-46, BC4-4,				
	BK10-2, BK10-28, BK11-19,				
	BK11-22, BK11-30, BK11-32,				
	BK11-33, BK11-43, BK12-15,				
	BK12-42, BC12-44, BC12-7,				
	BK7-31				
	BK6-13	Uncultured bacterium	FJ479120	477	98
	BC4-13	Uncultured bacterium	FJ479349	470	98
	E31	Uncultured bacterium	FJ479510	467	93
	F40	Uncultured bacterium	FJ479512	470	99
	BK4-33	Uncultured bacterium	FJ479550	466	100
	BC12-32	Uncultured bacterium	FJ895064	465	94
	C38	Uncultured bacterium	FN565562	471	100
	BC5-8	Uncultured bacterium	GQ021451	494	98
	BK10-7	Uncultured bacterium	GQ093936	470	97
	D31	Uncultured bacterium	GQ397037	470	100
	BK4-9	Uncultured bacterium	GQ860235	465	95
	BK10-16	Uncultured <i>Geodermatophilaceae</i> bacterium	AM936742	468	98
	A19, BC7-43	Uncultured soil bacterium	AY988680	471	99
	BC3-20	Uncultured soil bacterium	DQ643694	463	99
Firmicutes	A8	Uncultured bacterium	EF516634	492	95
	BK5-14	<i>Bacillus</i> sp.	GQ386842	485	100
Planctomycetes	BK10-29	Uncultured bacterium	AB179502	475	86
33 clones	BC7-23	Uncultured bacterium	AB238007	446	94
	BK4-12, BK8-34	Uncultured bacterium	AM945498	481	98–97
	E52	Uncultured bacterium	AY963398	480	98
	BK7-16	Uncultured bacterium	DQ404719	466	96
	B62	Uncultured bacterium	DQ664070	466	95
	BC8-40	Uncultured bacterium	EF018763	478	97
	BK11-11, BK11-37	Uncultured bacterium	EF580943	454	97
	BK4-50	Uncultured bacterium	EU135081	463	98
	BC7-8	Uncultured bacterium	EU135162	481	97
	BC8-18	Uncultured bacterium	EU135262	468	98
	BC3-16	Uncultured bacterium	EU385780	505	82
	BC4-41	Uncultured bacterium	EU881143	485	98
	C15	Uncultured bacterium	EU881251	482	94
	G12	Uncultured bacterium	FJ409382	452	99
	BC8-26	Uncultured bacterium	FJ479541	463	98

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BC3-8	Uncultured bacterium	FJ561539	444	96
	BK5-40	Uncultured bacterium	FN434034	479	99
	BK11-9	Uncultured bacterium	GQ500843	465	96
	C59	Uncultured bacterium	GQ500856	450	98
	G32	Uncultured bacterium	GU172185	478	93
	BC8-6	Uncultured <i>Planctomycetaceae</i> bacterium	AM935629	462	98
	A23	Uncultured <i>Planctomycete</i>	AF271339	475	98
	B42	Uncultured <i>Planctomycete</i>	DQ828721	480	98
	BC9-13	Uncultured <i>Planctomycete</i>	EF188390	582	93
	BC5-1	Uncultured <i>Planctomycete</i>	DQ334903	455	95
	B67	Uncultured <i>Planctomycetes</i> bacterium	CU922082	481	92
	G23	Uncultured soil bacterium	AY989608	473	96
	BC3-4	Uncultured soil bacterium	DQ154378	463	96
	BK7-1	Uncultured soil bacterium	EF455372	478	99
	E25	Unidentified bacterium	X64377	476	93
Nitrospirae	C32	Uncultured bacterium	DQ664134	488	99
10 clones	BC9-10, BK8-15	Uncultured bacterium	FN565533	488	99
	BC10-32	Uncultured bacterium	GQ500740	488	97
	A40	Uncultured bacterium	GU359089	486	100
	G16	Uncultured <i>Nitrospirae</i> bacterium	EU374034	464	97
	E35, BC5-28	Uncultured <i>Nitrospirae</i> bacterium	FJ535101	488	99
	BC8-44	Uncultured <i>Polyangiaceae</i> bacterium	EF020217	497	94
	BK8-4	Uncultured soil bacterium	DQ154451	488	99
ODI	A46	Uncultured bacterium	AY532554	483	89
	BK12-40	Uncultured bacterium	EF516449	438	89
	BK10-36	Uncultured candidate division ODI bacterium	FJ482173	488	87
OPI0	BC10-1	Uncultured bacterium	EF430426	470	100
	G67	Uncultured bacterium	EU134998	453	96
	BC5-35	Uncultured bacterium	GQ859782	453	96
	D4	Uncultured bacterium	GU183929	453	93
	G54	Uncultured bacterium	AJ271048	450	95
OPI1	BC8-28	Uncultured candidate division OPI1 bacterium	FJ482190	438	91
TM7	BC8-41	Uncultured bacterium	AM991262	533	90
	BC9-32	Uncultured bacterium	FJ478502	493	96
	BK5-10	Uncultured bacterium	FN297914	613	94
	BK9-41	Uncultured bacterium	GQ396865	528	93
WS3	BC6-20	Uncultured bacterium	DQ828640	504	99
	BC7-7	Uncultured soil bacterium	DQ154428	505	99
Gemmatimonadetes	G35	Uncultured bacterium	AF432633	469	98
34 clones	BK6-27	Uncultured bacterium	EF393220	469	96
	BK12-11	Uncultured bacterium	EU134803	470	93
	BK8-28	Uncultured bacterium	EU134827	472	95
	BC6-32	Uncultured bacterium	EU134844	476	94
	BC8-21	Uncultured bacterium	EU134844	470	98
	BK6-4	Uncultured bacterium	EU160218	461	98
	BK10-24, BK7-42	Uncultured bacterium	EU335213	493	98
	B34	Uncultured bacterium	EU335434	498	99
	BC11-24, BC9-39, BK8-5	Uncultured bacterium	EU881161	472	100
	E13	Uncultured bacterium	FN565521	470	100
	C8	Uncultured bacterium	GQ263125	475	98
	BK9-6	Uncultured bacterium	GQ402821	468	89
	BC10-27	Uncultured <i>Gemmatimonadales</i> bacterium	FM209166	468	96
	G38	Uncultured <i>Gemmatimonadales</i> bacterium	AY217502	493	99
	BK7-19	Uncultured <i>Gemmatimonadales</i> bacterium	AY921858	475	99

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	E9	Uncultured <i>Gemmatimonadales</i> bacterium	DQ451476	472	93
	BC6-37	Uncultured <i>Gemmatimonadales</i> bacterium	DQ828232	482	95
	BK11-17, BK3-43	Uncultured <i>Gemmatimonadales</i> bacterium	EF019245	475	99
	BC9-1	Uncultured <i>Gemmatimonadales</i> bacterium	EF664012	386	97
	BK3-30	Uncultured <i>Gemmatimonas</i> sp	FJ946547	468	97
	BK6-43	Uncultured soil bacterium	DQ128528	470	94
	BC6-25, BK9-19	Uncultured soil bacterium	DQ128836	482	98
	BK8-23	Uncultured soil bacterium	DQ128836	479	93
	BK7-10	Uncultured soil bacterium	DQ154587	472	99
	BC11-15	Uncultured soil bacterium	EF540397	467	97
	B12, B4	Uncultured soil bacterium	FJ621029	493	97
	BC9-20	Uncultured soil bacterium	FJ621069	469	99
Chloroflexi 43 clones	BK5-43, BK7-26	Uncultured <i>Anaerolineae</i> bacterium	AM935741	470	99–98
	BC12-18, BC6-13	Uncultured bacterium	AB240474	470	100
	BK11-36	Uncultured bacterium	AM158349	444	95
	G2	Uncultured bacterium	AM162480	470	97
	BC10-12	Uncultured bacterium	AY221060	461	98
	D43	Uncultured bacterium	DQ067035	470	97
	BC4-44, F46	Uncultured bacterium	DQ093879	470	99
	BC12-34	Uncultured bacterium	DQ444024	470	99
	BK3-7	Uncultured bacterium	DQ444105	470	100
	BK7-9	Uncultured bacterium	DQ828928	468	97
	BC5-4, BC8-2	Uncultured bacterium	EF667828	470	99
	BC4-27	Uncultured bacterium	EU134021	444	94
	BK3-28	Uncultured bacterium	EU134025	470	98
	BC4-26, E18	Uncultured bacterium	EU134065	451	93–92
	BK4-34	Uncultured bacterium	EU134147	470	99
	BK11-20	Uncultured bacterium	EU280640	470	99
	BC12-24	Uncultured bacterium	EU335201	448	95
	BK7-17	Uncultured bacterium	FJ175011	470	99
	BC7-4	Uncultured bacterium	FJ415684	470	98
	BC4-20	Uncultured bacterium	FJ478597	470	99
	BK10-31, BK10-32	Uncultured bacterium	FJ478654	470	98
	BC11-16, BC12-48, BC3-15, BC7-39, BK6-38	Uncultured bacterium	FJ479268	470	99–98
	BC7-27	Uncultured bacterium	GQ859931	470	98
	BK5-22	Uncultured bacterium	GU325870	470	98
	BC10-40	Uncultured bacterium	GU390324	470	99
	BC8-34, BC8-35, BK6-5	Uncultured <i>Chloroflexi</i> bacterium	EF220771	470	99
	BK5-23	Uncultured <i>Chloroflexi</i> bacterium	GQ267091	471	90
	BC6-43	Uncultured soil bacterium	DQ154387	470	99
	BC4-2, BC4-40, BK5-41	Uncultured soil bacterium	DQ154570	470	100–99
Cyanobacteria	BC4-15	Uncultured bacterium	GQ109114	438	100
	BC12-37	Uncultured bacterium	FJ390538	438	100
Spirochaetes	BC12-22	<i>Turneriella parva</i>	AY398688	445	93
Unclassified bacteria 238 clones	BK3-11	<i>Mortierella verticillata</i>	AY863211	409	94
	A51	Uncultured <i>Acidobacteria</i> bacterium	AM935472	506	96
	BC10-34, BK4-24	Uncultured <i>Acidobacteria</i> bacterium	DQ827894	506	94–93
	BC4-48	Uncultured <i>Acidobacteria</i> bacterium	DQ828059	506	97
	BC6-15, BC6-18	Uncultured <i>Acidobacteria</i> bacterium	DQ828073	506	97
	BC10-4, BK6-3	Uncultured <i>Actinobacteria</i> bacterium	AY922024	493	99
	BC5-40	Uncultured bacterium	AB237977	462	97
	A21	Uncultured bacterium	AF234118	485	93
	BC9-30	Uncultured bacterium	AF392792	490	94
	F18	Uncultured bacterium	AF432840	505	98

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BC3-2	Uncultured bacterium	AM085466	492	95
	A26	Uncultured bacterium	AM181963	501	83
	BK4-16	Uncultured bacterium	AM410034	492	95
	BC11-14, BK10-26	Uncultured bacterium	AM905671	500	96–95
	BC7-25, BC8-46	Uncultured bacterium	AM997454	455	85–84
	C55	Uncultured bacterium	AY221070	445	98
	BK12-23	Uncultured bacterium	DQ017911	459	97
	BK4-30	Uncultured bacterium	DQ310742	484	95
	F52	Uncultured bacterium	DQ444020	457	95
	BC11-8	Uncultured bacterium	DQ444114	480	89
	BK6-12	Uncultured bacterium	DQ510031	491	90
	C35, C54	Uncultured bacterium	DQ664088	481	98
	BC4-33	Uncultured bacterium	DQ791253	488	78
	BK10-4	Uncultured bacterium	DQ828137	463	95
	BK4-2, D40	Uncultured bacterium	DQ828297	453	94
	BC9-35	Uncultured bacterium	DQ829107	477	93
	E4	Uncultured bacterium	DQ829487	505	99
	C29	Uncultured bacterium	DQ906801	482	95
	G61	Uncultured bacterium	DQ906875	506	95
	BC4-23, BC5-32	Uncultured bacterium	EF018468	462	99–98
	BK7-24	Uncultured bacterium	EF019124	455	98
	BC5-36	Uncultured bacterium	EF019253	470	91
	BK5-50	Uncultured bacterium	EF020070	501	98
	BC7-3	Uncultured bacterium	EF020290	433	94
	BC5-41	Uncultured bacterium	EF393365	456	98
	D7	Uncultured bacterium	EF494294	475	88
	E40	Uncultured bacterium	EF515989	456	96
	BK10-46	Uncultured bacterium	EF516008	496	93
	BK6-48	Uncultured bacterium	EF516242	462	97
	BK4-8	Uncultured bacterium	EF516747	433	91
	B8	Uncultured bacterium	EF588346	473	98
	BC10-17, BC6-44	Uncultured bacterium	EF632929	482	96
	BK12-38	Uncultured bacterium	EU015112	446	90
	A58	Uncultured bacterium	EU043640	494	96
	A31	Uncultured bacterium	EU131926	506	94
	D10	Uncultured bacterium	EU132345	505	98
	BC6-11	Uncultured bacterium	EU132690	493	98
	BK8-19	Uncultured bacterium	EU132730	493	100
	BC3-36	Uncultured bacterium	EU132994	480	95
	BK11-10	Uncultured bacterium	EU133191	493	99
	BC11-42, BC12-29, BC12-38, BC12-47	Uncultured bacterium	EU133988	449	95–94
	BC3-24	Uncultured bacterium	EU134012	453	98
	BC5-29	Uncultured bacterium	EU134013	455	98
	BK11-42	Uncultured bacterium	EU134018	455	93
	BK4-23	Uncultured bacterium	EU134026	463	97
	BC4-42, BC7-19	Uncultured bacterium	EU134079	448	97–95
	BC3-32, BK5-7	Uncultured bacterium	EU134126	485	95–93
	BK6-37, BK7-28, E1, E10	Uncultured bacterium	EU134126	462	100–97
	BC7-1, BK8-17	Uncultured bacterium	EU134153	459	97
	A22, B69	Uncultured bacterium	EU134231	449	96–95
	BC9-22	Uncultured bacterium	EU134315	512	97
	F8	Uncultured bacterium	EU134491	504	95
	BK5-32	Uncultured bacterium	EU134688	482	88
	BC4-19	Uncultured bacterium	EU134816	562	95

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK11-7	Uncultured bacterium	EU134836	493	96
	BC9-14, BK4-6	Uncultured bacterium	EU135006	452	98
	BC8-23	Uncultured bacterium	EU135008	467	90
	BK7-15	Uncultured bacterium	EU135025	471	90
	BC8-16	Uncultured bacterium	EU135055	446	95
	BC8-30	Uncultured bacterium	EU135195	489	96
	BC11-30	Uncultured bacterium	EU135283	451	96
	BC12-40	Uncultured bacterium	EU135294	519	99
	BK3-10	Uncultured bacterium	EU135325	496	93
	A38	Uncultured bacterium	EU135338	505	96
	C13	Uncultured bacterium	EU135355	500	94
	A3, D15	Uncultured bacterium	EU135357	506	97–96
	BC10-23	Uncultured bacterium	EU160080	503	93
	BK12-30	Uncultured bacterium	EU335177	495	96
	F29	Uncultured bacterium	EU335213	493	98
	BC4-16, BC5-37, BK5-13	Uncultured bacterium	EU335214	506	98–97
	BC6-31	Uncultured bacterium	EU335271	488	98
	BC3-27	Uncultured bacterium	EU335413	464	96
	B3	Uncultured bacterium	EU335434	495	97
	BK10-22	Uncultured bacterium	EU385780	506	82
	B15	Uncultured bacterium	EU386030	616	95
	B53	Uncultured bacterium	EU386045	462	92
	G47	Uncultured bacterium	EU557952	455	98
	BC3-28	Uncultured bacterium	EU644764	537	85
	BC11-26	Uncultured bacterium	EU669607	460	92
	BC6-39	Uncultured bacterium	EU680389	482	97
	E36	Uncultured bacterium	EU881132	505	97
	BC11-31	Uncultured bacterium	EU881151	484	98
	A9	Uncultured bacterium	EU881154	505	98
	BC8-22	Uncultured bacterium	EU881212	445	98
	G9	Uncultured bacterium	EU881279	467	99
	A42	Uncultured bacterium	EU881343	505	98
	BC11-21, BC4-22, BK11-5	Uncultured bacterium	EU881358	485	94
	B19	Uncultured bacterium	EU917737	471	88
	BK3-6	Uncultured bacterium	FJ409340	474	93
	BK8-20	Uncultured bacterium	FJ409340	471	77
	G25	Uncultured bacterium	FJ409355	453	100
	E34	Uncultured bacterium	FJ478646	505	97
	BC7-12	Uncultured bacterium	FJ478663	461	96
	F45	Uncultured bacterium	FJ478699	444	95
	BK10-6	Uncultured bacterium	FJ478736	431	92
	BC6-1	Uncultured bacterium	FJ478869	505	99
	BK11-35	Uncultured bacterium	FJ479014	477	97
	BC4-47	Uncultured bacterium	FJ479108	467	94
	BK5-8, F41	Uncultured bacterium	FJ479108	467	98–97
	B59	Uncultured bacterium	FJ479177	466	94
	BK7-35	Uncultured bacterium	FJ479204	472	89
	BK8-1	Uncultured bacterium	FJ479317	493	99
	BK5-27	Uncultured bacterium	FJ479355	450	98
	G18	Uncultured bacterium	FJ479422	505	97
	E8	Uncultured bacterium	FJ592933	483	97
	G11	Uncultured bacterium	FJ615979	446	99
	A44	Uncultured bacterium	FJ625343	491	92
	BK12-25	Uncultured bacterium	FJ649511	463	85
	BC5-17	Uncultured bacterium	FJ748815	495	89
	D46	Uncultured bacterium	FJ902567	478	94
	BK10-19	Uncultured bacterium	FM956309	490	87

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK3-34	Uncultured bacterium	FM956978	469	92
	BC4-24	Uncultured bacterium	FN434032	463	90
	BC7-18	Uncultured bacterium	GQ015431	465	98
	BK6-46	Uncultured bacterium	GQ339116	457	98
	BK12-33	Uncultured bacterium	GQ347152	453	85
	G36	Uncultured bacterium	GQ359399	462	86
	D1	Uncultured bacterium	GQ376896	493	98
	C47	Uncultured bacterium	GQ376969	506	98
	B23, D45	Uncultured bacterium	GQ376998	470	99–98
	C62	Uncultured bacterium	GQ402727	505	87
	BC8-10	Uncultured bacterium	GQ402788	481	97
	BC10-9, BK9-25	Uncultured bacterium	GQ472345	514	94
	BC7-13	Uncultured bacterium	GQ500715	484	92
	BK10-34	Uncultured bacterium	GQ859793	488	97
	BK11-39, BK11-40, BK8-13	Uncultured bacterium	GQ859817	462	96
	G27	Uncultured bacterium	GQ860162	502	97
	BK11-18	Uncultured bacterium	GQ860182	463	95
	F17	Uncultured bacterium	GU326072	475	92
	BC6-36	Uncultured bacterium	GU366869	504	93
	F30, F39	Uncultured candidate division SAM bacterium	AY192283	484	92
	BK7-2, BK7-3, C14	Uncultured candidate division SPAM bacterium	AM936223	503	99–98
	BC11-12	Uncultured candidate division WS3 bacterium	AM935389	466	99
	BC5-23	Uncultured <i>Chloroflexi</i> bacterium	EF188525	459	96
	C31, C33	Uncultured <i>Chloroflexi</i> bacterium	FJ175075	455	91
	BK7-5	Uncultured <i>Chloroflexi</i> bacterium	FJ535096	463	98
	BK12-34	Uncultured <i>Cystobacteraceae</i> bacterium	FM176285	487	92
	BC8-32	Uncultured earthworm cast bacterium	AY037734	470	87
	BK10-37, BK10-40	Uncultured <i>Firmicutes</i> bacterium	EF662862	507	97
	BC6-33	Uncultured <i>Gemmatimonadetes</i> bacterium	AY795667	517	94
	BC9-26	Uncultured <i>Gemmatimonadetes</i> bacterium	AY921682	499	96
	BC4-45, BK4-7	Uncultured <i>Gemmatimonadetes</i> bacterium	AY921705	486	94–93
	B60, D12, G34	Uncultured <i>Gemmatimonadetes</i> bacterium	DQ828970	497	96
	BC8-12	Uncultured <i>Geothermobacter</i> sp.	FM176362	462	98
	BK5-34	Uncultured <i>Methylothermus</i> sp.	FM176282	435	97
	BK5-44	Uncultured organism	GQ391452	441	91
	BK8-10	Uncultured planctomycete	AF271338	468	82
	BC9-5	Uncultured planctomycete	AY921932	446	99
	BC4-29	Uncultured planctomycete	EF447090	489	96
	BC10-21	Uncultured prokaryote	GU208269	506	97
	BC7-38	Uncultured prokaryote	GU208285	434	98
	BC4-21	Uncultured proteobacterium	EU297116	447	97
	BC7-5, BC7-6	Uncultured proteobacterium	EU297681	434	98–97
	B37	Uncultured soil bacterium	AF507682	451	86
	B39	Uncultured soil bacterium	AF507700	466	93
	BC8-45	Uncultured soil bacterium	AY037564	471	92
	BK12-35	Uncultured soil bacterium	AY037564	471	99
	BC7-40	Uncultured soil bacterium	AY493960	457	89
	BK10-30	Uncultured soil bacterium	AY988880	480	94
	BC12-17	Uncultured soil bacterium	AY989142	489	90
	B18	Uncultured soil bacterium	AY989303	505	97

(Continued)

Table S2 (Continued)

Phylum	Clone name	Blast match	Accession number	16S rDNA size (bp)	Identity (%) ^a
	BK12-16	Uncultured soil bacterium	AY989453	501	95
	BC11-9, BC8-47, E39, BK11-46	Uncultured soil bacterium	DQ128836	479	93–92
	BC5-13, BK10-10, BK12-21, BK7-4	Uncultured soil bacterium	DQ128836	482	98–97
	BK8-18	Uncultured soil bacterium	DQ154470	503	97
	C21	Uncultured soil bacterium	DQ298008	477	87
	B68	Uncultured soil bacterium	EU861851	455	90
	BK10-17, E21	Uncultured soil bacterium	FJ621029	493	98
	D44	Uncultured soil bacterium	GU082820	446	99
	BC8-39	Uncultured soil bacterium	GU375142	455	98
	E6	Uncultured soil bacterium	GU375191	503	88
	BK4-25	Uncultured soil bacterium	GU375444	500	98
	BK6-16	Uncultured soil bacterium	GU375444	500	95
	BC10-31, BC10-41	Uncultured soil bacterium	GU375616	486	97
	BC3-22	Uncultured soil bacterium	GU375628	579	91
	E32	Uncultured soil bacterium	GU375654	489	96
	BK5-28	Uncultured <i>Thiorhodospira</i> sp	FM177025	483	95
	BK11-14	Unidentified bacterium	EF220517	503	99
	BC3-13	Unidentified bacterium	EF605627	445	92
	BK8-16	Unidentified bacterium	EF605970	470	97
	BK9-24	Unidentified bacterium	EF605978	443	94

Note: ^a16S rDNA gene sequences with >97% similarity are considered to be the same species in phylogenetic position.

Abbreviation: bp, base pair.

International Journal of Wine Research

Dovepress

Publish your work in this journal

The International Journal of Wine Research is an international, peer-reviewed open-access, online journal focusing on all scientific aspects of wine, including: vine growing; wine elaboration; human interaction with wine; and health aspects of wine. The journal provides an open access platform for the reporting

of evidence based studies on these topics. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from some of our published authors.

Submit your manuscript here: <http://www.dovepress.com/international-journal-of-wine-research-journal>