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Microbial Communities and Fecal Indicator Bacteria Associated with *Cladophora* Mats on Beach Sites along Lake Michigan Shores

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A high biomasses of *Cladophora*, a filamentous green alga, is found mainly during the summer along the shores of Lake Michigan. In this study, the abundance and persistence of the fecal indicator bacterium *Escherichia coli* and sulfate-reducing bacteria (SRB) on *Cladophora* mats collected at Lake Michigan beaches were evaluated using both culture-based and molecular analyses. Additionally, 16S rRNA gene cloning and sequencing were used to examine the bacterial community composition. Overall, *E. coli* was detected in all 63 samples obtained from 11 sites, and the average levels at most beaches ranged from 2,700 CFU/100 g (wet weight) of *Cladophora* to 7,500 CFU/100 g of *Cladophora*. However, three beaches were found to have site average *E. coli* densities of 12,800, 21,130, and 27,950 CFU/100 g of *Cladophora*. The *E. coli* levels in the lake water collected at the same time from these three sites were less than the recommended U.S. Environmental Protection Agency limit, 235 CFU/100 ml. *E. coli* also persisted on *Cladophora* mats in microcosms at room temperature for more than 7 days, and in some experiments it persisted for as long as 28 days. The SRB densities on *Cladophora* mats were relatively high, ranging from 4.4×10^6 cells/g (6.64 log CFU/g) to 5.73×10^6 cells/g (6.76 log CFU/g) and accounting for between 20% and 27% of the total bacterial counts. Partial sequences of the 16S rRNA gene clones revealed a phylogenetically diverse community, in which the *Cytophaga-Flavobacterium-Bacteroides* cluster and the low-G+C-content gram-positive bacteria were the dominant organisms, accounting for 40% and 12.8%, respectively, of the total clone library. These results further reveal the potential public health and ecological significance of *Cladophora* mats that are commonly found along the shoreline of Lake Michigan, especially with regard to the potential to harbor microorganisms associated with fecal pollution and odor-causing bacteria.

Cladophora spp. are filamentous green algae that commonly grow attached to hard substrates in the littoral zone of freshwater environments and are widely distributed throughout the Laurentian Great Lakes (7, 8, 11). The excessive growth of algae in the Great Lakes was previously linked to increases in phosphorus levels that resulted primarily from urban sewage inflow and agricultural runoff (21, 39). However, despite the fact that the external phosphorus load has decreased in the Great Lakes in recent years (4, 40), there has been a resurgence of *Cladophora* growth in the nearshore zone (13).

The occurrence of *Cladophora* in nearshore Lake Michigan habitats may have significant public health and ecological importance (18, 43) and could also potentially decrease recreational activities on beaches. There is evidence that the algae provide food and shelter for epiphytes, invertebrates, and small fish (7, 33, 40, 43) and could provide a niche for pathogenic bacteria from gull droppings, sewage overflow, or runoff from urban and agricultural sources (14, 43). The presence of *Cladophora* has been associated with high levels of *Escherichia coli* and enterococcal bacteria in beach sand and swimming waters of the Great Lakes (18, 43), which may diminish the relationship between indicator organisms and actual pollution and potentially complicate beach-monitoring efforts.

Cladophora mats that become stranded on beaches produce a noxious odor as the organic material decays. It has been suggested that activities of sulfate-reducing bacterial (SRB) populations on algae play a dominant role in the anaerobic processes in aquatic environments (15, 16), accounting for about 50% of the biomineralization of organic matter (17, 31). In general, the abundance and activities of SRB have been observed to increase with an increase in the supply of organic nutrients (34, 41). Sulfate-reducing bacteria belonging to the δ subdivision of the *Proteobacteria* have been shown to account for significant portions of bacterial populations on microbial mats in lakes (34, 35). In a study of Lake Cadagno (Switzerland), Tonolla et al. (35) found that 24% of the 4′,6′-diamidino-2-phenylindole (DAPI)-stained cells were sulfate reducers by using fluorescent in situ hybridization, which corroborated the results of previous studies that documented the dominance of these bacterial groups in freshwater systems (12, 25).

Although the public health and ecological importance of microbial communities on *Cladophora* mats in freshwater environments have been documented, the structure and composition of such communities remain largely uncharacterized. Therefore, in this study, we employed both culture-based and molecular analyses to examine and characterize microbial communities associated with *Cladophora* mats collected along the shoreline of Lake Michigan. The primary objective of our study was to determine the abundance and persistence of the fecal indicator bacterium *E. coli* and the SRB phylogenetic group on *Cladophora* mats. Additionally, this study was also designed to examine the compositions of microbial communities associated with *Cladophora* mats using 16S rRNA gene sequencing.

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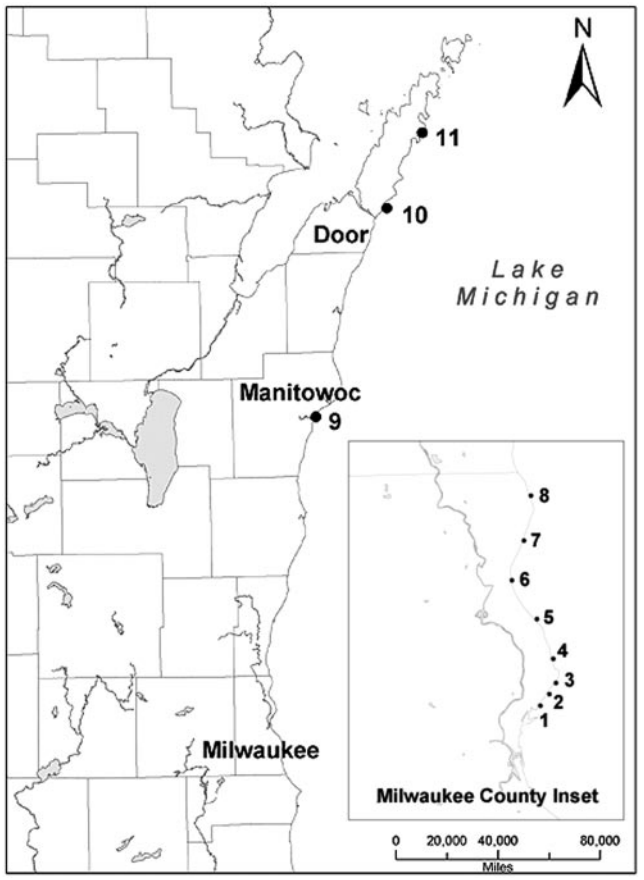

FIG. 1. Map of counties along Lake Michigan where *Cladophora* mats and water samples were collected from beaches from July to November 2004 and from June to September 2005.

MATERIALS AND METHODS

Site description. *Cladophora* was collected at 11 beach sites in Wisconsin along the western shore of Lake Michigan to determine the prevalence of *E. coli* on stranded mats (Fig. 1). The sites were predominately in highly urbanized areas (McKinley and Bradford) and suburban areas (Atwater, Big Bay, and Doctors Park), but they also included sites located in an agricultural watershed (Fischer Beach) and in less developed communities (Portage Park).

Three of the beach sites in Milwaukee County (Fig. 1) chosen for *Cladophora* collection were used in microcosm studies and for characterization of the microbial community by sequencing of the 16S rRNA gene. McKinley Beach is located immediately north of Milwaukee Harbor and is characterized by a steep littoral zone and a relatively deep (3 to 4 m) benthic zone 30 m from shore. Bradford Beach is located 2 km north of McKinley Beach and, in general, experiences a higher occurrence of *Cladophora* strandings than McKinley Beach. Bradford Beach has a low sloping littoral zone and shallow benthic zone (<2 m) that extends 400 m from the shore. At both McKinley Beach and Bradford Beach there are multiple sources of fecal pollution, including stormwater discharged directly into beach areas and high numbers of ring-billed gulls (*Larus delawarensis*). Atwater Beach is located approximately 5 km north of Bradford Beach and is the beach that is least affected by pollution sources. The littoral zone is similar to that at Bradford Beach, and the benthic zone is 3 m deep within 100 m from the shore. Two jetties flank the beach site and act as catchments for *Cladophora*, potentially increasing the residence time of *Cladophora* stranded at the shoreline.

Sample collection. *Cladophora* samples were collected on 10 sampling days from July through November 2004 and on six sampling days from June through September 2005 to determine the prevalence of *E. coli* on stranded mats along the shores of Lake Michigan. In all, 63 samples were collected at 11 beach sites. *Cladophora* mats were obtained from the waterline at each beach site and placed in sterile Whirl-Pak bags. At Bradford Beach, McKinley Beach, and Atwater Beach, the three beach sites used for microcosm studies, as well as several other beach sites, water samples were also collected using a 1-liter grab sampler and transferred to autoclaved sample bottles. Both the *Cladophora* mats and matching water samples were placed on ice and taken immediately to the laboratory, where they were stored at 4°C until they were analyzed.

Enumeration of *E. coli* on *Cladophora* mats from beaches. *E. coli* densities on the *Cladophora* mats and in the matching water samples were determined by membrane filtration using a U.S. Environmental Protection Agency (USEPA) modified method (36). For *Cladophora* samples, 50 g of algal material was placed in a sterile 1-liter beaker with 600 ml of 0.85% sterile saline and vigorously stirred for 10 min to elute the bacteria from the mats. Then 1 and 10 ml of the supernatant were removed for membrane filtration; for samples in which colonies were too numerous to count, 1 ml of a 1:10 or 1:100 dilution was used. For analysis of water samples, 10 ml and 100 ml were filtered through 0.45-μm nitrocellulose membrane filters, placed on modified m-TEC medium (BD Diagnostics, Sparks, MD), and incubated at 44.5°C overnight. Colonies that exhibited β-glucuronidase activity, as indicated by a purple color, were counted as *E. coli* colonies.

Determination of bacterial abundance and persistence on *Cladophora* mats in microcosm studies. Microcosm experiments were carried out with freshly collected *Cladophora* mats to specifically evaluate the persistence of *E. coli*, sulfate-reducing bacterial, and total bacterial populations in microbial communities in the mats. Approximately 100-g (wet weight) portions of mats were placed in sterile beakers along with 800 ml of lake water collected at the site. Matching water samples that contained no *Cladophora* were analyzed concurrently to examine the persistence of *E. coli* over time. The beakers were kept in the dark at room temperature to reduce or prevent photosynthetic activity. Subsamples were collected and examined for the presence of different bacterial populations. For *E. coli*, microcosms were vigorously stirred, and aliquots were analyzed as described above. Approximately 1 g of algal mat from each sampling site was weighed and added to 5 ml of sterile deionized water. Bacterial cells on the *Cladophora* mats were detached by sonication (model 2210 sonicator; Branson, Danbury, CT) at 40 kHz for 5 min in 30 ml of 0.1% tetrasodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) before total bacteria and SRB were counted as described below.

DAPI staining. The total number of bacteria was determined by concentrating samples under a 15-kPa vacuum onto 0.2-μm-pore-size, black polycarbonate filters (GE Osmonics, Minnetonka, MN), which were stained with 200 μl of 15-μg/μl DAPI for 3 min, rinsed with sterile water, and mounted on glass slides with type FF immersion oil (28). Cells in 10 fields were counted using epifluorescence microscopy (model BX60 microscope; Olympus, Melville, NY).

Fluorescent in situ hybridization. The abundance of SRB on the *Cladophora* mats was determined using fluorescent in situ hybridization as described by Manz et al. (20) and Olapade and Leff (23). SRB populations were detected using the SRB385 probe designed to specifically target the δ subdivision of the *Proteobacteria*, as described by Amann et al. (2), which is the phylogenetic group containing most sulfur-reducing bacteria (6, 31, 35); however, this probe also hybridizes with several gram-positive bacteria, such as *Clostridium* (30). Briefly, samples were concentrated onto 0.2-μm-pore-size polycarbonate filters, rinsed with deionized water, and treated with 1 ml of 0.1% Nonidet P-40 (Sigma-Aldrich, St. Louis, MO). The filters were placed in petri plates and treated with 40 μl of the Texas Red-labeled probe, which was used at a concentration of 5 ng/μl in hybridization buffer consisting of 6× SSC, 0.02 M TRIZMA base (pH 7.0), 0.1% sodium dodecyl sulfate, and 0.01% poly(A) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The filters and probe were incubated at 53°C for 4 h. The sequence of the SRB385 probe was 5′CGGCGTCGCTGCGTCAGG3′ (Sigma Genosys, The Woodlands, TX). After incubation, the filters were washed twice with 400 μl of wash buffer, incubated with 80 μl of wash buffer for 10 min at the hybridization temperature, and then rinsed twice with 400 μl sterile deionized water. Cells that hybridized to the SRB385 probe were enumerated using epifluorescence microscopy by counting between 50 and 100 fields on duplicate slides.

DNA extraction and PCR amplification. Total DNA was extracted from the *Cladophora* samples in microcosm studies using a QIAamp DNA extraction kit, as described by the manufacturer (QIAGEN, Valencia, CA). DNA was extracted from multiple beakers representing the three beach sites at the start of the experiments. DNA was eluted in 75 μl of sterile, deionized water before the concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Bacterial DNA in a sample was amplified by PCR by targeting the (approximately) full-length 16S rRNA gene with the universal bacterial primers 8F (5′AGAGTTTGATCCTGGCTCAG3′) and 1492R (5′ GGTTACCTT GTTACGACTT 3′) (Sigma Genosys, The Woodlands, TX). The PCR amplification protocol included DNA denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension 72°C for 1 min for 30 cycles and a final extension for 5 min at 72°C, using a thermal cycler from MJ Research (Watertown, MA). The amplification

TABLE 1. *E. coli* densities on *Cladophora* mats collected from beach sites on Lake Michigan

Site no.[a]	Beach	No. of days sampled	No. of samples	CFU/100 g of *Cladophora*	
				Mean	Range
1	McKinley	2	2	27,950	14,400–41,500
2	Bradford	7	9	21,130	1,680–60,000
3	Lake Drive	1	1	7,500	
4	Atwater	10	21	12,800	155–54,600
5	Big Bay	1	1	4,200	
6	Klode	1	1	2,700	
7	Beach Drive	1	1	38,400	
8	Doctors	1	2	6,100	3,000–5,100
9	Fischer	2	13	6,900	500–28,000
10	Portage Park	1	2	3,570	2,320–4,825
11	Bailey's Harbor	1	10	6,180	1,920–10,040

[a] See Fig. 1.

products were evaluated on a 1.5% agarose gel. The PCR products were purified with a QIAquick PCR purification kit (QIAGEN, Valencia, CA) before they were utilized for cloning and sequencing assays.

Cloning and sequencing. Clone libraries from 16S rRNA gene amplification were constructed using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Plasmid DNA was isolated using a QIAGEN plasmid mini kit (QIAGEN, Valencia, CA). Sequencing of the 16S rRNA gene was performed with a CEQ 8000 automated sequencer, using the chain terminator technique with dye-labeled dideoxynucleotides, according to the manufacturer's instructions (Beckman Coulter, Fullerton, CA).

Phylogenetic analysis. Approximately 200 clones from three 16S rRNA clone libraries were randomly chosen for sequencing. The sequences obtained were trimmed for quality before alignment, using the Vector NTI software (Invitrogen, Carlsbad, CA). Neighbor-joining trees were constructed using ClustalW with 1,000 bootstrap iterations and NJ plot (27). The clone sequences were compared with previously published GenBank sequences using the BLAST system (1) in order to determine close relatives.

Statistical analyses. Statistical analyses were carried out using SPSS, version 10.01 (SPSS Inc., Chicago, IL). Differences between numbers of bacteria were determined by Student *t* test analysis after normalization of all data sets by $\log_{10}$ transformation.

Nucleotide sequence accession numbers. The partial 16S rRNA gene sequences have been deposited in the GenBank database under accession numbers DQ228212 to DQ228252.

RESULTS

***E. coli* abundance on *Cladophora* mats and in water samples at beach sites.** The *E. coli* levels on the *Cladophora* mats collected from 11 beach sites far exceeded the USEPA threshold for *E. coli* in recreational water for more than 97% of the mats. The average *E. coli* level found on *Cladophora* mats at each site is shown in Table 1. The average values for several sites were between 2,700 and 7,500 CFU/100 g (wet weight) of *Cladophora*. However, *E. coli* levels that were almost 1 order of magnitude

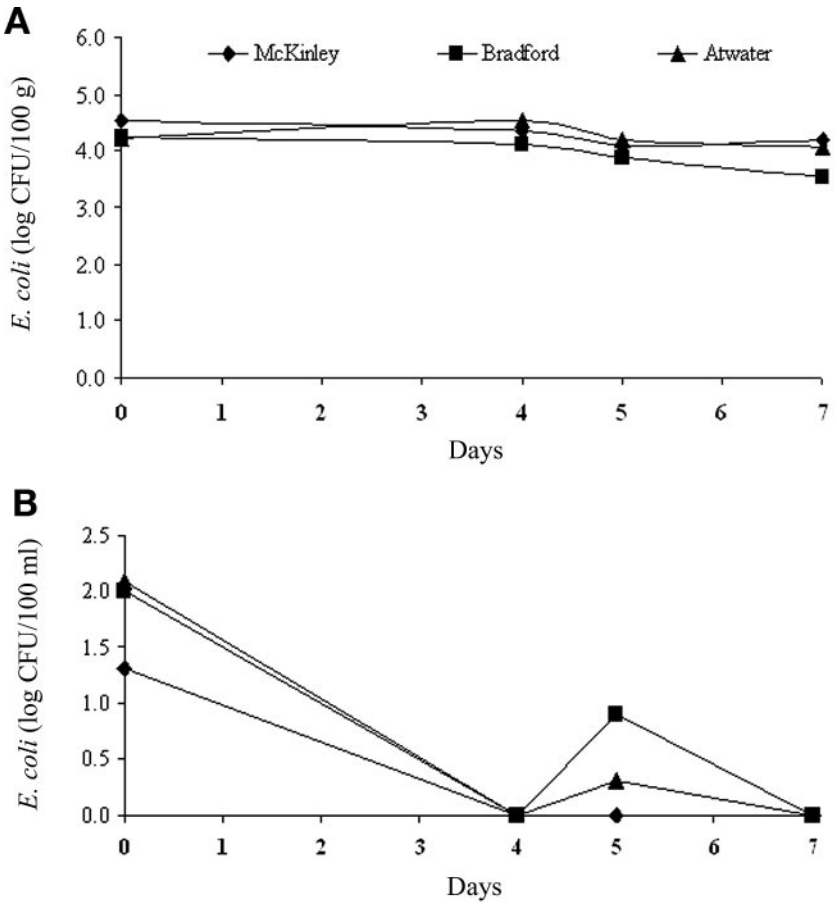

FIG. 2. Numbers of *E. coli* (log transformed) on *Cladophora* mats (A) and in lake water samples (B) collected from beach sites (McKinley Beach, Bradford Beach, and Atwater Beach) along the Lake Michigan shore. The values are averages for replicate samples from each microcosm. The standard errors were less than 6% of the *E. coli* values for *Cladophora* samples and less than 10% of the *E. coli* values for water samples.

higher were detected at McKinley Beach (27,950 CFU/100 g of *Cladophora*), Bradford Beach (21,130 CFU/100 g of *Cladophora*), and Atwater Beach (12,800 CFU/100 g of *Cladophora*). The *Cladophora* mats from the Beach Drive site had the highest density of *E. coli*, more than 38,000 CFU/100 g; however, only one sample was collected at this site. Water samples ($n = 29$) collected from a subset of the beach sites contained levels of *E. coli* ranging from 0 to 6,900 CFU/100 ml. The *E. coli* levels in the majority of the samples (more than 87%) were below the recommended USEPA limit, 235 CFU/100 ml. Overall, the *E. coli* levels on the *Cladophora* mats were significantly higher than the levels in the adjacent water ($P < 0.001$).

***E. coli* persistence on *Cladophora* mats in microcosm studies.** The results of microcosm studies demonstrated that the initial *E. coli* levels on the *Cladophora* mats (Fig. 2A) were higher than the levels in the matching water samples collected at the same beach sites (Fig. 2B). The *E. coli* levels on *Cladophora* mats in microcosms were similar for the first 4 days of the survival experiment, and slight decreases in density were observed on day 5. Also, it appeared that the persistence of *E. coli* on the *Cladophora* mats was similar for the three beach sites examined. For the lake water samples from the three sites, the *E. coli* levels decreased to levels below the detection level by day 4. These results indicate that *E. coli* populations could potentially survive on algal mats far longer than the 4- to 6 day die-off period observed in water samples containing no *Cladophora*.

In experiments performed for 28 days a similar trend was observed; the *E. coli* concentrations in microcosms containing only lake water decreased from 240 CFU/100 ml to 20 CFU/100 ml within 4 days, but the *E. coli* densities in microcosms with water and *Cladophora* remained steady for the first 7 days, and this was followed by gradual decreases in the number of *E. coli* until day 28, when the *E. coli* levels were found to be approximately 10% of the original concentration.

Numbers of total and sulfate-reducing bacteria. In general, the numbers of total and sulfate-reducing bacteria on the *Cladophora* mats in microcosms were fairly similar for the three sites (Fig. 3A and 3B). The numbers of sulfate-reducing bacteria that hybridized to the SRB385 probe on freshly collected *Cladophora* mats ranged from 4.4×10^6 cells/g (6.64 log CFU/g) to 5.73×10^6 cells/g (6.76 log CFU/g) and remained consistently high after 7 days in microcosms (Fig. 3B). The SRB in the microbial communities accounted for between 20% and 27% of the total bacteria at the three sites.

Phylogenetic diversity of phylotypes on *Cladophora* mats. Approximately 200 clone sequences were obtained from 16S rRNA gene libraries constructed from the microcosm experiment and used for phylogenetic analysis. The phylogenetic tree could be divided into seven clusters based on similarities to previously published sequences (Fig. 4). Clusters 1 and 2 consisted of bacteria belonging to the α- and β-*Proteobacteria* and accounted for 2.4% and 6.4% of the clone libraries (Table 2). Cluster 3 contained only three clones belonging to the δ-*Proteobacteria*, representing <1.0% of the clone libraries, while the ε subdivision of the *Proteobacteria* (cluster 4) accounted for 3.2% of the clone libraries. Clusters 5 and 6 accounted for 12.8% and 4.8% of the clone library and belonged to the low-G+C-content and high-G+C-content gram-positive bacteria, respectively. The largest cluster was the *Cytophaga-Flavobacterium-Bacteroides* (CFB) bacterial group, which accounted for about 40% of the total clone library.

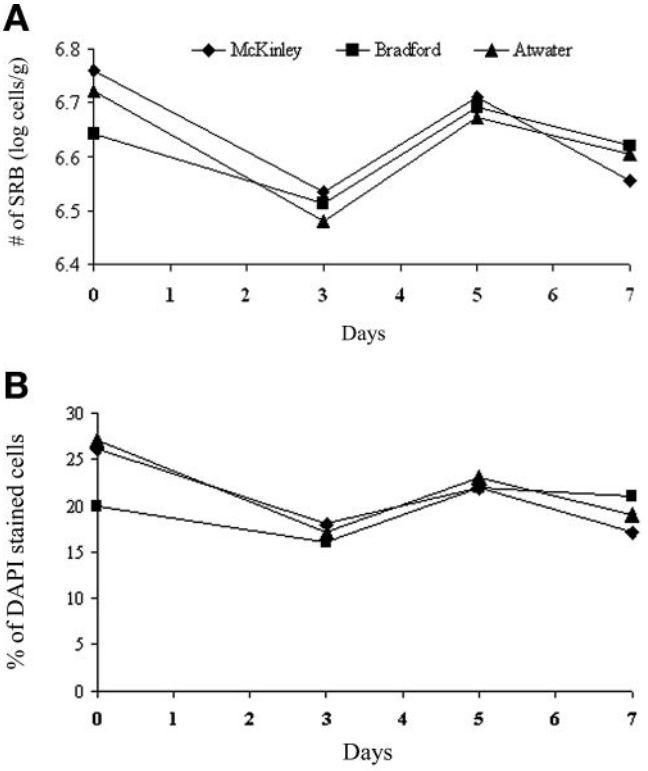

FIG. 3. Numbers of SRB (A) and percentages of DAPI-stained cells positive for the SRB385 probe (B) on *Cladophora* mats collected from McKinley Beach, Bradford Beach, and Atwater Beach in Wisconsin during survival studies conducted in microcosms. The values are averages for replicate samples from each microcosm.

DISCUSSION

***E. coli* abundance and persistence on *Cladophora* mats.** Our findings revealed that *E. coli* was frequently present at extremely high densities on *Cladophora* mats stranded at the shoreline of beach sites and that the levels far exceeded the level that is acceptable for recreational water (37). The widespread occurrence at the 11 beach sites further supports the hypothesis that this might be common at Great Lakes beaches, as documented previously (43). Furthermore, microcosm experiments suggested that *Cladophora* prolongs the survival of *E. coli*. These findings highlight the public health significance of conditions that could prolong the survival of nonindigenous bacteria (18, 43), including pathogens that might be carried with fecal pollution. Both of the previous studies (18, 43) cautioned against the sole use of either *E. coli* or enteroccoci as indicators of the quality of recreational freshwater, since the origin of these organisms might be not only fecal but also associated with drifts of *Cladophora* mats that potentially serve as secondary habitats and therefore affect the water quality at affected Lake Michigan beaches.

Abundance and persistence of sulfate-reducing bacteria on *Cladophora* mats. Quantitative analysis of the microbial communities on the *Cladophora* mats using the SRB385 fluores-

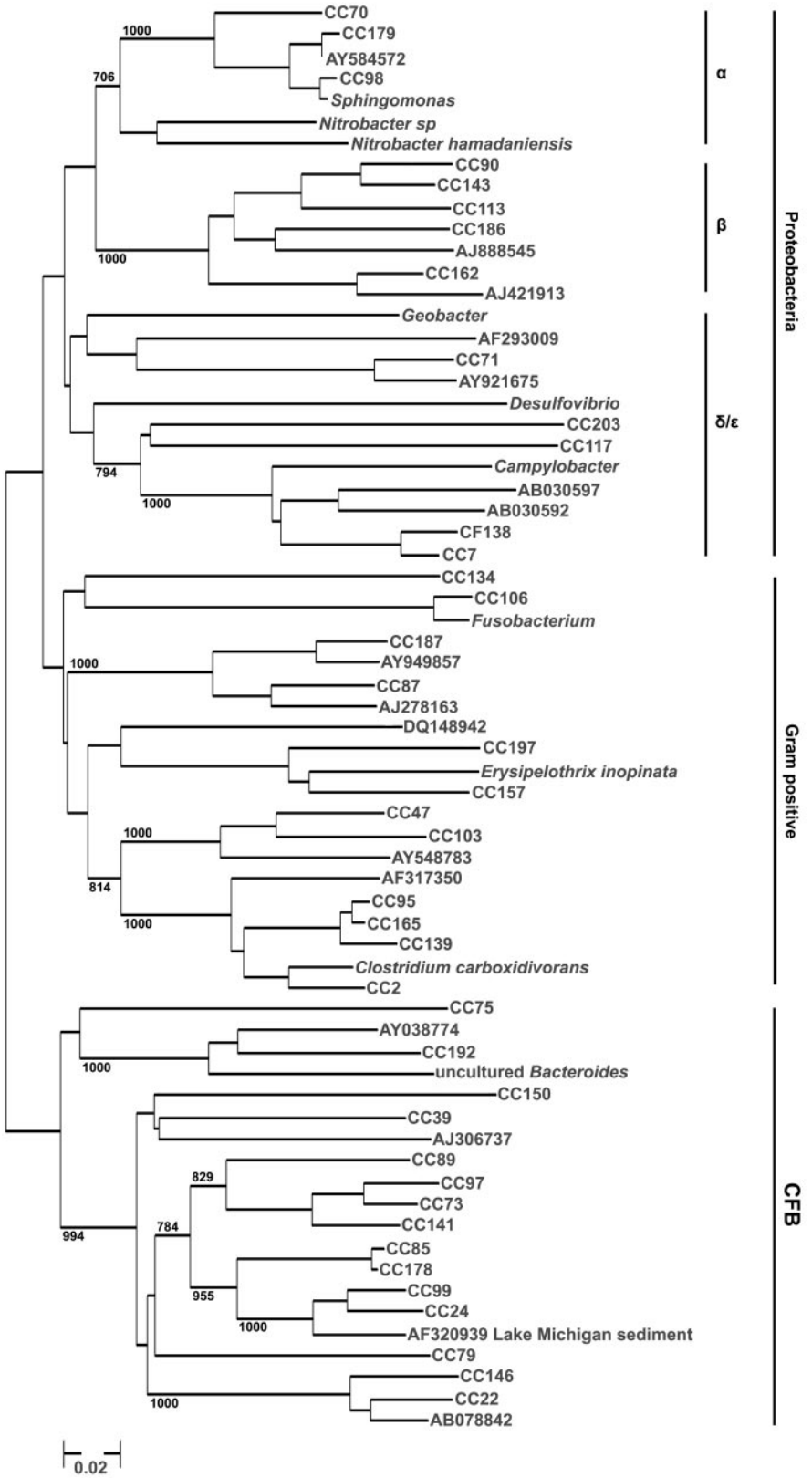

FIG. 4. Phylogenetic consensus tree based on neighbor-joining analyses of partial 16S rRNA gene sequences, showing the representative clones of predominant bacterial taxa.

TABLE 2. Distribution of 200 cloned 16S rRNA gene sequences obtained from the epiphytic bacterial communities on *Cladophora* mats collected from three beach sites along Lake Michigan shores

Taxonomic group	No. of clones	%	Closest relative	Accession no.
Bacteroides	80	40	Uncultured-feedlot manure bacterium A13	AF320939
High-G+C-content gram-positive bacteria	9	4.8	Actinobacteria	AY642054
Low-G+C-content gram-positive bacteria	26	12.8	*Clostridium carboxidivorans* 16S rRNA gene	AY170379
α-*Proteobacteria*	5	2.4	*Sphingomonas* sp. strain HTCC503	AY584572
β-*Proteobacteria*	13	6.4	β-Proteobacterium clone TH487 partial 16S rRNA gene	AJ888545
δ-*Proteobacteria*	2	0.8	δ-Proteobacterium clone AKYG859 16S rRNA gene	AY921675
ε-*Proteobacteria*	6	3.2	ε-Proteobacterium 1006 gene for 16S rRNA	AB030592
Unclassified bacteria	59	29.6	Uncultured bacterium clone SHA-13 partial 16S rRNA gene	AJ306737

cently labeled probe indicated that the numbers of sulfate-reducing bacteria were relatively high, perhaps higher than the numbers based on results obtained from clone libraries. The obvious disparity between the two methodological approaches could be attributed to the comparatively low coverage of this phylogenetic group by the sequencing approach. Overall, the sizes of the populations of bacteria belonging to the SRB functional group in the microbial communities examined in our study fell in the range that was previously documented in similar studies (31, 34). In a study in which a traditional most-probable-number approach was used, Teske et al. (34) found an average of 4.5×10^6 SRB cells/ml on the surface layer of cyanobacterial mats of Solar Lake (Sinai, Egypt). Also, Santegoeds et al. (31), using the same SRB385 probe that was used in our study, found an average of 64×10^6 SRB cells/ml on the surface of a 2-week-old artificially constructed bacterial biofilm. Cross-hybridization of the SRB385 probe with non-SRB members of the community also should be considered, as this probe has been shown to detect gram-positive bacteria, such as *Clostridium* spp. (30). The occurrence of bacteria belonging to the SRB functional group on *Cladophora* mats examined in this study may further corroborate the well-documented alga-bacterium association found in previous studies, based in part on the reliance of heterotrophic bacterial populations on alga-released dissolved organic carbon in surface-associated microbial communities (10, 23).

Bacterial community composition on the *Cladophora* mats. The cloned sequences obtained from the epiphytic bacterial communities on *Cladophora* mats belonged to various phylogenetic groups in the domain *Bacteria*, including the CFB cluster, the high- and low-G+C-content gram-positive bacteria, and the α, β, δ, and ε subdivisions of the *Proteobacteria*. Several of the clones used in this study were most closely related to sequences that were characterized as previously unclassified or uncultured bacteria based on sequences in the GenBank database, suggesting that a high number of phenotypically undescribed bacterial species were present in the community, as observed in other studies of bacterium-alga interactions (9).

The CFB phylogenetic group was the most abundant clone group obtained from the *Cladophora* mats, which corroborated the results of previous studies that documented that there were high levels of bacterial species belonging to the CFB cluster in similar alga-bacterium associations in aquatic systems (9, 42). In general, bacterial species belonging to the CFB cluster have been reported to account for a high percentage of biofilm bacteria in aquatic systems (24) because they possess unique phenotypic characteristics that enable them to attach to particles, exhibit surface-dependent gliding motility, and utilize various complex macromolecules (44). The high percentage of clones in this phylogenetic group perhaps reflects the active degradation of high-molecular-weight organic carbon (i.e., cellulose) and various other macromolecules on the *Cladophora* mats.

The relatively high numbers of bacteria belonging to the high-G+C-content gram-positive group in the microbial communities on the algal mats were not too surprising, despite the fact that this group usually occurs at fairly low levels in aquatic systems (3). We expected high levels of bacteria belonging to both the CFB cluster and the gram-positive bacteria in the microbial communities on the *Cladophora* mats, since both of these bacterial groups typically comprise species that can thrive under both the oxic and anoxic conditions (29) commonly found on cyanobacterial mats located in freshwater environments (34).

The four subdivisions of *Proteobacteria* (i.e., α, β, δ, and ε subdivisions) that were found in this study accounted for a relatively high proportion of the total cloned sequences in the microbial communities on the algal mats. Among these bacterial groups, bacteria belonging to the β-*Proteobacteria* generally predominated; this finding is consistent with the results of previous studies that showed that there were high proportions of β-*Proteobacteria* in aquatic biofilms (5, 22, 32). Although bacteria belonging to the δ-*Proteobacteria* accounted for the lowest proportion of sequences in our study, a high number of bacterial species with the ability to reduce sulfate belong to this subdivision and have been found previously in bacterial biofilms in other studies (31, 34).

Ecological and public health significance and future directions. There has been increased emphasis on beach water quality in recent years by both the USEPA and the public health sector, and as a result, more beaches are being monitored for fecal pollution (38). *Cladophora* accumulation at the shoreline, a common occurrence at Great Lakes beach sites, might be a major complicating factor in assessing beach sites for fecal pollution. The potential for *Cladophora* mats to harbor pathogens, as well as indicator bacteria, warrants further study.

Typically, a person's initial perception of water quality is based entirely on the esthetic characteristics of the water and the surrounding environment (26). Decaying *Cladophora* emits a noxious odor which frequently can be mistaken for sewage contamination. The bacteria belonging to the CFB cluster in the microbial communities are most likely responsible for the

majority of cell material breakdown on the algal mats and may produce the anoxic environment required for SRB, which are mainly obligate anaerobes (19). The metabolic activity of members of the SRB functional group, especially organisms belonging to the δ-*Proteobacteria*, may contribute the most to the noxious odor that is characteristic of the beach sites along Lake Michigan. Additionally, the presence of SRB on the *Cladophora* mats suggests that the availability of sulfate is high and that the sulfate is metabolized, releasing hydrogen sulfide gas, which has a characteristic pungent odor. There is a need for studies examining this and establishing a direct correlation between populations of bacteria belonging to the SRB group on *Cladophora* mats and hydrogen sulfide production in order to further understand the ecological importance of the mats.

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