J. Microbiol. Biotechnol. (2007), **17**(1), 168–175

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

"Bring to Lab" of 19 Novel Species Among 60 Isolates Retrieved from a Freshwater Pond

SONG, JAEHO, SEUNG-JO YANG, AND JANG-CHEON CHO*

Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Korea

Received: July 12, 2006
Accepted: September 19, 2006

Abstract We report here on the cultivation of numerous novel bacterial species from a eutrophic freshwater pond. A total of 60 strains, 15 strains per each culture medium, were obtained from the surface of a eutrophic freshwater pond by employing a conventional dilution-plating method with four different kinds of culture media, including R2A, 1/10R2A, PCA, and 1/10PCA. Among the 60 strains isolated, 27 strains showed less than 97% 16S rRNA gene sequence similarities to validly published species, and thus they are considered to comprise 19 novel species. Of the 27 strains assigned to the novel species, the majority of the strains (20 strains) were affiliated with the *Alphaproteobacteria* and *Betaproteobacteria*. The remaining 7 strains were affiliated with the *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Deinococci*. Because we have isolated 19 novel species from a usual freshwater pond using a conventional culturing technique, our results suggest that an unexplored ecosystem, even if it looks like a common ecosystem found elsewhere, harbors diverse unidentified microbes, which will be definitely further characterized.

Key words: Cultivation, freshwater, novel species, 16S rRNA gene, bacterial diversity

Owing to the increasing number of studies using molecular ecological and phylogenetic analyses based on 16S rRNA genes, it is now widely accepted that only a small fraction of the existing microbial diversity can be cultured by conventional cultivation techniques [8, 12, 13, 17, 18]. In this sense, cultivation approaches alone cannot reveal the whole microbial community in a given ecosystem. There is, however, still a need to cultivate previously uncultured bacteria because the ecophysiological role and ecosystem function of bacterial populations can be fully understood only when they are successfully brought to the laboratory.

*Corresponding author
Phone: 82-32-860-7711; Fax: 82-32-876-5541;
E-mail: chojc@inha.ac.kr

Although freshwater microbial ecology and limnology have a relatively long history of studies, most 16S rRNA gene based studies have been mainly focused on marine and soil ecosystems [2, 5, 13, 14, 16, 17, 20, 34]. The 16S rRNA gene sequence analyses without cultivation, in spite of limited information on bacterial composition in freshwater systems, have revealed that the previously uncultured bacterial fractions comprise ecologically significant freshwater bacterial populations [9, 11, 23, 35, 36].

High throughput cultivation approaches based on dilution-to-extinction [4, 7] and the Microdrop microdispenser system [3] have a great potential to isolate previously uncultured and non-colony-forming bacteria [19, 24, 27]. The approaches, however, have a shortcoming in the typical bacterial classification system if extinction cultures do not form colonies on the agar surface, because standard bacterial characterization requires a large quantity of biomass for biochemical characterizations. Although the routine cultivation method using solid agar plates is out of fashion, many freshwater isolates have been isolated continuously using different types of agar media containing relevant carbon concentrations [10, 15]. Here, we report on the cultivation of multiple novel species from a eutrophic freshwater pond. A total of 60 isolates were recovered from a freshwater sample. Among them, 19 novel species were phylogenetically identified.

The study site, Inkyoung pond, is a small artificial pond located inside Inha University, Incheon, Korea (37° 26.59' N, 126° 39.21' E). The pond is a shallow (maximum depth, 1.5 m; average 1.0 m) eutrophic lake receiving an urban input. A freshwater sample was collected from the surface of the freshwater pond in August 2005. The temperature, pH, conductivity, and Secchi depth at the sampling site were 24.3°C, 8.5, 407.1 μS, and 0.45 m, respectively. The freshwater was serially diluted in sterile deionized water, and 100 μl of the serially diluted samples were spread onto four different agar media, including R2A agar (Difco Laboratories) [25], R2A agar diluted in deionized water

Table 1. Number of isolates representing novel species affiliated with major bacterial phyla or classes.

Phylum or class	No. of isolates (No. of strains representing novel species)				
	R2A	1/10R2A	PCA	1/10PCA	Total
Alphaproteobacteria	5 (2)	3 (2)	6 (2)	3 (3)	17 (9)
Betaproteobacteria	4 (3)	6 (5)	3 (2)	2 (1)	15 (11)
Gammaproteobacteria	2 (1)	3 (0)	0	0	5 (1)
Bacteroidetes	0	1 (0)	0	2 (0)	3 (0)
Firmicutes	2 (0)	2 (0)	4 (0)	5 (2)	13 (2)
Actinobacteria	0	0	2 (0)	2 (2)	4 (2)
Deinococci	2 (2)	0	0	1 (0)	3 (2)
Total	15 (8)	15 (7)	15 (4)	15 (8)	60 (27)

1:10 (v/v) (1/10 R2A), plate count agar (PCA, Difco Laboratories, digest of casein 5.0 g, yeast extract 2.5 g, dextrose 1 g, agar 15 g per 1 l of deionized water), and PCA diluted in deionized water 1:10 (v/v) (1/10 PCA). All the agar plates were aerobically incubated at 20°C for 5 days. Well-isolated 15–16 colonies per each medium were randomly selected to be identified.

Bacterial genomic DNA was extracted from single colonies using a Core-one bacterial genomic DNA extraction kit (Corebio, Korea). The 16S rRNA genes were amplified from all the colonies isolated by polymerase chain reaction (PCR) using the primers of 27F-B and 1492R as described previously [4]. The PCR products were grouped by restriction fragment length polymorphism (RFLP) analyses employing HaeIII restriction. All the isolates (finally 60 isolates) were basically subjected to be partially sequenced using the 27F-B primer, and the 16S rRNA genes of the isolates showing unique RFLP patterns were almost fully sequenced using the primers of 27F-B, 519F (5'-CAGCMGCCGCGGTAATWC-3'), 926F (5'-AAACT-YAAAKGAATTGACGG-3'), and 1492R. The 16S rRNA gene sequences of freshwater isolates sharing over 97% sequence similarity were grouped in the same operational taxonomic unit as the same species, based on the practical value for species demarcation [28]. The 16S rRNA gene sequences were aligned automatically and corrected manually using the ARB software package [21] against ca. 52,400 aligned reference 16S rRNA gene sequences. Aligned sequences including partial sequences were extracted from the ARB package and transported to PAUP* 4.0 beta 10 [26]. The neighbor-joining trees were generated using Jukes-Cantor distance parameter in PAUP and evaluated by bootstrap analyses based on 1,000 resamplings.

The 16S rRNA gene sequence analyses grouped the freshwater isolates into 38 distinct species, according to the practical cut-off value of 97% 16S rRNA gene sequence similarity [28]. A total of 27 strains, including 8 strains from R2A, 7 strains from 1/10R2A, 4 strains from PCA, and 8 strains from 1/10PCA, were found to comprise 19 novel species (Table 1, Table 2). Only 4 strains categorized as novel species were isolated from the highest organic

Table 2. Phylogenetic affiliations and novelty of isolates cultivated on different media from a freshwater pond.

Phylum or class	Medium	Isolate (Accession No.)	Closest related and validly published species	Similarity (%)	Novelty[a]
Alphaproteobacteria	R2A	IMCC1702 (DQ664257)	*Catellibacterium nectariphilum* (AB101543)	94.0	sp. nov. (13)
Alphaproteobacteria	R2A	IMCC1706 (DQ664200)	*Caulobacter vibrioides* (AJ227756)	99.7	-
Alphaproteobacteria	R2A	IMCC1708 (DQ664215)	*Bosea massiliensis* (AF288309)	99.2	-
Alphaproteobacteria	R2A	IMCC1709 (DQ664232)	*Caulobacter fusiformis* (AJ227759)	99.8	-
Alphaproteobacteria	R2A	IMCC1710 (DQ664216)	*Novosphingobium stygium* (U20775)	95.6	sp. nov. (11)
Betaproteobacteria	R2A	IMCC1705 (DQ664251)	*Panaciterramonas fulva* (AB245357)	95.8	sp. nov. (1)
Betaproteobacteria	R2A	IMCC1707 (DQ664214)	*Hydrogenophaga atypical* (AJ585992)	99.7	-
Betaproteobacteria	R2A	IMCC1713 (DQ664238)	*Roseateles depolymerans* (AB003625)	96.1	sp. nov. (6)
Betaproteobacteria	R2A	IMCC1716 (DQ664239)	*Azonexus fungiphilus* (AF011350)	95.6	sp. nov. (8)
Gammaproteobacteria	R2A	IMCC1703 (DQ664206)	*Stenotrophomonas maltophilia* (X95923)	98.5	-
Gammaproteobacteria	R2A	IMCC1704 (DQ664237)	*Alkanindiges illinoisensis* (AF513979)	92.2	sp. nov. (10)
Firmicutes	R2A	IMCC1701 (DQ664199)	*Staphylococcus saprophyticus* (L37596)	99.9	-
Firmicutes	R2A	IMCC1714 (DQ664217)	*Staphylococcus saprophyticus* (L37596)	99.9	-
Deinococci	R2A	IMCC1711 (DQ664256)	*Deinococcus radiophilus*(Y11333)	93.6	sp. nov. (19)
Deinococci	R2A	IMCC1715 (DQ664213)	*Deinococcus grandis* (Y11329)	95.3	sp. nov. (18)

Table 2. Continued.

Phylum or class	Medium	Isolate (Accession No.)	Closest related and validly published species	Similarity (%)	Novelty[a]
Alphaproteobacteria	1/10R2A	IMCC1720 (DQ664202)	*Xanthobacter flavus*(X94199)	99.6	-
Alphaproteobacteria	1/10R2A	IMCC1725 (DQ664243)	*Novosphingobium stygium* (U20775)	95.3	sp. nov. (11)
Alphaproteobacteria	1/10R2A	IMCC1730 (DQ664218)	*Novosphingobium stygium* (U20775)	95.5	sp. nov. (11)
Betaproteobacteria	1/10R2A	IMCC1718 (DQ664252)	*Mitsuaria chitosanitabida* (AB006851)	96.6	sp. nov. (3)
Betaproteobacteria	1/10R2A	IMCC1721 (DQ664240)	*Aquabacterium parvum* (AF035052)	96.8	sp. nov. (2)
Betaproteobacteria	1/10R2A	IMCC1722 (DQ664241)	*Ideonella dechloratans* (X72724)	98.2	-
Betaproteobacteria	1/10R2A	IMCC1723 (DQ664242)	*Rhodoferax fermentans* (D16211)	96.9	sp. nov. (7)
Betaproteobacteria	1/10R2A	IMCC1728 (DQ664244)	*Panaciterramonas fulva* (AB245357)	95.9	sp. nov. (1)
Betaproteobacteria	1/10R2A	IMCC1729 (DQ664245)	*Zoogloea resiniphila* (AJ011506)	93.2	sp. nov. (9)
Gammaproteobacteria	1/10R2A	IMCC1719 (DQ664207)	*Aeromonas veronii* (x74684)	99.1	-
Gammaproteobacteria	1/10R2A	IMCC1724 (DQ664210)	*Aeromonas veronii* (x74684)	99.4	-
Gammaproteobacteria	1/10R2A	IMCC1727 (DQ664209)	*Aeromonas veronii* (x74684)	99.6	-
Bacteroidetes	1/10R2A	IMCC1731 (DQ664246)	*Kaistomonas ginsengisoli* (AB245370)	98.0	-
Firmicutes	1/10R2A	IMCC1717 (DQ664201)	*Staphylococcus sciuri* (AJ421446)	99.8	-
Firmicutes	1/10R2A	IMCC1732 (DQ664219)	*Staphylococcus saprophyticus* (L37596)	99.3	-
Alphaproteobacteria	PCA	IMCC1738 (DQ664253)	*Bosea massiliensis* (AF288309)	99.3	-
Alphaproteobacteria	PCA	IMCC1740 (DQ664255)	*Ancalomicrobium adetum* (AB095950)	97.4	-
Alphaproteobacteria	PCA	IMCC1741 (DQ664248)	*Azorhizobium doebereinerae* (AY655487)	99.6	-
Alphaproteobacteria	PCA	IMCC1742 (DQ664223)	*Azospirillum irakense* (X79737)	96.6	sp. nov. (14)
Alphaproteobacteria	PCA	IMCC1743 (DQ664249)	*Sphingomonas suberifaciens* (D13737)	95.2	sp. nov. (12)
Alphaproteobacteria	PCA	IMCC1744 (DQ664236)	*Bosea massiliensis* (AF288309)	99.3	-
Betaproteobacteria	PCA	IMCC1733 (DQ664247)	*Mitsuaria chitosanitabida* (AB006851)	96.3	sp. nov. (3)
Betaproteobacteria	PCA	IMCC1734 (DQ664220)	*Mitsuaria chitosanitabida* (AB006851)	97.7	-
Betaproteobacteria	PCA	IMCC1745 (DQ664204)	*Mitsuaria chitosanitabida* (AB006851)	96.6	sp. nov. (4)
Firmicutes	PCA	IMCC1735 (DQ664221)	*Staphylococcus sciuri* (AJ421446)	99.2	-
Firmicutes	PCA	IMCC1736 (DQ664203)	*Lactobacillus satsumensis* (AB154519)	97.9	-
Firmicutes	PCA	IMCC1737 (DQ664222)	*Staphylococcus saprophyticus* (L37596)	99.5	-
Firmicutes	PCA	IMCC1748 (DQ664205)	*Bacillus subtilis* subsp. *spizizenii* (AF074970)	99.7	-
Actinobacteria	PCA	IMCC1739 (DQ664254)	*Microbacterium schleiferi* (AB004723)	98.8	-
Actinobacteria	PCA	IMCC1746 (DQ664211)	*Aeromicrobium erythreum* (AF005021)	98.0	-
Alphaproteobacteria	1/10PCA	IMCC1753 (DQ664250)	*Novosphingobium stygium* (U20775)	95.3	sp. nov. (11)
Alphaproteobacteria	1/10PCA	IMCC1755 (DQ664227)	*Novosphingobium stygium* (U20775)	95.5	sp. nov. (11)
Alphaproteobacteria	1/10PCA	IMCC1763 (DQ664231)	*Novosphingobium stygium* (U20775)	95.7	sp. nov. (11)
Betaproteobacteria	1/10PCA	IMCC1754 (DQ664226)	*Roseateles depolymerans* (AB003625)	93.8	sp. nov. (5)
Betaproteobacteria	1/10PCA	IMCC1762 (DQ664233)	*Ideonella dechloratans* (X72724)	98.0	-
Bacteroidetes	1/10PCA	IMCC1758 (DQ664228)	*Flavobacterium columnare* (D12659)	97.7	-
Bacteroidetes	1/10PCA	IMCC1765 (DQ664234)	*Flavobacterium columnare* (D12659)	97.5	-
Firmicutes	1/10PCA	IMCC1749 (DQ664235)	*Paenibacillus panaciterrae* (AB245385)	96.3	sp. nov. (15)
Firmicutes	1/10PCA	IMCC1750 (DQ664212)	*Paenibacillus panaciterrae* (AB245385)	96.0	sp. nov. (15)
Firmicutes	1/10PCA	IMCC1751 (DQ664224)	*Staphylococcus saprophitycus* (L37596)	99.6	-
Firmicutes	1/10PCA	IMCC1752 (DQ664225)	*Staphylococcus saprophitycus* (L37596)	99.8	-
Firmicutes	1/10PCA	IMCC1760 (DQ664229)	*Bacillus cereus* (AF290547)	99.7	-
Actinobacteria	1/10PCA	IMCC1756 (DQ664258)	*Rhodococcus opacus* (X80630)	95.6	sp. nov. (17)
Actinobacteria	1/10PCA	IMCC1764 (DQ664208)	*Nocardioides ganghwensis* (AY423718)	96.8	sp. nov. (16)
Deinococci	1/10PCA	IMCC1761 (DQ664230)	*Deinococcus ficus* (AY941086)	99.8	-

[a]A number in parenthesis indicates a serial number of novel species. *See* Figs. 1 and 2.

material-containing culture medium, PCA. Lower-range organic material-containing culture media (R2A, 1/10 R2A, 1/10 PCA) were better than PCA medium in isolating novel species from the study site. Because historically many freshwater bacteria have been isolated from organic-rich media [15, 32] and the organic contents of PCA medium are much higher than those of natural freshwater, it can be postulated that use of PCA medium might not bring many novel species into the cultures. A recent report by Tamaki *et al.* [30] showed that the use of diluted R2A

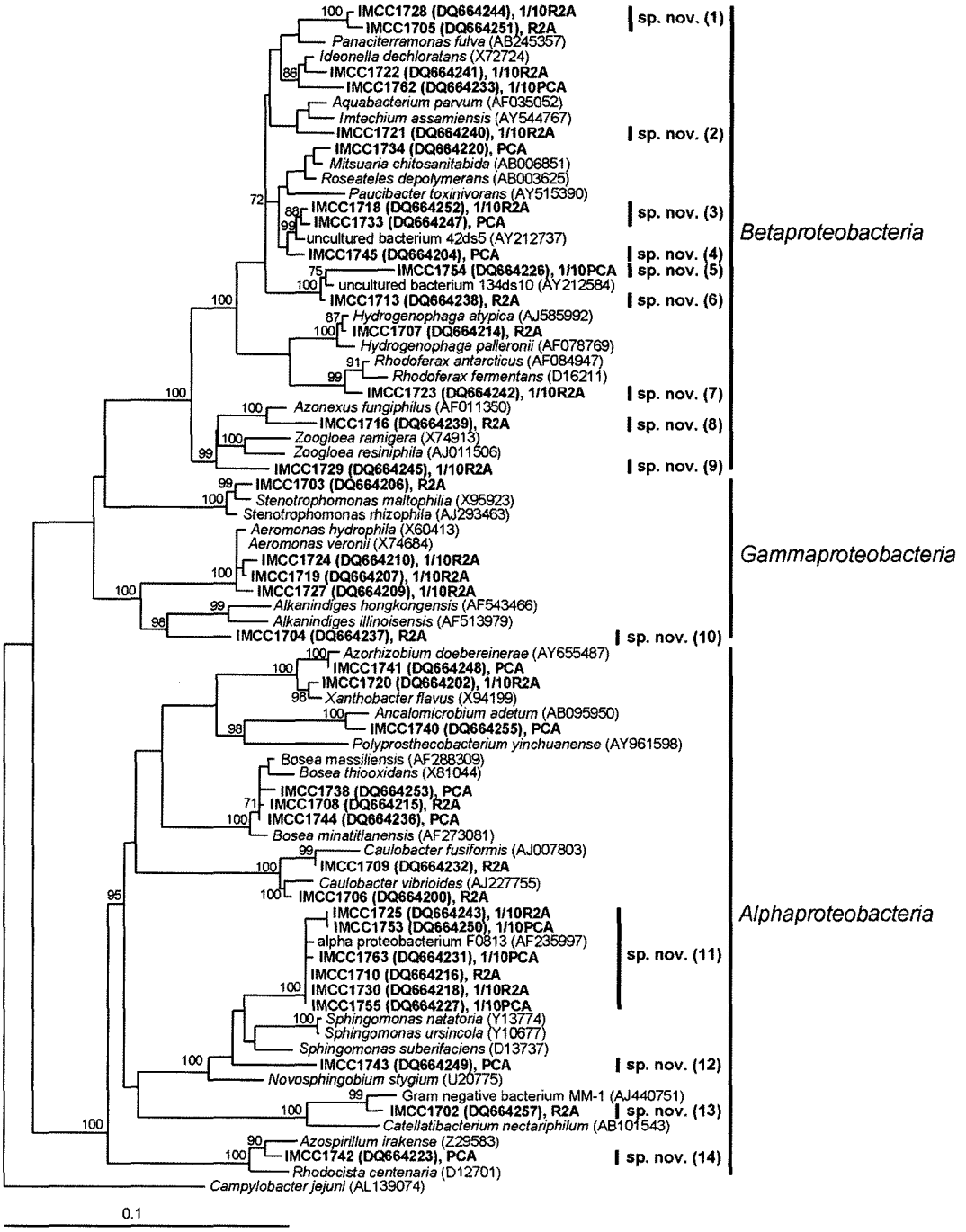

Fig. 1. Neighbor-joining 16S rRNA phylogenetic tree showing relationships between the IMCC isolates and validly published species or environmental sequences in the phylum *Proteobacteria*.

Bootstrap proportions over 70% from the neighbor-joining analysis are shown. Bold vertical lines indicate the boundary of each novel species or classes. The serial numbers of the novel species are shown in parenthesis. *Campylobacter jejuni* (AL139074) served as the outgroup organism. Scale bar, 0.1 substitutions per nucleotide position.

media together with gellan gum as a solidifying agent resulted in isolating numerous novel species from a shallow eutrophic freshwater sediment. Although the sample size of the isolates per medium in our study was too small to draw a generalized conclusion, our results showing the recovery of numerous novel species from the eutrophic freshwater using low nutrient media also support the previous findings.

The freshwater isolates analyzed, occurrence in the culture media, phylogenetic affiliations, and cumulative

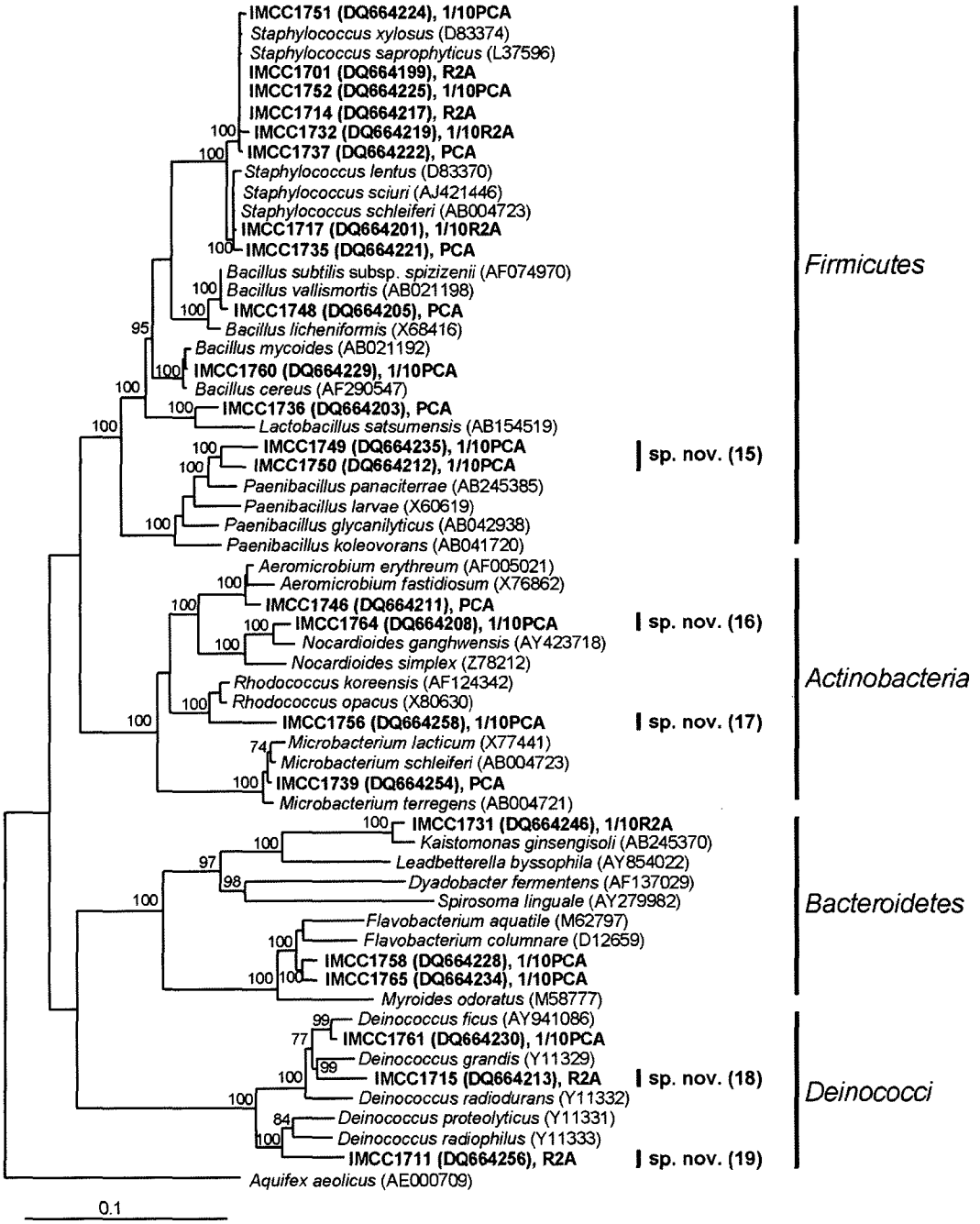

Fig. 2. Neighbor-joining 16S rRNA phylogenetic tree showing relationships between the IMCC isolates and validly published species or environmental sequences in the phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Deinococci*.
Bootstrap proportions over 70% from the neighbor-joining analysis are shown. Bold vertical lines indicate the boundary of each novel species or classes. The serial numbers of the novel species are shown in parenthesis. *Aquifex aeolicus* (AE000709) served as the outgroup organism. Scale bar, 0.1 substitutions per nucleotide position.

numbers of novel species are represented in Table 2, Fig. 1, and Fig. 2. Of the 27 strains assigned to the novel species, the majority of the strains (20 strains) were affiliated with the *Alphaproteobacteria* and *Betaproteobacteria* (Table 1). In contrast, among the 13 strains assigned to the *Firmicutes*, only two strains comprising one novel species were isolated. Phylogenetic positions of the isolates assigned to the phylum *Proteobacteria* are represented in Fig. 1. Of the 15 strains assigned to the *Betaproteobacteria*, 11 strains formed 9 novel species. Five strains, designated IMCC1728, IMCC1705, IMCC1721, IMCC1723, and IMCC1716 (IMCC stands for Inha Microbe Culture

Collection, which is maintained by Inha University), had a relatively high sequence similarity (95.6–96.9%) to validly published species. Six strains (IMCC1718, IMCC1733, IMCC1745, IMCC1754, IMCC1713, and IMCC1729), however, could not be assigned to any previous known genera. Based on phylogenetic analyses, it seems that these 6 strains comprise three novel genera; a genus for strains IMCC1718, IMCC1733 and IMCC1745, a genus for IMCC1754 and IMCC1713, and a genus for IMCC1729 (Fig. 1). Of the 5 isolates assigned to the *Gammaproteobacteria*, three strains were closely related to *Aeromonas veronii* with 99.1–99.6% 16S rRNA gene similarity, and strain IMCC1703 showed 98.5% sequence similarity to *Stenotrophomonas maltophilia*. Strain IMCC1704 showed only 92.2% sequence similarity to *Alkanindiges illinoisensis* and therefore it was assigned to novel species. A total of 17 strains isolated from all the four kinds of culture media were affiliated with the *Alphaproteobacteria*. Eight strains affiliated with the orders *Rhizobiales* and *Caulobacterales* had sequence similarities greater than 97% to validly published species within the orders. However, 9 strains affiliated to the orders *Sphingomonadales*, *Rhodobacterales*, and *Rhodospirillales* did not have any closely related and validly published species that share over 97% 16S rRNA gene sequence similarity to those strains. Based on sequence similarity and phylogenetic analyses, those 9 strains could be assigned to 4 novel species.

The isolates assigned to the phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Deinococci* are shown in Fig. 2. Of the 23 strains affiliated with the phyla, only 6 strains were found to comprise novel species. There were no novel species lineages among the three strains within the *Bacteroidetes*. Two strains (IMCC1749 and IMCC1750) showing 96.0–96.3% sequence similarity to *Paenibacillus panaciterrae* within the *Firmicutes* were assigned to the genus *Paenibacillus* as novel species. The remaining eleven strains in the *Firmicutes* were very closely related to predefined species of the genera *Staphylococcus*, *Bacillus*, and *Lactobacillus*. Strains IMCC1764 and IMCC1756 were assigned to the genera *Nocardioides* and *Rhodococcus* in the *Actinobacteria*, respectively. Two strains, designated IMCC1715 and IMCC1711, formed two separate novel species lineages in the genus *Deinococcus*.

In this study, we applied the practical value "97%" to demarcate bacterial species, which assumes that different species share less than 97% 16S RNA gene sequence similarity [28], because we are practically unable to perform DNA:DNA hybridization with all the isolates against related species identified. However, numerous reports support that there are different bacterial genotypes (*e.g.*, ecospecies [6]) belonging to the same species-like phylogenetic group (sharing >97% similarity), such as marine *Prochlorococcus*, *Vibrio*, and the SAR11 clade [1, 22, 26, 31]. It is also empirically defined that the bacterial genomic species is "the strains sharing greater than 70% of overall DNA-DNA relatedness" [33]. This means that species appearing to be similar at the rRNA gene level could be different species according to DNA-DNA relatedness. In this context, the number of novel species identified in this study using only 16S rRNA gene information may be underestimated. Further study through measuring DNA-DNA relatedness for the strains sharing over 97% 16S rRNA gene sequence similarity will be definitely required to reveal the more true bacterial richness in the freshwater sample.

In conclusion, we have isolated 19 novel species among 60 isolates using a conventional culturing technique with four usual media, from a usual eutrophic pond found in Korea, suggesting that an unexplored ecosystem, even if it looks like a common ecosystem found elsewhere, harbors diverse uncharacterized microbes. We are continuously maintaining these novel strains to further identify them by polyphasic approaches and to further investigate their eco-physiological roles in eutrophic freshwater ponds.

The sequences of the freshwater isolates cultured in this study have been deposited in GenBank under accession number DQ664199 to DQ664258.

Acknowledgment

This work was supported by an Ihha University research grant.

REFERENCES

1. Acinas, S. G., V. Klepac-Ceraj, D. E. Hunt, C. Pharino, I. Ceraj, D. L. Distel, and M. F. Polz. 2004. Fine-scale phylogenetic architecture of a complex bacterial community. *Nature* **430:** 551–554.
2. Ahn, J.-H., M.-S. Kim, M.-C. Kim, J.-S. Lim, G.-T. Lee, J. K. Yun, T. Kim, T. Kim, and J.-O. Ka. 2006. Analysis of bacterial diversity and community structure in forest soils contaminated with fuel hydrocarbon. *J. Microbiol. Biotechnol.* **16:** 704–715.
3. Bruns, A., H. Hoffelner, and J. Overmann. 2003. A novel approach for high throughput cultivation assays and the isolation of planktonic bacteria. *FEMS Microbiol. Ecol.* **45:** 161–171.
4. Cho, J.-C. and S. J. Giovannoni. 2004. Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. *Appl. Environ. Microbiol.* **70:** 432–440.
5. Cho, W., E.-H. Lee, E.-H. Shim, J. Kim, H. W. Ryu, and K.-S. Cho. 2005. Bacterial communities of biofilms sampled from seepage groundwater contaminated with petroleum oil. *J. Microbiol. Biotechnol.* **15:** 952–964.
6. Cohan, F. M. 2002. What are bacterial species? *Annu. Rev. Microbiol.* **56:** 457–487.

7. Connon, S. A. and S. J. Giovannoni. 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl. Environ. Microbiol.* **68:** 3878–3885.
8. DeLong, E. F., C. M. Preston, T. Mincer, V. Rich, S. J. Hallam, N. U. Frigaard, A. Martinez, M. B. Sullivan, R. Edwards, B. R. Brito, S. W. Chisholm, and D. M. Karl. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* **311:** 496–503.
9. Gich, F., J. Garcia-Gil, and J. Overmann. 2001. Previously unknown and phylogenetically diverse members of the green nonsulfur bacteria are indigenous to freshwater lakes. *Arch. Microbiol.* **177:** 1–10.
10. Gich, F. and J. Overmann. 2006. *Sandarakinorhabdus limnophila* gen. nov., sp. nov., a novel bacteriochlorophyll a-containing, obligately aerobic bacterium isolated from freshwater lakes. *Int. J. Syst. Evol. Microbiol.* **56:** 847–854.
11. Gich, F., K. Schubert, A. Bruns, H. Hoffelner, and J. Overmann. 2005. Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. *Appl. Environ. Microbiol.* **71:** 5908–5919.
12. Giovannoni, S. and M. Rappé. 2000. Evolution, diversity and molecular ecology of marine prokaryotes, pp. 47–84. *In* D. L. Kirchman (ed.), *Microbial Ecology of the Oceans.* John Wiley & Sons, Inc., New York.
13. Hugenholtz, P., B. M. Goebel, and N. R. Pace. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J. Bacteriol.* **180:** 4765–4774.
14. Hugenholtz, P., C. Pitulle, K. Hershberger, and N. R. Pace. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. *J. Bacteriol.* **180:** 366–376.
15. Hwang, C. Y. and B. C. Cho. 2006. *Flectobacillus lacus* sp. nov., isolated from a highly eutrophic pond in Korea. *Int. J. Syst. Evol. Microbiol.* **56:** 1197–1201.
16. Kim, B. S., H. M. Oh, H. J. Kang, S. S. Park, and J. S. Chun. 2004. Remarkable bacterial diversity in the tidal flat sediment as revealed by 16S rDNA analysis. *J. Microbiol. Biotechnol.* **14:** 205–211.
17. Kim, M.-S., J.-H. Ahn, M.-K. Jung, J.-H. Yu, D.-H. Joo, M.-C. Kim, H.-C. Shin, T. Kim, T.-H. Ryu, S.-J. Kweon, T. Kim, D.-H. Kim, and J.-O. Ka. 2005. Molecular and cultivation-based characterization of bacterial community structure in rice field soil. *J. Microbiol. Biotechnol.* **15:** 1087–1093.
18. Kim, W.-K., R. Cui, and D. Jahng. 2005. Enrichment of ammonia-oxidizing bacteria for efficient nitrification of wastewater. *J. Microbiol. Biotechnol.* **15:** 772–779.
19. Konneke, M., A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury, and D. A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437:** 543–546.
20. Lee, S.-H., H.-R. Oh, J.-H. Lee, S.-J. Kim, and J.-C. Cho. 2004. Cold-seep sediment harbors phylogenetically diverse uncultured bacteria. *J. Microbiol. Biotechnol.* **14:** 906–913.
21. Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Forster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. Konig, T. Liss, R. Lussmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K. H. Schleifer. 2004. ARB: A software environment for sequence data. *Nucleic Acids Res.* **32:** 1363–1371.
22. Moore, L. R., G. Rocap, and S. W. Chisholm. 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* **393:** 464–467.
23. Page, K. A., S. A. Connon, and S. J. Giovannoni. 2004. Representative freshwater bacterioplankton isolated from Crater Lake, Oregon. *Appl. Environ. Microbiol.* **70:** 6542–6550.
24. Rappe, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovannoni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418:** 630–633.
25. Reasoner, D. J. and E. E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* **49:** 1–7.
26. Rocap, G., F. W. Larimer, J. Lamerdin, S. Malfatti, P. Chain, N. A. Ahlgren, A. Arellano, M. Coleman, L. Hauser, W. R. Hess, Z. I. Johnson, M. Land, D. Lindell, A. F. Post, W. Regala, M. Shah, S. L. Shaw, C. Steglich, M. B. Sullivan, C. S. Ting, A. Tolonen, E. A. Webb, E. R. Zinser, and S. W. Chisholm. 2003. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature* **424:** 1042–1047.
27. Simu, K. and A. Hagstrom. 2004. Oligotrophic bacterioplankton with a novel single-cell life strategy. *Appl. Environ. Microbiol.* **70:** 2445–2451.
28. Stackebrandt, E. and B. M. Göebel. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44:** 846–849.
29. Swofford, D. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* 4b8 Ed. Sinauer Associates, Sunderland, Massachusetts.
30. Tamaki, H., Y. Sekiguchi, S. Hanada, K. Nakamura, N. Nomura, M. Matsumura, and Y. Kamagata. 2005. Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. *Appl. Environ. Microbiol.* **71:** 2162–2169.
31. Thompson, J. R., M. A. Randa, L. A. Marcelino, A. Tomita-Mitchell, E. Lim, and M. F. Polz. 2004. Diversity and dynamics of a north atlantic coastal *Vibrio* community. *Appl. Environ. Microbiol.* **70:** 4103–4110.
32. Wang, Z.-W., Y.-H. Liu, X. Dai, B.-J. Wang, C.-Y. Jiang, and S.-J. Liu. 2006. *Flavobacterium saliperosum* sp. nov., isolated from freshwater lake sediment. *Int. J. Syst. Evol. Microbiol.* **56:** 439–442.
33. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüfer. 1987. Report of the *ad hoc* committee on reconciliation

of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37:** 463–464.
34. Xiang, Z., X. Xiao, P. Wang, and F. P. Wang. 2004. Screening and characterization of psychrotrophic, lipolytic bacteria from deep-sea sediments. *J. Microbiol. Biotechnol.* **14:** 952–958.
35. Zwart, G., W. D. Hiorns, B. A. Methe, M. P. van Agterveld, R. Huismans, S. C. Nold, J. P. Zehr, and H. J. Laanbroek. 1998. Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. *Syst. Appl. Microbiol.* **21:** 546–556.
36. Zwart, G., R. Huismans, M. P. van Agterveld, Y. Van de Peer, P. De Rijk, H. Eenhoorn, G. Muyzer, E. J. van Hannen, H. J. Gons, and H. J. Laanbroek. 1998. Divergent members of the bacterial division *Verrucomicrobiales* in a temperate freshwater lake. *FEMS Microbiol. Ecol.* **25:** 159–169.