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Phylum-targeted pyrosequencing reveals diverse planctomycete populations in a eutrophic lake

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ABSTRACT: Although populations of planctomycete bacteria are nearly ubiquitous in aquatic habitats, we lack a detailed understanding of their diversity and structure. This is due to difficulty in obtaining cultured representatives, but also to low recovery rates for planctomycete 16S rRNA genes when universal PCR primers are employed for sequencing studies. In an attempt to expand recoverable planctomycete diversity, we investigated the use of primers targeting the *Planctomycetes* phylum. Planctomycete populations present during an algal bloom in a eutrophic lake were characterized by clone library sequencing and pyrosequencing of 16S rRNA genes, using planctomycete-targeted primers. We analyzed samples recovered from the sediment, water column, and algal mats within the lake's littoral zone. Sequences related to 9 planctomycete genera were identified within the 6287 planctomycete sequences and 1730 operational taxonomic units (OTUs) recovered. We observed variation in the specificity of the planctomycete primers, with more non-planctomycete sequences recovered through pyrosequencing than through cloning-based sequencing. Nevertheless, the results of our study suggest that phylum-targeted pyrosequencing is a useful tool for better describing the diversity of bacterial sub-populations. This methodology could be employed for future testing of hypotheses regarding spatial and temporal differences in planctomycete diversity and abundance. Candidate hypotheses arising from this preliminary study include (1) members of certain planctomycete genera (particularly *Rhodopirellula*) are enriched in algal mats relative to other planctomycete genera; (2) sediments harbor the most diverse planctomycete populations; and (3) most planctomycete OTUs are not shared between lake habitats.

KEY WORDS: Planctomycetes · Freshwater lake · Algal bloom

INTRODUCTION

The phylum *Planctomycetes* represents a widely distributed, but relatively understudied bacterial group. Planctomycete bacteria have been isolated from environments such as freshwater (Staley 1973, Franzmann & Skerman 1984, Wang et al. 2002), ocean water (Schlesner & Stackebrandt 1986), soil (Wang et al. 2002), and hot springs (Giovannoni et al. 1987, Kahan 1961). Culture-independent surveys have detected planctomycete bacteria in environments as diverse as human (Andersson et al. 2008) and termite (Köhler et al. 2008) guts, permafrost (Steven et al. 2007), and a wastewater treatment plant (Chouari et al. 2003). The detection of uncultured lineages of planctomycete bacteria in these studies suggests that the phylogenetic, metabolic, and physiological diversity of these organisms has not yet been adequately described. The *Planctomycetes* share a compartmentalized cell plan with the *Verrucomicrobia* (Fuerst 2005, Lee et al. 2009); this organization appears to be unique among the bacteria. The consequences of this compartmentalization for the biology of the cell remain undetermined, although

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the recent remarkable finding of endocytosis in the planctomycete *Gemmata obscuriglobus* (Lonhienne et al. 2010) naturally leads to speculation over whether this may occur in other planctomycetes and in *Verrucomicrobia*. Planctomycetes exhibit other unusual phenotypic properties, such as a cell wall that lacks peptidoglycan, crateriform structures on the cell surface, and diverse cellular appendages (Ward et al. 2006, and the references within). All axenic cultures of planctomycetes are heterotrophic, but the physiology of the uncultivated majority is unclear. There is, however, evidence that planctomycetes possess unique and ecologically important physiologies; for example, certain planctomycete bacteria are uniquely capable of anaerobic ammonium oxidation (anammox reaction) (Strous et al. 1999).

Several lines of evidence suggest that planctomycete bacteria form ecologically relevant associations with algae. The presence of ambient light and development of phototroph populations favor successful laboratory enrichment (Starr & Schmidt 1989), and freshwater *Pirellula* spp. are observed as epiphytes of sheathed cyanobacteria (Staley et al. 1992). Kristiansen (1971) suggested that increased iron, manganese, and hydrogen sulfide concentrations resulting from decomposition of phytoplankton support proliferation of *Planctomyces bekefii* blooms. Blooms of organisms morphologically resembling *P. bekefii* (Schmidt & Starr 1980) and *P. guttaeformis* have been observed in a Louisiana lake during and subsequent to a bloom of *Pithophora* spp. (N. L. Ward unpubl. obs.). Budding bacteria resembling planctomycetes were among the dominant organisms enumerated from several lakes in Australia, but were most abundant in eutrophic lakes (Staley et al. 1980). Recently, planctomycete bacteria related to the genus *Pirellula* were reported to be among the dominant bacteria in an Oregon coast seawater diatom bloom (Morris et al. 2006).

Despite the apparent ubiquity of planctomycetes in aquatic habitats, we lack a detailed understanding of their population diversity and structure. This can be partly attributed to low recovery rates for planctomycete 16S rRNA genes when universal polymerase chain reaction (PCR) primers are employed for sequencing studies. In an attempt to expand recoverable planctomycete diversity, we investigated the use of primers targeting the phylum *Planctomycetes*. To our knowledge the present study represents the first application of targeted high-throughput pyrosequencing to describe the diversity of planctomycete bacterial populations. Given the reported associations of planctomycetes with eutrophic conditions and algal blooms (described above), we chose to pursue our study in a small eutrophic lake in which an algal bloom was occurring.

MATERIALS AND METHODS

Sample collection and field measurements. LaBonte Lake, located at 41° 19′ 17.53″ N, 105° 35′ 12.96″ W, elevation 2177 m in Laramie, Wyoming, USA, is a highly impacted urban lake. Samples were collected from LaBonte Lake on June 16, 2008, after the development of macroscopically visible algal mats. The lake has been observed to undergo annual algal blooms that are associated with noxious odors. The algal bloom in 2008 appeared as a mat covering the shallow surface water. Microscopic investigation of the algal mat revealed a community predominantly composed of the filamentous eukaryotic genera *Spirogyra* and *Zygnema*, with a minor contribution of *Rhizoclonium*. A small population of cyanobacterial cells resembling *Nostoc* was also observed. Algal identification was performed as described by Prescott (1978).

Samples were collected from the lake littoral zone (~0.5 m depth) from the south shore of the lake, near the water inlet. This area was free from tree cover, allowing for maximal sun exposure. Water temperature and pH were measured with an Ecosense water quality monitor (YSI Inc.) and dissolved oxygen was determined with a YSI 55 dissolved oxygen meter (YSI Inc.). Mixed sediment samples were collected with a sterile 50 ml Falcon tube from the upper 10 cm of sediment and stored in a sterile Whirlpak bag. A 500 ml sample of near-shore lake water (up to 0.5 m in depth) was collected in a sterile graduated cylinder. This method of water sample collection resulted in a mixing of the water column. Finally, algal mat samples were collected with sterile forceps and placed in 50 ml Falcon tubes. All samples were immediately transported (5 min driving time) to the laboratory for immediate processing.

Laboratory sample processing and chemical analysis. Water samples were filtered through a 2.0 µm and then a 0.2 µm pore filter, and the 0.2 µm retentate was subsequently used for DNA extraction (described in the following subsection). Chemical characterization was performed on the 0.2 µm filtrate, which was stored at −20°C until assayed. Total nitrogen, phosphorus (Method 4500-P, B, E) and organic carbon (Method 5310 C) were analyzed according to standard methods of the American Public Health Association (1995). Total nitrogen was calculated as the sum of total Kjeldahl nitrogen (Method 4500-Norg B) and nitrate (Methods EPA 300.0 & 300.1). The Wyoming Department of Agriculture Analytical Services performed all chemical analyses.

Community DNA extraction. Total DNA was extracted from a single sample taken from each lake habitat (sediment, water column, and algal mat) using the MoBio PowerSoil kit (MoBio Laboratories), according to the manufacturer's instructions. DNA was ex-

tracted from 0.5 g of sediment, 0.2 g of algal mat material, or the retentate from one-half of the 0.2 µm filter. Processing of samples was standardized as much as possible to limit the effects of DNA extraction bias.

Construction of planctomycete 16S rRNA gene clone libraries. Planctomycete 16S rRNA genes were amplified with primers PLA40F (Derakshani et al. 2001) and 1492R (Lane 1991), the same primers used to describe planctomycete-related sequences from soil (Derakshani et al. 2001). The ~1400 bp amplification products were gel purified from 0.8% agarose gels using the QIAquick kit (Qiagen) with manufacturer-supplied protocols. Eluted DNA was cloned into the TOPO-TA vector system (Invitrogen) and transformed into supplied competent DH5α *Escherichia coli* cells. Approximately 50 white colonies from each library were selected for plasmid isolation using the Qiagen plasmid mini kit with standard protocols. Sequencing of plasmid DNA was performed with primer PLA40 at the Washington University Genome Sequencing Center.

Editing and analysis of clone library sequences. Sequences from the clone libraries were selected based on 2 criteria: sequence length of at least 500 bp (to ensure overlap with the pyrosequencing results) and quality scores of the sequence. Sequences were edited, and low-scoring bases were trimmed in the Sequencher software package (Gene Codes Corporation). High-quality sequences were aligned using the fast, secondary-structure aware infernal aligner available through the RDP database (Cole et al. 2009) and screened for possible chimeras using the Mallard software package (Ashelford et al. 2006). Potential chimeras were discarded, and the resulting sequences were realigned as above. The sequence alignment was 489 bp, and terminal gaps were not considered in the analysis. Sequences were classified using the Greengenes Classify software (DeSantis et al. 2006) and all 3 available models (RDP, NCBI, and Hugenholtz).

Pyrosequencing of planctomycete 16S rRNA genes. Planctomycete tag-encoded FLX-Titanium amplicon pyrosequencing (TETAP) was performed as described previously (Dowd et al. 2008) using the PLA40F and 1492R primers and PCR conditions described above (with the addition of required adaptor sequences). This planctomycete TETAP approach utilized Roche FLX Titanium reagents and procedures and a 1-step PCR. A mixture of Hot Start and HotStar high-fidelity *Taq* polymerases (Qiagen) was used. Sequencing was performed at the Research and Testing Laboratory (RTL, Lubbock, TX, USA) based upon RTL protocols (www.researchandtesting.com).

16S rRNA gene pyrosequencing analyses. Initial quality filtering, noise removal, and chimera removal were performed on the sequence reads using the PyroTagger software (Kunin & Hugenholtz 2010). Further trimming and selection of high-quality sequences were performed using the Mothur software package (Schloss et al. 2009) to select sequences ≥200 bp in length, with no ambiguous bases and no homopolymer stretches of >7 bp. Sequences were aligned over the 200 bp, and terminal end gaps were removed from the analysis. Sequence libraries were classified as described for the clone library sequences. After classification, planctomycete-related sequences were collected and retained for further analysis. Planctomycete-related sequences were binned into operational taxonomic units (OTUs) at 97% sequence identity using the average neighbor algorithm, and these groupings were used for all inter-library comparisons and statistical tests. The 97% sequence identity threshold was selected because it has long been proposed to represent species-level differences, originally through correlation with genomic DNA:DNA hybridization values (Stackebrandt & Goebel 1994). It has also been suggested that a 97% sequence identity threshold minimizes potential inflations of sequence diversity associated with massively parallel tag sequencing (Kunin et al. 2010). Venn diagrams, H' (non-parametric), ChaoI (Chao et al. 2005), and coverage (Good's coverage) were all calculated within the Mothur software environment, using OTUs defined at 97% sequence identity (Schloss et al. 2009). These indices were chosen as they are commonly employed in microbial diversity studies and offer a basis for comparison to other ecosystems. LIBSHUFF statistical comparisons were also performed within Mothur using 10 000 reiterations.

Phylogenetic analysis of 16S rRNA sequences. For phylogenetic analysis, sequences from the pyrosequencing libraries were aligned against sequences from the clone libraries and clustered into OTUs (97% sequence identity) as described above. Representative sequences were extracted from each OTU that was populated by at least 10 sequences, in order to limit the analysis to sequences that were well represented in the library. The representative sequences were then aligned to sequences from type strains of the 11 validly described species within the phylum *Planctomycetes*, as well as to type strains of representative organisms outside the *Planctomycetes* but within the *Planctomycetes*/*Verrucomicrobia*/*Chlamydiae* (PVC) superphylum. The 16S rRNA gene sequence of *Escherichia coli* (ATCC 11775^T) was used as an outgroup to root the tree. Alignments were performed with the RDP as described above. The alignment was used to generate a neighbor-joining tree (BioNJ, observed substitution model) within the SeaView software package (Gouy et al. 2009). The alignment spanned 176 bp, corresponding to positions 31 to 212 of the *E. coli* 16S rRNA gene sequence.

Sequence accession numbers. Planctomycete tag sequences were submitted to the GenBank Short Read Archive and appear under Accession Number SRA012453.6. Clone library sequences were deposited in GenBank and are archived under the Accession Numbers GQ994712 to GQ99493.

RESULTS

Physical/chemical characterization of the littoral zone of LaBonte Lake

The physical and chemical characteristics of the littoral zone of LaBonte Lake on June 16, 2008, were as follows: temperature, 25.0°C; pH, 9.4; total nitrogen, 2.1 mg l^{-1}; total phosphorus, 0.13 mg l^{-1}; and total organic carbon, 17.0 mg l^{-1}.

Planctomycete diversity in LaBonte Lake

We first conducted small-scale cloning-based sequencing to evaluate the effectiveness and specificity of planctomycete-targeted primers. Within the clone libraries produced from the sediment, water column, and algal mat, a total of 133 sequences were recovered, of which 113 (85%) were identified as being related to the phylum *Planctomycetes* (Table 1). Non-planctomycete sequences were related to the bacterial phyla *Verrucomicrobia*, *Lentisphaerae*, *Chlamydiae*, and *Proteobacteria* (data not shown). The *Verrucomicrobia*, *Lentisphaerae*, and *Chlamydiae* have been placed in the same superphylum as the phylum *Planctomycetes*; this group is referred to as the PVC superphylum (Wagner & Horn 2006). The majority (81) of the 113 planctomycete-related sequences could not be assigned with confidence to any of the known planctomycete genera, but the remaining 32 sequences were affiliated with 5 out of the 9 recognized planctomycete genera (Table 1). The results of this clone library sequencing, therefore, suggested that planctomycete-targeted sequencing, although not perfectly specific, was capable of recovering considerable diversity at the genus level. The estimated coverage of the planctomycete sequence diversity ranged from 7 to 12% (Table 1), indicating that only a small fraction of the planctomycete diversity in the different environments was described within the small number of clones sequenced. The low coverage obtained with cloning-based sequencing prompted us to next combine the planctomycete-targeted primers with high-throughput pyrosequencing as an alternative sequencing platform.

In total, 19119 16S rRNA gene sequences were obtained from pyrosequencing, of which 6287 were classified as belonging to the phylum *Planctomycetes* (Table 2). Non-planctomycete sequences were detected at various frequencies in all of the samples. All non-planctomycete sequences were related to the bacterial phyla *Actinobacteria*, *Alphaproteobacteria*, BRC1, or to members of the PVC superphylum (data not shown). The estimated coverage of the planctomycete 16S rRNA gene diversity in this pyrosequenc-

Table 1. Description of 16S rRNA gene clone libraries. Good's coverage was calculated using sequences identified as belonging to the planctomycetes, with an operational taxonomic unit definition of 97% sequence identity

	Sequences	Planctomycete-related sequences	*Pirellula*	*Blasto-pirellula*	*Rhodo-pirellula*	*Iso-sphaera*	*Planctomyces*	Unclassified	Coverage (%)
Sediment	46	39	4	1	4	–	3	27	9
Water	44	36	–	–	–	1	1	34	12
Algal mat	43	38	7	–	9	–	2	20	7
Compiled	133	113	11	1	13	1	6	81	6

Table 2. Description of 16S rRNA gene pyrosequencing libraries. OTUs: operational taxonomic units; *H'*: Shannon diversity index

	Total sequences	Planctomycete sequences	Proportion planctomycetes (%)	OTUs[a]	Coverage[a] (%)	*H'*[a]	Chao1[a]
Sediment	6552	2181	33.3	1006	68.3	6.7	2610
Water	7624	641	8.4	337	63.1	5.8	981
Algal mat	4943	3465	70.1	749	86.3	5.4	1772
Compiled	19119	6287	32.8	1730	81.6	6.2	4539

[a]Calculated only using planctomycete-related sequences, with OTUs defined at 97% sequence identity

ing collection ranged from 63 to 86%, suggesting that the majority of the planctomycete sequences expected to occur in LaBonte Lake were recovered in this survey. Planctomycete-related sequences were assigned to OTUs at 97% sequence identity, resulting in the identification of a total of 1730 OTUs (Table 2). Based on the ChaoI estimate, over 4500 planctomycete OTUs are expected to be present in LaBonte Lake (Table 2). The highest planctomycete diversity, as described by the Shannon diversity index (*H'*), was observed in the sediment sample, followed by the water sample, and the lowest diversity was observed in the algal mat (Table 2).

Only 6 OTUs (>1% of planctomycete-related OTUs) showed a common distribution in the lake, in that they contained sequences present in the sediment, water, and algal mat environments, while the majority of OTUs identified in LaBonte Lake appeared to contain sequences unique to a single environment (Fig. 1). Pairwise LIBSHUFF (Schloss et al. 2009) analysis of the sequence libraries indicated a significant difference between the composition of all of the libraries ($p < 0.0001$ for all combinations with 10 000 iterations; data not shown). The clone library sequences were also used as query sequences for a BLASTALL search against the pyrosequencing library. For all but 7 clone library sequences, at least 1 sequence in the pyrosequencing library that was at least 99% identical to the query sequence was identified. For the remaining sequences, all from the water sample, the identity scores ranged from 88 to 97% and were within the unclassified sequences (Table 1). This suggests a significant overlap between the sequences reliably classified as planctomycete-related between clone and pyrosequencing libraries, suggesting that the 2 methods primarily differed in their identification of sequences that are potentially non-target organisms.

Within the 6287 planctomycete-related clones obtained from LaBonte Lake, sequences related to all 9 currently described planctomycete genera were identified (Table 3). These included the long-established genera *Planctomyces*, *Pirellula*, *Gemmata*, and *Isosphaera*, and the more recently reclassified *Rhodopirellula* and *Blastopirellula* (formerly contained within *Pirellula*). Also detected were sequences assigned to the newly described genera *Schlesneria* (Kulichevskaya et al. 2007), *Singulisphaera* (Kulichevskaya et al. 2008), and *Zavarzinella* (Kulichevskaya et al. 2009). Sequences that could not be assigned to any characterized genus were also found in all samples, and in 2 out of 3 sampled lake habitats represented the majority of the planctomycete sequences. Comparison of the planctomycete populations between the different environments suggested a higher relative abundance of the genus *Rhodopirellula* in the algal mat (32.7%) compared to the sediment (5.5%) and water (0.6%) samples. In the water, 10% of the sequences were related to the genus *Isosphaera*, while these organisms

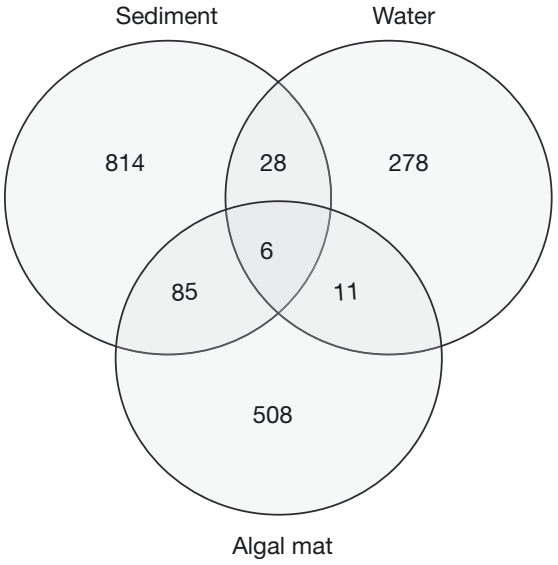

Fig. 1. Operational taxonomic unit (OTU) overlap between different libraries. The number of OTUs unique to each library is indicated within each circle, whereas the numbers of OTUs that were common to multiple libraries are indicated in the overlapping regions. OTU definition = 97% sequence identity

Table 3. Relative abundance (% of planctomycete sequences) of planctomycete genera within the pyrosequencing libraries. All classifications to the genus level were based on agreement between all 3 models (RDP, Hugenholtz and NCBI) in the Greengenes classifier software; unclassified sequences were all classified as planctomycete bacteria, but were not resolved to genus. Unclass.: unclassified planctomycete; Other: sequences not classified as planctomycete sequences by at least 1 of the models; ND: not detected

	Blastopirellula	*Gemmata*	*Isosphaera*	*Pirellula*	*Planctomyces*	*Rhodopirellula*	*Schlesneria*	*Singulisphaera*	*Zavarzinella*	Unclass.	Other
Sediment	0.1	0.2	ND	8.7	2.9	5.5	0.2	0.2	0.1	51.0	30.8
Water	ND	ND	10.0	0.9	4.1	0.6	0.2	ND	ND	2.3	85.9
Algal mat	ND	0.2	ND	5.5	1.4	32.7	0.4	ND	0.1	52.0	7.8

were not detected in either the sediment or algal mat. Sequences allocated to other genera were so similar or rare that comparison of distributions is not expected to be meaningful.

In order to determine the phylogenetic relationships between our lake sequences and described planctomycete species, we constructed a phylogenetic tree containing reference sequences from the 11 validly described planctomycete species and representative sequences from OTUs containing at least 10 sequences (Fig. 2). The tree obtained was constructed from an alignment of only 176 nucleotides, which probably explains some incongruencies between our tree and the established planctomycete phylogeny based on

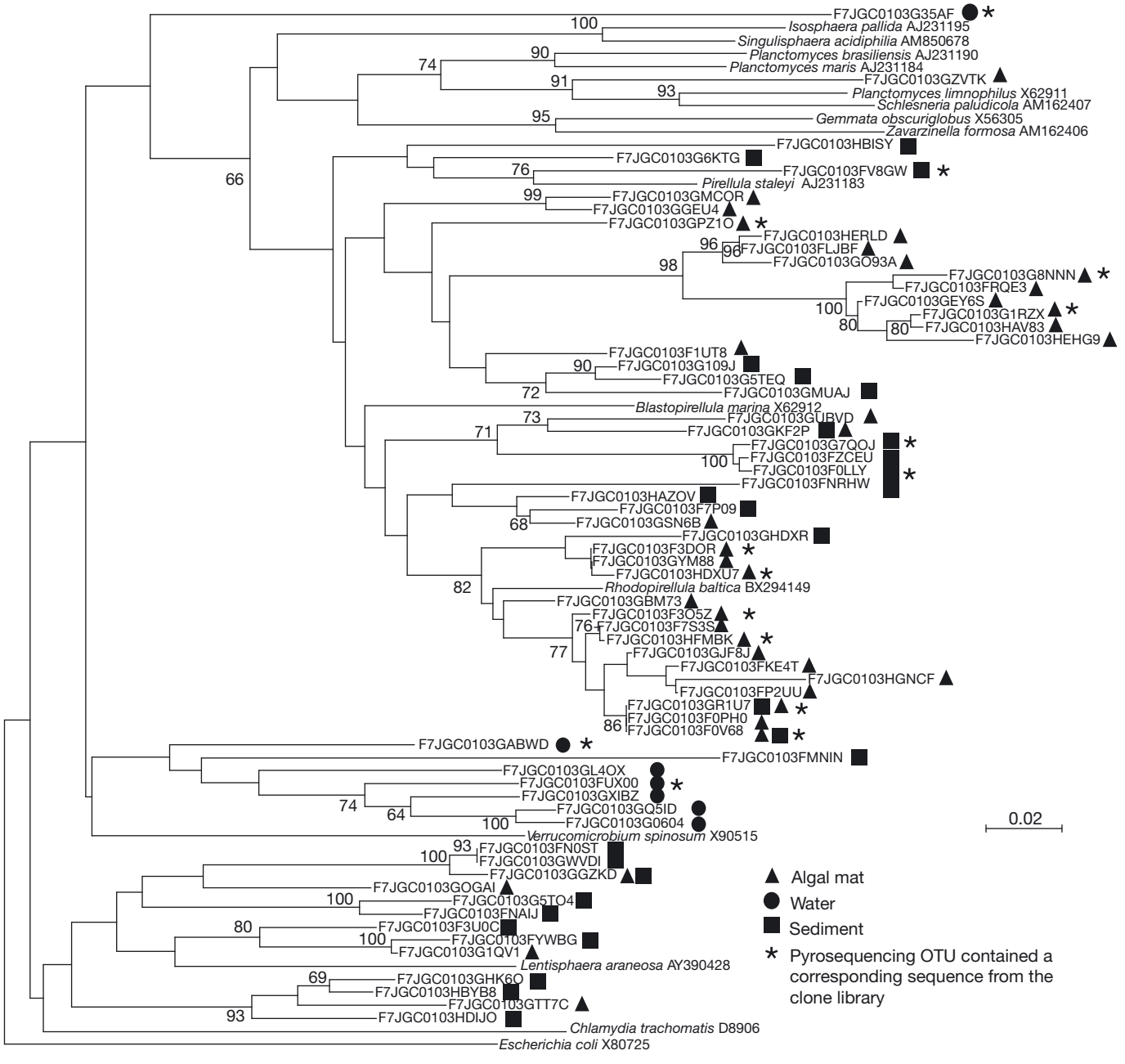

Fig. 2. Neighbor-joining distance tree showing the relationship between representative operational taxonomic unit (OTU) sequences and type strains of related genera. Bootstrap values (1000 resamplings) with ≥60% support are shown at the nodes. *Escherichia coli* is included as an outgroup to root the tree. Symbols at the leaves indicate the lake environment with which the OTU is associated. In cases where the branch has 2 symbols, the OTU contains sequences from 2 lake environments. Branches labeled with an asterisk indicate OTUs that also include sequences from the clone libraries. Bar = 0.02 substitutions per site. GenBank accession numbers of reference strains are indicated

full-length sequences. For example, we were able to recover the previously reported sister relationships between the genera *Isosphaera* and *Singulisphaera* (Kulichevskaya et al. 2008), *Gemmata* and *Zavarzinella* (Kulichevskaya et al. 2009), and *Planctomyces* and *Schlesneria* (Kulichevskaya et al. 2007). *Blastopirellula* and *Rhodopirellula* also clustered with *Pirellula*, as expected. However, there were some discrepancies in our tree, such as the lack of monophyly for the *Planctomyces* species, which were interspersed with *S. paludicola*.

Despite these discrepancies, which are probably attributable to the use of short sequences, the phylogenetic relationships of the recovered sequences generally verified the taxonomic classification presented in Tables 1 & 3, with the majority of sequences clustering with *Rhodopirellula*, *Blastopirellula*, and *Pirellula* species. Few sequences clustered with the type strains from the genera *Planctomyces*, *Gemmata*, *Singulisphaera*, *Schlesneria*, or *Zavarzinella*. This was likely due to the low relative abundance of sequences affiliated to these genera in the libraries, leading to their exclusion from the set of OTUs used for phylogenetic reconstruction (i.e. OTUs with >10 sequences). In most cases, our lake sequences were not highly related to any of the described, cultured planctomycete species. Several of the clones were more closely related to other sequences from the PVC superphylum than to any planctomycete sequences. On further analysis, these sequences were all within the set of sequences not considered to be planctomycete by at least 1 classifier (Table 3). There was some clustering of planctomycete populations by environment type, although there were also some interspersed sequences from different environments.

DISCUSSION

To our knowledge, this report presents the largest culture-independent study of planctomycete diversity performed to date. In total 6287 planctomycete sequences were identified via targeted pyrosequencing. These sequences could be assigned to 9 planctomycete genera, indicating the ability of targeted pyrosequencing to recover a diverse planctomycete population. Of these sequences, 3270 (52%) shared <97% sequence identity to any database sequences, suggesting that many originate from previously undescribed planctomycete lineages. It seems likely that subsequent studies employing targeted pyrosequencing could uncover a similar level of planctomycete diversity from other environments. BLASTALL comparison between data obtained from cloning-based and pyrosequencing approaches indicated that the 2 methods detected an overlapping set of planctomycete sequences, with the exception of a small number of clone library sequences that were not detected in the pyrosequencing library. However, the non-overlapping sequences were all among the sequences that could not reliably be classified to any known planctomycete genus, suggesting that the methods differed mainly in the amplification of organisms distantly related to known planctomycete-related sequences.

Planctomycete-related 16S rRNA genes were identified in all 3 lake environments sampled. However, non-planctomycete sequences were also detected in all of the samples, in both the clone and pyrosequencing libraries. Similar amplification of non-target organisms with the PLA40 targeted primer has been reported in other studies using clone libraries (Derakshani et al. 2001, Köhler et al. 2008). Currently, we cannot account for the increased detection of non-target organisms in the pyrosequencing survey relative to the clone library sequencing. Differences in PCR product amplification and purification are inherent in preparing material for the different library constructions and may account for the differences in the ratio of non-target organisms. For example, pyrosequencing requires adding adaptors to the primers during the initial PCR to allow for bead immobilization (http://454.com/products-solutions/how-it-works/sequencing-chemistry.asp). This modification to the pyrosequencing primer sequence may have slightly altered the thermal properties of the primer as compared to the primers used to generate the clone libraries, accounting for differential amplification. Further studies to characterize these biases will need to be conducted in order to optimize targeted pyrosequencing.

We observed a lack of overlapping OTUs between pyrosequencing libraries obtained from different lake environments (Fig. 1) and clustering of sequences based on the environment of isolation (Fig. 2), suggesting that the populations of planctomycetes differ between the different lake habitats. However, samples from the water column were collected by pre-filtration through 2.0 µm pore filters, which would have excluded large particles. Therefore, the planctomycete 16S rRNA gene sequences described in the water likely represent free-living bacterial cells. This physical separation was not applied to the sediment and algal mat samples and so may explain some of the differences seen between the water planctomycete populations compared to the sediment and algal mat populations. The microbial communities associated with particulate matter, or 'lake snow', which were excluded from the present study, have been shown to differ from the free-living microbial community (Riemann & Winding 2001, Lemarchand et al. 2006). As

this particulate matter is composed of planktonic or senescent algal cells, bacteria associated with this material may represent an important bacterial–algal association not described in the present study (Tang et al. 2009).

Pyrosequencing suggested an enrichment of sequences related to *Rhodopirellula* in the algal mat (6-fold greater relative abundance) relative to the other samples. The genome sequence of the marine isolate *R. baltica* encodes a large number of predicted sulfatase genes that may aid in the metabolism of sulfated polymers, such as sulfated fucans, produced by algae (Glöckner et al. 2003). Similar genes may be present in other *Rhodopirellula*-related organisms. In the water sample, sequences related to *Isosphaera* were detected, but were not found in the sediment or algal mat. *Isosphaera* species were first described as thermophiles (Giovannoni et al. 1987), but have also been recovered from non-thermal habitats (Wang et al. 2002). Given that the lake temperature at the time of sampling was 25°C, *Isosphaera* sequences recovered in the present study likely represent non-thermophilic organisms. Analysis of a larger number of samples is needed to determine whether these genus-level patterns of distribution are specifically associated with these different habitats.

Our data indicate that pyrosequencing with phylum-targeted primers could be a useful approach for describing the distribution and abundance of organisms that are recalcitrant to laboratory cultivation or are difficult to study due to their relatively low abundance in environmental samples. Refinement of the planctomycete-targeted primers, and determination of the basis for differences between pyrosequencing and clone library sequencing in recovery rates of target organisms, may reduce the recovery of non-planctomycete sequences and further optimize our ability to describe planctomycete populations in environmental samples. This methodology could be employed for future testing of hypotheses regarding spatial and temporal differences in planctomycete diversity and abundance. Candidate hypotheses arising from this preliminary study include (1) the relative abundance of *Rhodopirellula* species is higher in algal mats than in other lake habitats; (2) sediments harbor the most diverse planctomycete populations; and (3) most planctomycete OTUs are not shared between habitats.

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