Detection of Prochlorothrix in Brackish Waters by Specific Amplification of pcb Genes

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Received 21 April 2003/Accepted 21 July 2003

Prochlorothrix hollandica is the only filamentous chlorophyll b (Chlb)-containing oxyphotobacterium that has been found in freshwater habitats to date. Chlb serves as a light-harvesting pigment which is bound to special binding proteins (Pcb). Even though Prochlorothrix was initially characterized as a highly salt-sensitive species, we detected it in a brackish water environment that is characterized by salinities of up to 12 practical salinity units. Using PCR and reverse transcription, we amplified pcb genes fragments of phytoplankton samples taken along a salinity gradient in the eutrophic Darss-Zingst estuary (southern Baltic Sea). After sequencing, high levels of homology to the pcbB and pcbC genes of P. hollandica were found. Furthermore, autofluorescence of Prochlorothrix-like filaments that indicated that Chlb was present was detected in enrichment cultures prepared from the estuarine phytoplankton. The detection of Chlb-containing filaments, as well as the pcb and 16S rRNA sequences, suggests that Prochlorothrix is an indigenous genus in the Darss-Zingst estuary and may also inhabit many other brackish water environments. The potential of using pcb gene detection to differentiate Prochlorothrix from morphologically indistinguishable species belonging to the genera Pseudanabaena and Planktothrix (Oscillatoria) in phytoplankton analyses is discussed.

The Prochlorophyta is an unusual group among the oxyphotobacteria. Like the chloroplasts in higher plants, the members of this group contain chlorophyll b (Chlb) as an accessory pigment. For many years, the symbiont Prochloron didemni was the only known representative of the Prochlorophyta. The first filamentous strain (5) in the group of Chlb-synthesizing prokaryotes, the free-living species Prochlorothrix hollandica, was isolated in 1984 from a lake in The Netherlands (4). Prochlorothrix sp. strain NIVA-8/90, tentatively named Prochlorothrix Scandinava, has been proposed as a second species (28). In contrast to the salt-sensitive filamentous oxyphotobacteria, coccolid Chlb-containing species of the genus Prochlorococcus are very abundant in the central oceans (7, 26). However, phylogenetic analyses of 16S rRNA sequences, have clearly indicated that the oxyphotobacteria do not form a separate bacterial lineage, but are specially pigmented members of the old cyanobacterial evolutionary radiation (39).

Many investigations have concentrated on the evolutionary importance of the Chlb-containing oxyphotobacteria and the particularity of their photosynthetic machinery (3, 12, 19, 23, 37, 38). However, since most attention has been paid to the biology of the globally important genus Prochlorococcus in marine ecosystems (8, 11, 21, 22, 26, 33), our understanding of Prochlorothrix ecology is limited. The isolation of Prochlorothrix strains from the Loosdrecht lakes (6) and Lake Malaren (28) and the results of laboratory studies (5) imply that Prochlorothrix spp. are freshwater organisms with a preference for shallow eutrophic water. The highest levels of Prochlorothrix were found during the summer in shallow, phosphate-limited regions of the Loosdrecht lakes (5). The oxygenic photosynthetic activity was found to be highly resistant to inhibition by sulfide (30). Experiments focusing on phosphorus nutrition resulted in characterization of P. hollandica as a high-affinity storage strategist (9).

While Prochlorococcus cells dominate phytoplankton communities in wide areas of the oceans (27), Prochlorothrix occurs at most times of the year at rather low levels, while cyanobacteria that are very similar morphologically seem to dominate. It is particularly difficult to distinguish Prochlorothrix from members of the cyanobacterial genera Pseudanabaena and Planktothrix (Oscillatoria). In the past, epifluorescence microscopy was used to investigate phytoplankton from the Loosdrecht lakes (43), the original source of P. hollandica (5). This technique is based on the phycobilisome autofluorescence that is characteristic of filamentous cyanobacteria but is missing in Prochlorothrix because of its different pigmentation. Detecting Prochlorothrix based on the absence of fluorescent trichomes is not a very reliable method (5, 43). Modern molecular techniques may be much more suitable for this purpose. The currently used methods, such as 16S rRNA denaturing gradient gel electrophoresis and sequence analysis, are restricted to analysis of species that occur at rather high levels in the environment (42). Methods based on genes which are restricted to specific bacterial groups could provide greater sensitivity. Genes encoding Chla/b binding proteins (pcb) may be good candidates for specific detection of Chlb-containing oxyphotobacteria. These genes exhibit only low levels of similarity to members of the extended gene family encoding eukaryotic Chla/b and Chla/g light-harvesting proteins (18). The antenna polypeptides are encoded by three genes in P. hollandica.

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was estimated by using 11.25% carbon per fresh weight (15) at a specific cell number, the total number of cells per species was calculated. Cell diameters (the organism proposed as P. scandica) were obtained as a frozen pellet. Samples for RNA extraction were quickly frozen on board. An aqueous solution of KCl was made by adding 68.4 mM (4 PSU) to 15°C seawater. It is much more widespread than the previous occasional occurrences of P. hollandica (15) and increases in salinity (Fig. 1). Samples for RNA extraction were isolated by using a High Pure RNA isolation kit (Roche Biochemicals). All PCR were performed using Taq PCR Master Mix (Qiagen), and reverse transcription (RT) reactions were carried out with SuperScriptII RNase H reverse transcriptase (Gibco BRL Life Technologies). Cyanobacterial 16S rDNA fragments (424 bp) were amplified with degenerate standard primers CYA359F and CYA781R (25). The 16S rDNA primers that specifically targeted P. hollandica (16S-Pholl-fw [5'-ACA CAG CTT AAC TGT GGG AGA-3'] and 16S-Pholl-rev [5'-AGT TGG CTC TTC GTC GTC-3']) were based on the alignment obtained with the software BIOEDIT (14). Besides the exact match with P. hollandica, the primer sequences are identical to sequences of the unidentified cyanobacterial clones LD16 and LD22 (accession no. AJ007866 and AJ006285) from theLoosdrecht lakes. These clones exhibit more than 99% identity to P. hollandica (44). All primers were used at an annealing temperature of 55°C. The pchB gene was obtained by first using primers optiis-fw (5'-AAT TGY TGG TAG GC-3') and hlwha-l-rev (5'-GCG TGC CAS AGR TGA CC-3') and then using reverse primer pyfadt-rev (5'-CGT TTC GGC AAA RTG GGC-3') in a second seminested PCR. Specific amplification of the pchB gene was obtained with primers pchB-fw (5'-GTA ATG TGC TGC TAGGC-3') and pchB-rev (5'-CTA ACC GTC AGA CCT TAA CC-3'). The PCR program and all other procedures have been described previously (13). Sequencing of cloned fragments was done at least in triplicate by using a capillary sequencer (Beckman-Coulter).

Computer analysis. We searched for sequence similarities in databases with the assistance of the BLAST software (1). Sequence alignment was performed with the software BIOEDIT (14). A consensus tree was constructed by using the multiple-sequence alignment and the software program PAUP (Phylogenetic Analysis Using Parsimony, version 4.0; David Swoford, Laboratory of Molecular Systematics, Smithsonian Institution). Rooted cladograms were constructed after phylogenetic analyses of the 16S rDNA sequences (maximum-parsimony method) and of the pchB and pchC sequences (neighbor-joining method) with 1,000 replications. The cladograms were constructed with TreeView (version 1.5; R. D. M. Page, 1998).

Cultivation and high-performance liquid chromatography (HPLC) analysis. Estuarine phytoplankton were enriched by cultivating 1 ml of the natural phytoplankton assemblage in 3 ml of BG11 medium (32) at 23°C. The enrichment cultures were exposed to daylight. P. hollandica SAG 10.89 cells were grown at 23°C in plates containing 15 ml of BG11 medium (32) supplemented with different concentrations of NaCl with constant illumination of 1 μmol of photons m−2 s−1 (photosynthetically active radiation; fluorescent tubes; color code 25; Philips). The NaCl concentrations used were 34.2 mM (2 PSU), 68.4 mM (4 PSU), and 102.6 mM (6 PSU). Cells were harvested after 10 days by filtration onto membrane filters (pore size, 2 μm) for determination of the net dry weight and compatible solutes. For analysis of compatible solutes by HPLC, the cells on filters were extracted in 2 ml of 80% ethanol for 3 h at 65°C. After an internal standard (50 μg of sorbitol) was added, the particulate material was removed by centrifugation, and the supernatant was filtered through a 0.2-μm filter. The filtrate was then analyzed by GC. The concentrations of low-molecular-weight carbohydrates were analyzed with an HPLC setup as described by Schoor et al. (35). All chromatographic experiments were performed with a chromatograph consisting of LC-9A pumps, SIL-9A autoinjector (1- to 50-μl sample loop), a CTO-6A column oven, and a 4×150-μm i.d., 5-μm, reverse-phase index detector (Shimadzu Corp., Kyoto, Japan). A reverse-phase column filled with Hypersil 120 ODS and a sugar-alcohol column (Aminex HPX-87C, Bio-Rad) were the columns used.
The nucleotide sequences of the 16S rDNA of *P. hollandica* (accession no. AJ007907), *Leptolyngbya* sp. strain PCC 7104 (AB039012), *Thermophycolosuccus elongatus* BP-1 (AP005376), *Prochlorococcus marinus* SSW5 (X63140), *Prochlororosa* sp. (X63141), uncultured cyanobacterial clones LD7 (AJ007864) and LD16 (AJ007866), *Escherichia coli* PK3 (X80731), *Glaciebacter violaceus* PCC 8105 (AF132791), *Prochlorococcus marinus* subsp. *pastoris* NATL2 (AF311219), *Prochlororosa* marinus MIT 9503 (AF001469), *Planktothrix agardhii* NIVA CYAS (AB045939), *Synechococcus* sp. strain PCC 6802 (AB041938), *Synechococcus* sp. strain PCC 7002 (AJ000716), *Prochlororosa* marinus (X63140), *Nostoc* sp. strain PCC 7120 (AP003595), *Synechococcus* sp. strain WH7805 (AF001478), and *Trichodesmium erythreum* IMS 101 (NZ_AA01000054) and the nucleotide sequences of the *pcbC* genes of *P. hollandica* (X97043) and *Prochlororosa* marinus CCMP 1375 (AF198526 and AF198528) were obtained from databases. Partial 16S rDNA and *pcbC* gene sequences of *Prochlororosa* sp. strain NIVA-A890 (accession no. AJ534944 and AJ534947) and of the uncultured estuarine *Prochlororosa* (AJ534945 and AJ534946) were obtained in this study and were deposited in the databases.

**RESULTS**

Detection of *pcb*-like and 16S rDNA sequences in samples from the Darss-Zingst estuary. Gene fragments that exhibited high levels of sequence similarity (99.9%) to *pcbB* from *P. hollandica* were amplified by RT-PCR from sampling sites 1 to 9 (Fig. 2D). Until now, this gene has been described only for *P. hollandica* and *Prochlorococcus marinus*. In order to verify that *Prochlororix*-like organisms are present in the Darss-Zingst estuary, specific primers for amplification of the *pcbC* gene of *P. hollandica* were used in RT-PCR, as well as in PCR. Use of these *pcbC*-specific primers resulted in amplification of the expected 1-kb fragment with DNA from sampling sites 2 to 7 (Fig. 2C). Fragments of the same size were also obtained with cDNA, which were synthesized from total RNA from sampling sites 1, 3, 6, 7, and 9 (Fig. 2E). The similarities of the DNA fragments obtained to *pcbC* of *P. hollandica* were verified by Southern hybridization experiments, in which the fragments were recognized by a specific *pcbC* probe obtained from *P. hollandica* (data not shown). Restriction analyses with NcoI resulted in a fragment size pattern which was identical to that expected based on the *P. hollandica* *pcbC* sequence (data not shown). Several PCR fragments from all sampling sites were cloned and sequenced. All of the sequences were 99% identical to *pcbC* from *P. hollandica* regardless of the sampling site; i.e., just one genotype was found.

Finally, we searched for *P. hollandica*-like 16S rDNA sequences in environmental samples (Fig. 2A). This was done initially with cyanobacterium-specific primers (25), which amplified a 425-bp internal 16S rDNA fragment. Restriction analyses and sequencing of at least 10 randomly obtained 16S rDNA clones never resulted in a sequence similar to that of *P. hollandica*. In all cases, the sequences were similar to those of phycobilisome-containing cyanobacteria (data not shown). *Prochlororix*-type organisms could not be detected by this approach. However, the use of *P. hollandica*-specific 16S rDNA primers allowed amplification of the expected 650-bp fragments. The sequences of these fragments exhibited about 99% identity to sequences of *P. hollandica*. In addition, significant amounts of 16S rDNA fragments characteristic of *P. hollandica* were detected in *pcbC*-positive samples (Fig. 2B). Significant amounts of *P. hollandica*-like fragments were not detected in DNA from sampling sites 9 and 10. The partial sequences of the *pcbC*, *pcbB*, and 16S rDNA
genes were used for phylogenetic comparisons with similar sequences from the databases (Fig. 3A). Corresponding sequences were also obtained from the proposed new Prochlorothrix species, *P. scandica* (strain NIVA-9/80) (29). The *pcb* gene sequences from the estuarine phytoplankton samples clustered closely with those of *P. hollandica*, while the sequences of *Prochlorococcus marinus* were clearly not closely related (Fig. 3B). The *pcbC* sequence of *P. scandica* NIVA-8/90 was similar to the sequence of *P. hollandica*. In spite of minor differences, the resulting amino acid sequences were 99% identical (Table 1). Thus, the *pcbC* sequences of the Darss-Zingst estuary clones, *P. scandica*, and *P. hollandica* are almost identical. Analyzing the partial 16S rDNA sequences again led to close grouping of the 16S rDNA sequences in the genus *Prochlorothrix* (Fig. 3A). According to this alignment, the 16S rDNA sequence of the environmental samples exhibited a slightly higher level of similarity to the sequence of *P. scandica* NIVA-8/90 than to the sequence of *P. hollandica*.

**Detection of Prochlorothrix-like trichomes in estuarine phytoplankton.** Phytoplankton counts from the 10 sampling sites (Fig. 4) revealed that about one-half of the members of the Oscillatoriales could be morphologically attributed to the microscopically indistinguishable taxa *P. hollandica* and *Pseudanabaena*. This morphotype represented an important fraction of the phytoplankton biomass (mean, 12%) at stations 1 to 7, but for stations 8 to 10 the percentage was less than 1% (mean, 0.4%), which basically corresponds to the results of the PCR analyses.

**FIG. 3.** Rooted cladograms obtained after phylogenetic analyses by the maximum-parsimony method (performed with PAUP, beta version 4.0; Laboratory of Molecular Systematics, Smithsonian Institution) of a 527-bp fragment of the 16S rDNA sequences (maximum-parsimony method) (A) and of a 828-bp fragment of the *pcbB* and *pcbC* sequences (neighbor-joining method) (B). Organisms whose sequences were obtained from databases are indicated by asterisks. Selected bootstrap values based on 1,000 replications are shown at the nodes; only values greater than 50% are shown. The cladograms were constructed with TreeView (version 1.5; R. D. M. Page).
The molecular data strongly suggest that *P. hollandica*-like organisms are present in the Dars-Zingst estuary. This encouraged us to search for *P. hollandica*-like trichomes. Since the Dars-Zingst estuary is a highly eutrophic aquatic system, filamentous cyanobacteria are abundant, and many filamentous cyanobacteria of the *Planktothrix-Pseudoanabaena* type are present (Fig. 5A). This fact made it impossible to detect *P. hollandica* trichomes in water samples reliably. Culture experiments to enrich mixtures of filamentous cyanobacteria were started. The cultures were examined after 2 weeks by light microscopy and differential epifluorescence microscopy (Fig. 5B and C). Some trichomes produced clear autofluorescence signals after selective Chlb-exciting illumination, in contrast to other trichomes that had the same morphology and to colony-forming chroococcoid cyanobacteria. Some green algae proved that the Chlb excitation was specific. This finding supported the assumption that parts of the filamentous cyanoplankton contain Chlb, like *Prochlorothrix* filaments. Furthermore, PCR fragments from DNA extracted from the enrichment culture contained the same *Prochlorothrix*-like sequences as the environmental samples contained.

Our data suggest that *P. hollandica*-like organisms are able to live throughout the estuary under low-salinity conditions, although such organisms have been isolated previously only from freshwater. *P. hollandica* SAG 10.89 was cultivated in NaCl-supplemented BG11 medium to mimic estuarine conditions. Growth was observed in media containing NaCl at salinities up to 6 PSU. In salt-treated cells, accumulation of sucrose was detected, while this sugar did not accumulate in *P. hollandica* cells under the low-salt conditions of standard BG11 medium (data not shown). These data indicate that sucrose might be used for osmoregulation, which can support acclimation to the salinities present in the Dars-Zingst estuary.

**DISCUSSION**

We corroborated initial indications that *Prochlorothrix*-like organisms are present in the natural cyanoplankton community of coastal waters in the southern Baltic Sea. DNA fragments exhibiting high levels of sequence identity to *pcbB, pcbC*, or 16S rDNA of *P. hollandica* were amplified from DNA isolated from different sampling sites along a gradient of salinities ranging from 0.2 to 8.2 PSU. In particular, the *pcbC* sequence can be used as a specific marker for *P. hollandica*-like strains, since this gene is different from other *pcb* genes in oxyphotobacteria and from similar genes in eukaryotic algae (41). Furthermore, *P. hollandica*-like trichomes were enriched from the estuary and produced autofluorescence signatures that indicated that Chlb was present. Assuming that mRNA is a useful marker of cell viability because of its short half-life compared to that of DNA, *Prochlorothrix*-like cells are able to propagate at least in the enclosed parts of the Dars-Zingst estuary. Horizontal drifting of buoyant phytoplankton is caused by net horizontal water currents with velocities that are usually in the range from 0.1 to 0.5 m s⁻¹ (34) (broad and narrow parts). Therefore, 30 to 150 h is the minimum drifting time from sampling site 1 to site 10 in the estuary. These time estimates were obtained by excluding mixing in the larger basins and periods of salt water inflow with inverted drift direction. Addition of *Prochlorothrix* originating from freshwater input is unlikely, since *pcb* mRNA half-lives were shown to be in the range from minutes (in light) to hours (in darkness) (24).

The occurrence of viable *Prochlorothrix* at salinities up to 8 PSU was initially surprising, because Burger-Wiersma et al. (5) characterized *P. hollandica* as a freshwater organism with a very low salt tolerance. Growth of *P. hollandica* ceased completely in the presence of 100 mM NaCl (17% of the seawater concentration or 6 PSU) and was inhibited in the presence of >25 mM NaCl (ca. 1.5 PSU). In contrast, our growth experiments with *P. hollandica* SAG 10.89 did not show growth inhibition at such salinities, and optimum growth was detected in the presence of about 85 mM NaCl (14% of the seawater concentration or 5 PSU) under the conditions used in this study. We assume that our cultivation method, which decreased shearing forces and other parameters that are not known, led to these differences in salt tolerance. Mechanical destruction of cell surfaces in particular might be critical for regulation of osmotic pressure by accumulation of compatible solutes and ion export. Cells of *P. hollandica* SAG 10.89 accumulated sucrose upon exposure to increased NaCl concentrations. Sucrose accumulation was found to be characteristic of the cyanobacterial strains with the lowest halotolerance (31). The optimal growth conditions for *P. hollandica* in cultures at

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**TABLE 1. Levels of similarity of the DNA sequences from**

*Prochlorothrix* **field samples to sequences obtained from databases and from analyses in this study**

<table>
<thead>
<tr>
<th>Organism</th>
<th>% Similarity</th>
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<tbody>
<tr>
<td></td>
<td>16S rDNA</td>
</tr>
<tr>
<td><em>Prochlorothrix</em> sp. strain NIVA-8/90</td>
<td>99.6</td>
</tr>
<tr>
<td><em>Prochlorothrix</em> hollandica&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.9</td>
</tr>
<tr>
<td><em>Prochlorothrix</em> sp.&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.5</td>
</tr>
<tr>
<td><em>Prochlororococcus marinus</em> SSW5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.3</td>
</tr>
<tr>
<td><em>Prochlororococcus marinus</em> CCMP 1375&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Cyanobacterium clone LD7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.9</td>
</tr>
<tr>
<td>Cyanobacterium clone LD16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.7</td>
</tr>
<tr>
<td><em>Planktothrix agardhii</em> NIVA CYA53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> 16S rDNA sequences (527 bp) and sequences of chlorophyll binding protein genes (*pcbb* and *pcbC*; 826 bp) were compared.

<sup>b</sup> Sequence obtained from database.

<sup>c</sup> ND, not determined.

**FIG. 4. Relative biomasses of *Planktothrix*-like filaments in different fractions of the phytoplankton in samples from the Dars-Zingst estuary. The percentages of biomass were calculated for Oscillatoriales (open bars), total cyanobacteria (grey bars), and total phytoplankton (solid bars).**
On the 16S rDNA level the Prochlorothrix tuary in fact indicate that chlorothrix-like gene fragments found in the Darss-Zingst estuary. Even though pcbB and pcbC DNA fragments were found in the western to central part of the Darss-Zingst estuary at all sampling sites, undetectable amounts of these fragments in the northeastern transition zone to the open Baltic Sea (station 10) support the suggestion that the organisms are restricted to salinities below 10 PSU.

The high levels of similarity of the 16S rDNA and functional pcb sequences (more than 99%) put the Prochlorothrix field sample sequences into the Prochlorothrix cluster with the type strain of P. hollandica. Only a few additional isolates, like Prochlorothrix strain NIVA-8/90, provisionally named P. scandica (28), and some uncultured clones from Lake Loosdrecht, exhibited high levels of similarity to P. hollandica (44). Based on the pcbB and pcbC sequence comparisons, the levels of similarity are even higher. Therefore, we believe that the Prochlorothrix-like gene fragments found in the Darss-Zingst estuary in fact indicate that Prochlorothrix species are present. On the 16S rDNA level the Prochlorothrix representative from the Darss-Zingst estuary showed a slightly higher level similarity to P. scandica NIVA-8/90 from Lake Malaren. The pcbB-pcbC sequence comparison implied that there is a closer relationship to P. hollandica. Nevertheless, it should be considered how significant differences are when levels of 16S rDNA sequence identity in the range from 98.8 to 99.6% are used to distinguish different species. At this time we are reluctant to assign the sequences to different species, and generally we refer to P. hollandica. In the case of Prochlorococcus species, all comparisons of 16S rDNA sequence homologies showed high degrees of identity, but there are stable ecotypes with completely different genome sizes (16). Therefore, further investigations of the genus Prochlorothrix could reveal more detailed information.

Monthly phytoplankton monitoring in the estuary failed to detect Prochlorothrix because of its inconspicuous and somewhat unclear morphological features. It is necessary to distinguish this taxon from the frequently occurring, morphologically similar cyanobacteria (i.e., Pseudoanabaena limnetica [Oscillatoriaria limnetica]). These organisms can be distinguished by some ultrastructural features (29) or by different pigment-dependent autofluorescence signatures (42). However, electron microscopy is too costly to be used as a tool to search for phytoplankton species. In addition, acclimation to nutrient depletion and irradiance could lead to a significantly changed pigment content of Pseudoanabaena or Planktothrix (Oscillatoriaria) species (26). Phycobilisome fluorescence is not a reliable tool for distinguishing filamentous cyanobacterium-like organisms in the phytoplankton (5, 43). We used differential Chl/a/b excitation of long-wavelength autofluorescence to differentiate Prochlorothrix from Chlb-free cyanobacteria. This positive indication allowed us to prove that Prochlorothrix was present. However, a heavily reduced Chlb antenna per cell also could lead to incorrect identification. Therefore, prechecking phytoplankton samples for the presence of Prochlorothrix-like DNA sequences with PCR can be a useful technique for reducing the risk of misinterpreting microscopic investigations of natural phytoplankton samples. Even in samples in which Prochlorothrix-like trichomes accounted for less than 1% of the total phytoplankton biomass (Fig. 4), Prochlorothrix could be detected by PCR. In addition, potential errors of microscopic investigation can be excluded completely when a set of Prochlorothrix-like filaments can be verified to be Prochlorothrix by using a PCR approach at the level of single trichomes. The latter method has already been successfully applied to cyanobacterial filaments (2, 17). Possibly, Prochlorothrix is much more widely distributed than currently expected. In fact, Prochlorothrix-like filaments were suspected to be components of cyanobacterial blooms in the Baltic Sea (Gulf of Finland, Baltic Proper) during monitoring cruises (http://meri.fimr.fi/Algaline/eng/En/Algaline.nsf). Methods for the unambiguous identification of these organisms are necessary. Further investigations should lead to estimates of the ecological importance of Prochlorothrix in brackish phytoplankton.

ACKNOWLEDGMENTS

We thank H. Schubert (Department of Aquatic Ecology, University of Rostock) for the setup used for differential epifluorescence microscopy. B. Brzezinka is acknowledged for her technical support. We are grateful to the crew of the Zingst Biological Station (University of Rostock) for their kind support of the field work. Cells of P. scandica were kindly provided by H. C. P. Matthijs (Department of Microbiology, University of Amsterdam). Cells of P. hollandica were kindly provided by M. Lorenz (Sammlung von Algenkulturen, University of Göttingen).

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG).

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