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Molecular Characterization of Epiphytic Bacterial Communities on Charophycean Green Algae

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Epiphytic bacterial communities within the sheath material of three filamentous green algae, *Desmidium grevillii*, *Hyalotheca dissiliens*, and *Spondylosium pulchrum* (class Charophyceae, order Zygnematales), collected from a *Sphagnum* bog were characterized by PCR amplification, cloning, and sequencing of 16S ribosomal DNA. A total of 20 partial sequences and nine different sequence types were obtained, and one sequence type was recovered from the bacterial communities on all three algae. By phylogenetic analysis, the cloned sequences were placed into several major lineages of the *Bacteria* domain: the *Flexibacter*/*Cytophaga*/*Bacteroides* phylum and the α, β, and γ subdivisions of the phylum *Proteobacteria*. Analysis at the subphylum level revealed that the majority of our sequences were not closely affiliated with those of known, cultured taxa, although the estimated evolutionary distances between our sequences and their nearest neighbors were always less than 0.1 (i.e., greater than 90% similar). This result suggests that the majority of sequences obtained in this study represent as yet phenotypically undescribed bacterial species and that the range of bacterial-algal interactions that occur in nature has not yet been fully described.

Associations between microalgae and bacteria are commonly observed in both freshwater and marine ecosystems, and past culture and microscopy studies have documented a number of bacterial-algal interactions. Much attention has been focused on the release of dissolved organic carbon by algal cells and its support of bacterial growth (12, 20), and the surfaces of living cells may also provide microenvironmental conditions favorable for bacterial processes (e.g., nitrogen fixation) that otherwise could not occur under ambient water conditions (34). Heavy bacterial colonization of algae is generally considered a sign of algal senescence (6), but colonization of young, active algal cells or colonies is also observed (24, 35, 37) and benefits to algae of such associations have been frequently reported (16, 22, 26, 32). Bacteria and algae may also compete for inorganic nutrients (36, 44), and many algal taxa produce compounds that are potentially inhibitory to bacterial growth (21).

Although a variety of bacterial-algal interactions have been documented from previous studies, the ecological significance of most naturally occurring bacterial-algal associations is unclear and in most cases the bacterial species involved have not been identified (3, 28). Traditionally, the first step in investigations of microbial associations and identification of the organisms involved is to remove them into culture. However, it is well known that many microorganisms, especially many species of bacteria, resist cultivation because of their interdependencies with other microbes and the lack of knowledge concerning their specific growth requirements (29, 31).

Furthermore, although it is usual to think of an alga interacting with a single bacterial species (5) or with a homogeneous community of epiphytes with similar physiological attributes, surfaces in aquatic environments (including algal cells and mucilage) harbor complex and diverse bacterial communities (33). It is therefore possible that several interactions are occurring simultaneously between an alga and its bacterial flora. These interactions are important not only for the growth and survival of the microbes themselves but may have implications for ecosystem-level processes (4, 34).

In view of this, it would be useful to more fully describe the natural bacterial communities present on algae by using molecular methodologies based on PCR and the phylogenetics of the 16S rRNA gene (42). These approaches are particularly advantageous in instances where little is known of the bacterial community under study (31). In the case of bacterial-algal associations, determination of the genetic diversity of the bacterial community might provide insight into the diversity of interactions occurring between the two groups in the natural environment. Sequence analysis of 16S ribosomal DNA (rDNA) may also allow identification of readily culturable models (close relatives of unknown organisms) for use in future investigations.

In this study, we used 16S rDNA analysis to characterize bacterial epiphytes of members of the green algal group commonly known as desmids (class Charophyceae, order Zygnematales, family Desmidiaceae). These algae reach their greatest species diversity in acidic, soft waters such as *Sphagnum* bog pools and lakes (14), and certain desmid taxa (mainly filamentous forms) collected from *Sphagnum* bogs are consistently associated with bacteria (10). We suggest that desmid-bacterial associations represent a good system for molecular studies of bacterial communities on algae because (i) filamentous desmids are large and thus easily manipulated, (ii) filamentous desmids typically harbor large numbers of associated bacteria, and (iii) bacterial epiphytes are found within desmid mucilage, with very few being adherent to the sheath margin (see reference 10). Epiphytes of these algae are therefore more likely to be experiencing the microenvironmental conditions of the sheath rather than the water column and to be involved in some sort of interaction with their algal hosts.

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TABLE 1. Distribution of 20 cloned sequences obtained from the epiphytic bacterial communities on three filamentous desmid taxa[a]

Sequence type	No. of clones found on:			Total no. of clones by sequence type	Possible affiliation (phylum or group)
	Desmidium	*Hyalotheca*	*Spondylosium*		
DgEPI2[b]	4	1	4	9	*Flexibacter*/*Cytophaga*/*Bacteroides*
DgEPI1	3			3	α-*Proteobacteria*
DgEPI5	1			1	γ-*Proteobacteria*
DgEPI6	1			1	*Flexibacter*/*Cytophaga*/*Bacteroides*
HdEPI5		1		1	α-*Proteobacteria*
HdEPI3		1		1	β-*Proteobacteria*
HdEPI1		1		1	Group with high G+C content, gram positive
HdEPI2		2		2	β-*Proteobacteria*
HdEPI4		1		1	α-*Proteobacteria*
Total	9	7	4	20	

[a] A total of nine different sequence types were identified. One type was present on all three desmid genera examined. Four sequence types were found on *Desmidium*, six were found on *Hyalotheca*, and one was found on *Spondylosium*.

[b] DgEPI2 represents two sequence types that differed at only two to three positions.

MATERIALS AND METHODS

Sample collection. Whole-water samples containing filamentous desmids were collected in October 1994 from an unnamed peatland in Oneida County, Wisconsin (45° 48′N, 89° 39′W). "Bird Lake Road Bog" is a small, floating-mat *Sphagnum* peatland enclosing a humic lake measuring 0.68 ha in area. The algal populations sampled were growing benthically in moat (lagg) regions of the bog. Material was transported to the laboratory on ice and promptly refrigerated at 5 to 7°C with fluorescent lighting on a 12 h-12 h or 8 h-16 h light-dark cycle. Extraction of DNA was performed within 2 to 3 days of sample collection.

DNA extraction. Individual filaments of desmid taxa *Desmidium grevillii* (Kütz.) DeBary, *Hyalotheca dissiliens* (Smith) Bréb. ex Ralfs, and *Spondylosium pulchrum* (Bail.) Archer with attached bacteria were micropipetted from field samples, washed by pipetting through several drops of deionized, filter-sterilized water, and placed directly into 100 μl of UNSET lysis buffer (which contains 8 M urea and 2% sodium dodecyl sulfate) (13). Generally two to three filaments, composed of 50 to 200 desmid cells each, were placed into each tube of UNSET buffer. The mixtures were allowed to incubate for 15 to 30 min at 55°C with frequent vortexing, and DNA was extracted with phenol-chloroform (at 55°C), ethanol precipitated, and resuspended in Tris-EDTA buffer or water. After the extraction, desmid filaments were stained with the DNA fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) and viewed with an epifluorescence microscope to determine whether bacteria in the sheath were lysed by our extraction protocol. We observed that most bacterial fluorescence was removed by the lysis procedure, but we did not attempt to quantify the amount. In addition, algal cells were not lysed, which was advantageous because neither algal chloroplast nor mitochondrial 16S genes were amplified in subsequent PCRs.

PCR amplification. Primers corresponding to the universal primers A and C of Lane et al. (25) were used to amplify approximately 900 bp of the small-subunit (16S) rRNA gene between positions 536 and 1390 (*Escherichia coli* numbering). Standard 100-μl reaction mixtures with 20 pM concentrations of each primer were used, with the cycling parameters typically being 94°C for 45 s, 50°C for 1 min, and 72°C for 2 min (35 cycles). DNA extraction and amplification from the final rinse water used to wash desmid filaments were also attempted; we did not obtain amplification in any of these controls (data not shown). This indicated to us that the amplified 16S genes in our samples came from bacteria attached to or within desmid mucilage rather than from free-living bacteria in the bog water. After PCR, products from two or more PCRs were pooled and concentrated and purified by using GeneClean II (Bio 101).

Cloning and sequencing. PCR products were cloned into M13 sequencing vectors by using restriction sites engineered into the amplification primers. Two combinations of cloning enzymes were used: *Bam*HI-*Sal*I and *Pst*I-*Hin*dIII. PCR products were purified by using Geneclean, digested, ligated into M13, and transformed into *E. coli* DHαF′. Positive clones were verified by digestion of double-stranded M13 DNA with the cloning endonucleases. Single-stranded M13 DNA was isolated and purified for sequencing by using polyethylene glycol-NaCl precipitation, followed by a phenol-chloroform extraction and ethanol precipitation. M13mp19 DNA was sequenced by standard Sanger dideoxy chain-termination methods with Sequenase version 2.0 (U.S. Biochemicals) and [^{35}S]dATP. The entire PCR product (approximately 900 bp) was sequenced by using standard M13 sequencing primers and three rRNA primers.

Sequence analysis. For each cloned sequence, a query was made to the Similarity Rank analysis program of the Ribosomal Database Project (RDP) (27) and the BLAST (basic local alignment search tool) network (1) for an initial determination of the nearest phylogenetic neighbor sequences. Our sequences were then manually aligned by using Sequencher version 3.0 with sequences from representatives of the nearest neighbor groups along with sequences from taxa representative of all bacterial phyla (93 taxa total). The number of taxa in the analysis was eventually reduced to 40 to build the final trees. The RDP sequences we used are indicated by their RDP short identifications (ids), and the GenBank sequences are indicated by their accession numbers, in the phylogenetic trees. After the initial analysis, our sequences were sorted based on phylum and subphylum affiliations, and subanalyses were conducted with a larger number of representative sequences from the groups and subgroups into which our sequences had tentatively been placed.

PCR primer regions, and regions that could not be unambiguously aligned by eye (hypervariable regions), were excluded from phylogenetic analyses. Therefore, our analyses were based on 800 to 850 aligned positions, except for the phylum-level analysis, which was based on 751. Alignments are available upon request. Phylogenetic trees were constructed by using both distance and parsimony methods. Estimated evolutionary distances were calculated with the Kimura two-parameter model (23) and a transition-transversion ratio of 2.0, and trees were inferred from distance matrices by using the neighbor-joining method (38) and the Fitch-Margoliash algorithm (11) with PHYLIP version 3.5c (9). Parsimony trees were inferred by using the heuristic search option in PAUP 3.1.1 (40). For parsimony analyses, a weighting scheme was employed to correct for lack of independence in base-paired (double-stranded) regions of the 16S rRNA molecule (45). If among all taxa in a particular analysis there were three or fewer mismatches between base positions potentially involved in pairing (secondary structure was inferred from the model for *E. coli* [15]), the base position was down-weighted by one-half (weight = 1). Base positions showing four or greater mismatches were considered to be independently evolving and were given a weight of 2. Bootstrapping (100 resamplings) was also applied to both distance and parsimony trees (8).

Sequences were sent to the program Check Chimera of the RDP. We also looked for pairing mismatches in double-stranded regions of the 16S gene. Using these two approaches it was not immediately apparent that any of our sequences were chimeric.

Nucleotide sequence accession numbers. Partial sequences of 16S rRNA genes cloned from the epiphytic bacterial communities on filamentous desmids were submitted to GenBank and have the following accession numbers: AF059756, AF059757, AF059758, AF059759, AF059760, AF059761, AF059762, AF059763, AF059764, and AF059765.

RESULTS

We obtained 22 cloned sequences from the bacterial epiphytes on *D. grevillii*, *H. dissiliens*, and *S. pulchrum* (Table 1). Two of the sequences were alignable with each other but were not clearly so with the others, and by using the RDP's similarity rank function these sequences appeared to be fungal 18S rDNA sequences. Among the remaining 20 prokaryotic sequences, 10 different sequence types were identified, but 2 of the sequences differed at only two to three positions and consequently were grouped for analysis (see Table 1). The resulting nine sequence types are labeled Dg or Hd, indicating that they were obtained from *D. grevillii* or *H. dissiliens*, respectively. There was no overlap in the sequence types retrieved from these two algal species except for sequence DgEPI2, which was recovered from all three algal taxa and was the only sequence type obtained from *S. pulchrum*.

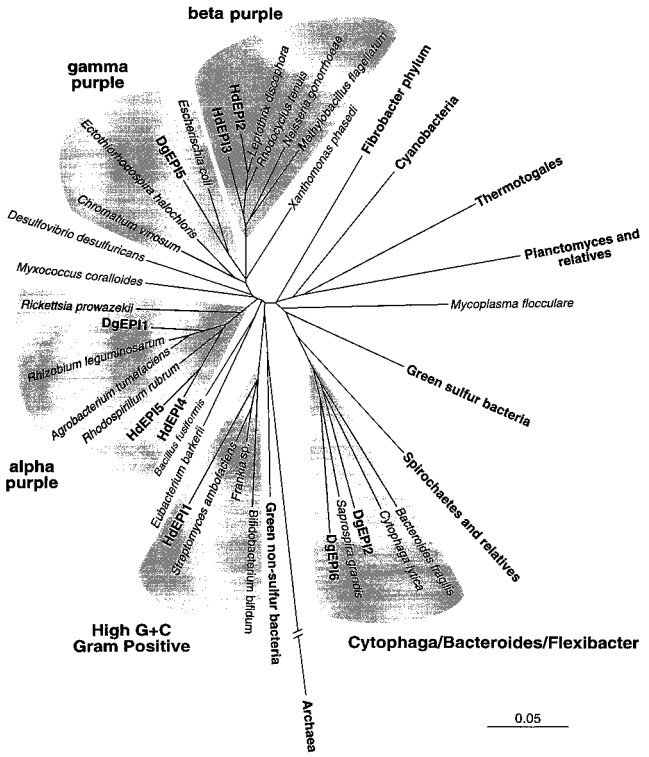

FIG. 1. Unrooted phylogenetic tree showing the relationships of all nine sequence types obtained in this study (listed in Table 1) to the major lines of radiation within the domain *Bacteria*. The tree was inferred from a matrix of pairwise distances by the Fitch-Margoliash algorithm using a total of 751 aligned positions. The scale bar represents 0.05 base changes per nucleotide position.

In addition to our attempt to amplify 16S genes from the final rinse water used to wash desmid filaments (results were negative), we also conducted a preliminary molecular assessment of the free-living bacterial community present in the same region of the moat from which *D. grevillii* and *H. dissiliens* were collected. No sequences retrieved from the free-living community (six sequence types and 23 clones total) were identical to sequences recovered from the algae (data not shown). These results together indicate that the 16S gene sequences we obtained came from epiphytic populations and not from the ambient water.

The phylogenetic placement of our sequence types in relation to the major bacterial phyla is shown in Fig. 1. Both parsimony and distance measures supported the placement of our clones into these phyla. In general, our results obtained by using parsimony and distance measures were highly congruent; that is, clustering of known taxa and the placement of our clones in relation to known taxa were similar by both measures, although branching orders sometimes differed. Our clones fell into five major lineages of the *Bacteria* domain: the α, β, and γ subdivisions of the *Proteobacteria* (purple bacteria and relatives), the *Flexibacter*/*Cytophaga*/*Bacteroides* phylum, and gram-positive organisms with high G+C content. The clone that grouped with the high G+C gram-positive organisms was subsequently determined to be closely affiliated with the *Propionibacterium* group (RDP designation 2.16.1.9) and specifically with *Propionibacterium acnes*, a cutaneous species in humans. The retrieval of this sequence may have been due to windborne contaminating DNA in the laboratory, and therefore it will not be discussed further.

After assigning our clones to their respective major bacterial phyla, we conducted subanalyses with additional representative members from subgroups within each phylum. The results of these analyses are shown in Fig. 2 and 3. None of our clones were identical to any 16S rDNA sequences from cultured organisms or environmental clones available through the RDP or GenBank. In some cases our sequences were deeply nested within defined subgroups of bacteria (e.g., Fig. 3). In other instances, as has been the case with many previous molecular assessments of natural bacterial communities, our sequences could be placed into subgroups but were not closely affiliated with sequences from any known taxa (e.g., Fig. 2c).

Both of the sequence types that fell into the *Flexibacter*/*Cytophaga*/*Bacteroides* phylum grouped in Subdivision II (RDP designation 2.7.2) of that phylum (Fig. 2a). DgEPI2, our most abundant clone, was placed with high bootstrap support (95 and 100% in parsimony and distance analyses, respectively) in the *Sphingobacterium* group (RDP designation 2.7.2.2). Evolutionary distances between DgEPI2 and known organisms *Sphingobacterium heparinum* (RDP short id, Sph.heparn), *S. thalpophilum* (Sph.thalpo), and *Flavobacterium mizutaii* (F.mizutaii) were 0.056, 0.072, and 0.079, respectively. DgEPI6 grouped in the *Saprospira* group (2.7.2.3) of Subdivision II, again with high bootstrap support [100% for its placement with *Cytophaga arvensicola* (Cy.arvensi), *Flexibacter filiformis* (Flx.filfor), *F. sancti* (Flx.sancti), and *Flavobacterium ferrugineum* (F.ferrugin)] (Fig. 2a). Distances between DgEPI6 and these taxa were 0.072 (*F. ferrugineum*), 0.086 (*C. arvensicola*), 0.090 (*F. sancti*), and 0.091 (*F. filiformis*). Interestingly, the *Saprospira* group also contains two environmental clones (env.agg41 and env.agg32) which were retrieved from particle-associated bacterial communities in the marine environment (7).

Of the three sequence types that grouped in the α subdivision of the *Proteobacteria*, two, HdEPI4 and HdEPI5, were more closely related to each other ($d = 0.062$, bootstrap 99 to 100%) than to any other taxa in the analysis (Fig. 2b). This result is common in phylogenetic studies of naturally occurring bacterial communities, and the degree of relatedness between these two sequences may represent variation between 16S genes in different rRNA operons within the same cell, clonal variation within a population, or speciation (30). These sequences were placed into the *Rhodospirillum rubrum* assemblage (2.14.1.1) of the α subdivision, but their position within this group was rather uncertain. They may be affiliated with members of the *Rhodospirillum fulvum* group (2.14.1.1.2), and specifically the genus *Azospirillum*, as both distance and parsimony methods gave the tree topology shown in Fig. 2b. However, membership within the *R. fulvum* group was not supported by bootstrapping. The evolutionary distances between HdEPI5 and *Azospirillum lipoferum* PA1 (Azs.lipof6) and VIP SpRG 20a (Azs.lipof4) were 0.091 and 0.093, respectively. HdEPI4 was similarly closest to *A. lipoferum* VIP sp59b (Azs.lipof) ($d = 0.079$) and *A. lipoferum* VIP SpRG 20a (Azs.lipof4) ($d = 0.084$).

The third sequence type in the α subdivision, DgEPI1, fell within the *Rhizobium-Agrobacterium* group (2.14.1.9), but again its position within this group was poorly resolved (Fig. 2c). Both distance and parsimony analyses placed this sequence with the genus *Methylobacterium* (Methylobacteria group, 2.14.1.9.2). However, bootstrap support was low (56% in the distance analysis), and based on pairwise distance values, its nearest neighbor sequences were *Beijerinckia indica* (Bei.indica, $d = 0.064$) and *Rhodopseudomonas acidophila* (Rps.acidop., $d = 0.062$), both of the *Beijerinckia* subgroup (2.14.1.9.4), and a *Methylosinus* species (Msi.spLAC, $d = 0.063$).

Two sequence types grouped in the β subdivision of the purple bacteria (Fig. 2d). One, HdEPI2, fell within the *Rubrivivax gelatinosus* group (2.14.2.2) and within this group, the

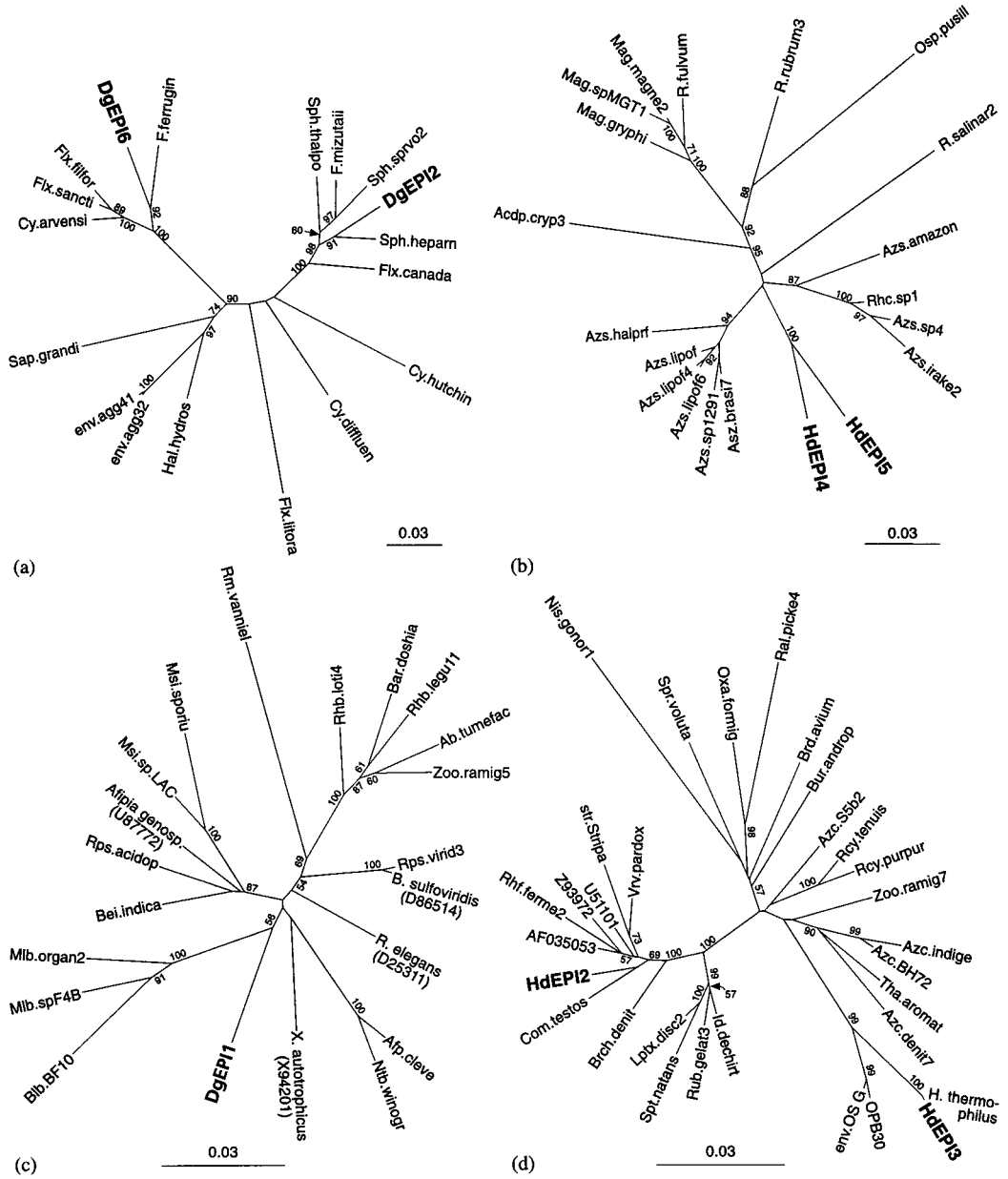

FIG. 2. Unrooted phylogenetic tree showing the relationships among our sequence types and representative members of Subdivision II of the *Flexibacter/Cytophaga/Bacteroides* phylum (2.7.2) (a), the *R. rubrum* assemblage (2.14.1.1) (b), and the *Rhizobium-Agrobacterium* group (2.14.1.9) (c) in the α subdivision of the *Proteobacteria* and the β subdivision of the *Proteobacteria* (2.14.2) (d). Trees were inferred from a matrix of pairwise distances by the Fitch-Margoliash algorithm using a total of 800, 826, and 818 aligned positions for panels a, b and c, and d, respectively. The numbers at the nodes of the trees indicate bootstrap values for each node of 100 bootstrap resamplings (values below 50 are not shown). Scale bars represent the number of base changes per nucleotide position. Sequences from the RDP are indicated by their RDP short ids; those from GenBank are indicated by their accession numbers.

Rubrivivax subgroup (2.14.2.2.6). Both distance and parsimony analyses placed it with the known taxa *Brachyomonas denitrificans* (Brch.denit), *Comamonas testosteronii* (Com.testos), *Rhodoferax fermentans* (Rhf.ferme2), *Variovorax paradoxus* (Vrv. pardox), along with several environmental clones (bootstrap support 99 to 100%). In our analysis, HdEPI2 showed the closest relationships to the described taxon *C. testosteronii* (d = 0.027), an environmental clone obtained from a drinking water biofilm (GenBank accession no. AF035053, d = 0.027), and an unidentified denitrifying, Fe[II]-oxidizing bacterium (U51101D, d = 0.027). Hiorns et al. (17) recovered a number of sequences from freshwater, planktonic bacterial communities that also cluster near the *Rubrivivax* subgroup (termed the ACK 2 cluster), but none of their clones were closely related to ours.

The placement of the other clone in the β subdivision, HdEPI3, was quite surprising (Fig. 2d). It was affiliated with members of the *Rhodocyclus* group (2.14.2.4) and was very closely related (d = 0.0038, bootstrap support 100% with both distance and parsimony) to the cultured organism *Hydrogenoluteola thermophilus* (AB009828), a facultative chemolithoautotroph that uses H_2 as an electron donor and CO_2 as a carbon source (2). In addition, HdEPI3 and *H. thermophilus* clustered with high bootstrap support (99%) with two environmental clones obtained from hot springs communities, env.OS G re-

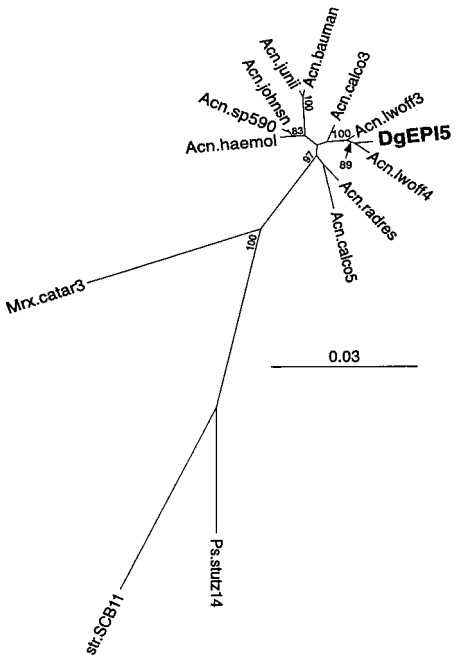

FIG. 3. Unrooted phylogenetic tree showing the relationships of our sequence type and representative members of the *Acinetobacter* subgroup (2.14.3.10.1) in the γ subdivision of the *Proteobacteria*. The tree was inferred as described in the legend for Fig. 2 using 849 aligned positions. The numbers at the nodes of the tree indicate bootstrap values for each node of 100 bootstrap resamplings (values below 50 are not shown). The scale bar represents the number of base changes per nucleotide position. Sequences are indicated by their RDP short ids.

trieved by Ward et al. (41) and OPB30 (AF026979) obtained by Hugenholtz et al. (18).

Finally, DgEPI5 of the γ subdivision of the *Proteobacteria* was found to be deeply nested within the genus *Acinetobacter* (within the *Acinetobacter* subgroup, RDP designation 2.14.3.10.1, of *Pseudomonas* and relatives) and bootstrapping supported this placement in both distance and parsimony analyses (97%) (Fig. 3). The estimated distance between DgEPI5 and *Acinetobacter lwoffii* (Acn.lwoffii4) was 0.0049.

DISCUSSION

In this investigation, we found that cloned sequences obtained from the epiphytic bacterial communities on three charophycean green algal genera fell into a number of major lineages within the *Bacteria* domain: the α, β, and γ subdivisions of the *Proteobacteria* and the *Flexibacter*/*Cytophaga*/*Bacteroides* phylum. As in other phylogenetic studies of naturally occurring bacterial communities, none of our sequences were identical to any sequences available through the RDP or GenBank, although the estimated evolutionary distances between our clones and their nearest neighbors were always less than 0.10 (i.e., roughly greater than 90% similar). Although we were able to place our clones into subgroups within phyla with a high degree of confidence, they were in many instances not closely related to known taxa. This result indicates that most of our sequences represent as yet phenotypically undescribed bacterial species. To our knowledge, the current versions of the RDP and GenBank databases contain few 16S rDNA sequences from bacteria isolated from communities on algae, so it is difficult to assess whether our results further indicate that most natural bacterial associates of algae are not readily cultured. However, it was recently demonstrated by Suzuki et al. (39) in the marine environment that 16S rDNA sequences from isolates cultured on typical nutrient rich media and from those obtained directly from the environment were different. If this is also true for bacterial associates of microalgae, our results imply that the full range of bacterial-algal interactions, and those that may be the most ecologically relevant, are yet to be determined, because most of what we know of bacterial-algal interactions comes from culture studies.

Suggestions as to the possible phenotypes of our clones based on their phylogenetic relationships to known organisms should be made with caution. Nonetheless, the phenotypic properties of some of the closest-described relatives to our clones matched with the environment (algal mucilage) from which they were obtained. For example, two of our sequences, DgEPI2 (obtained from all three algal genera) and DgEPI6, fell within the *Cytophaga*/*Flavobacteria* group, and DgEPI2 was also the most abundant clone recovered. Most organisms in the flavobacterial lineage share the common characteristics of surface-dependent gliding motility, attachment to particles, and ability to degrade complex macromolecules such as cellulose, nucleic acids, proteins, and chitin (46). The occurrence of *Flavobacterium* spp. in association with algae was previously documented (43), and one study suggested a mutualistic relationship between a *Flavobacterium* sp. and the diatom *Navicula muralis* (19).

Another of our clones, DgEPI5, was very closely related to *Acinetobacter*, which also possesses an aerobic, chemoorganotrophic mode of nutrition and is commonly found in both water and soil. Given the past research emphasis on bacterial use of algal dissolved organic carbon (12), we fully expected that most sequences we obtained would be related to known organisms having strictly heterotrophic modes of nutrition. However, several of our clones fell within the α or β subdivisions of the *Proteobacteria*, and those within the β subdivision were affiliated with either the *Rubrivivax* or *Rhodocyclus* groups. These lineages contain most of the nonsulfur purple photosynthetic bacteria, and most known members of the α subdivision also fix atmospheric nitrogen (e.g., *A. lipoferum*) (47). In addition, one of our clones was very closely related to *Hydrogenoluteola thermophilus*, a facultatively H_2-oxidizing bacterium which is usually found in high-temperature environments but has also been isolated from cold, nongeothermal habitats (2). Although we cannot state conclusively that bacteria capable of other than strictly heterotrophic growth are present in the bacterial communities on green algae, we believe this possibility warrants further investigation. Reduced microzones around living algal cells and filaments have been detected and may provide suitable conditions for bacterial processes such as nitrogen fixation (34).

Rather than demonstrate any particular interaction between filamentous desmids and their bacterial floras, our objective in this study was to more fully describe the bacterial communities present on green algae than had been done previously in culture studies. Molecular methods could be further used to investigate the distribution of bacterial epiphytes on other algal taxa, to compare the communities attached to detrital particles with those present on living algal cells, and to examine succession in epiphytic bacterial communities. Such comparisons would provide additional information as to the specificity of the association between algae and their epiphytes and aid researchers in focusing future studies into specific interactions in the natural environment.

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