Prochlorothrix hollandica gen. nov., sp. nov., a Filamentous Oxygenic Photoautotrophic Procaryote Containing Chlorophylls *a* and *b*: Assignment to *Prochlorotrichaceae* fam. nov. and Order *Prochlorales* Florenzano, Balloni, and Materassi 1986, with Emendation of the Ordinal Description

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The genus *Prochlorothrix* gen. nov., with one species, *Prochlorothrix hollandica* sp. nov., is proposed for filamentous oxygenic photoautotrophic procaryotes containing chlorophylls *a* and *b*. The cylindrical cells are arranged in trichomes which lack sheaths, are not motile, and do not possess differentiated cells. *P. hollandica* grows in liquid media or on solid mineral media and is incapable of dinitrogen fixation. Optimum growth occurs between 20 and 30°C and at pH 8.4. The deoxyribonucleic acid base composition of this organism is 53 mol% guanine plus cytosine. Emendation of the description of the order *Prochlorales* Florenzano, Balloni, and Materassi 1986 is proposed since the original definition of this taxon would exclude the genus *Prochlorothrix*. To accommodate the filamentous genera in the order *Prochlorales*, we propose the family *Prochlorotrichaceae* fam. nov.

The organisms belonging to the Prochlorophyta (16) are oxygenic, photoautotrophic procaryotes which contain chlorophylls *a* and *b*. Usually, this combination of pigments is associated with green algae and higher plants. However, the main criterion discriminating prochlorophytes from green algae is their typical procaryotic cellular ultrastructure. Therefore, Florenzano et al. (6) proposed that the photosynthetic procaryotes containing chlorophylls *a* and *b* should be under the jurisdiction of the *International Code of Nomenclature of Bacteria* (15). The property of oxygenic photosynthesis suggests a close relationship among this new group of bacteria, the oxychlorobacteria, and the cyanobacteria. Although studies on the homologies of the 16S ribosomal ribonucleic acid (rRNA) sequences (23, 24) confirmed this relationship, the conclusions of these studies were debated by Van Valen (29). A less close relationship can be hypothesized on the basis of the thylakoid structure. The lack of phycobilin pigments and the presence of chlorophyll *b* may have consequences for the structure of the thylakoid membranes. The accessory phycobilin pigments of cyanobacteria are located in complexes extrinsic to the thylakoid membranes, whereas the light-harvesting chlorophyll *a/b* complexes in plants and green algae are intrinsic to the photosynthetic membranes. To separate the oxychlorobacteria from the cyanobacteria, Florenzano et al. (6) suggested that an order should be created in the class *Photobacteria* Gibbons and Murray 1978 (7). In connection with this, these authors proposed descriptions for the order *Prochlorales*, the family *Prochloraceae*, and the genus *Prochloron*, as well as the type species *Prochloron didemni* (17).

Hitherto, *Prochloron didemni* (6, 15) was the only described species in this order. This organism is a unicellular, extracellular symbiont of certain marine invertebrates. Recently, Burger-Wiersma et al. (2) reported the isolation of a nonsymbiotic chlorophyll *a/b*-containing procaryote from the freshwater Loosdrecht Lakes in The Netherlands, where

it has been abundant in the summer phytoplankton over the last 5 years. In contrast to *Prochloron didemni*, the newly isolated strain is filamentous with long cylinder-shaped cells. All of the currently available results on ultrastructure (2), cell wall organization (13), and carotenoid (2) and lipid (30) compositions have established the procaryotic nature of the new strain and its resemblance to the cyanobacteria. According to a 16S rRNA-based phylogenetic analysis, this organism is a deeply branching member of the cyanobacterial line of descent (26a). However, there is a distinct difference between the cyanobacteria and the oxychlorobacteria. For both the organism described in this paper (20a) and *Prochloron* spp. (9), lateral heterogeneity of the thylakoid membranes has been reported. This feature is common in green algae and plants, but has never been observed in cyanobacteria. This supports the proposal that the oxychlorobacteria and the cyanobacteria should be separated at the ordinal level (6).

A close relationship between the photosynthetic apparatus of the new strain and that of *Prochloron* sp. has not been established yet, but is suggested by the fact that both organisms contain a chlorophyll *a/b* antenna apoprotein of comparable molecular weights, which is distinct from the protein that is usually observed in green algae and plants (1).

In this paper, we formally describe the new isolate and propose the name *Prochlorothrix hollandica*. Since the ordinal definition of Florenzano et al. (6) would exclude *Prochlorothrix hollandica*, we propose emendation of the original description of the order *Prochlorales*.

MATERIALS AND METHODS

Strain. The new strain was isolated from a sample from a mixed water column from eutrophic Lake Loosdrecht, The Netherlands (52°20' North latitude, 5°5' East longitude). The temperature and pH of the lake water on the sampling date (9 July 1984) were 18°C and 8.8, respectively.

Growth media. The liquid medium contained NaNO₃ (500 mg/liter), MgSO₄ · 7H₂O (50 mg/liter), CaCl₂ · 2H₂O (13

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mg/liter), K_2HPO_4 (25 mg/liter), $NaHCO_3$ (100 mg/liter), disodium ethylenediaminetetraacetate (0.3 mg/liter), $FeCl_2 \cdot 2H_2O$ (0.3 mg/liter), and the following trace elements: H_3BO_3 (2.68 mg/liter), $MnCl_2 \cdot 4H_2O$ (1.81 mg/liter), $ZnSO_4 \cdot 7H_2O$ (0.22 mg/liter), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.002 mg/liter), $CuSO_4 \cdot 5H_2O$ (0.08 mg/liter), $Co(NO_3)_2 \cdot 6H_2O$ (0.08 mg/liter), and NH_4VO_3 (0.01 mg/liter). Filter-sterilized solutions of the trace elements, $NaHCO_3$, and ethylenediaminetetraacetate- $FeCl_2$ were added separately after autoclaving. This medium was referred to as medium FPG. Growth was also tested in medium BG-11 (25).

Solid media were prepared by adding agar to medium FPG or BG-11. The agar was washed three times with distilled water, successively rinsed with 96% ethanol and 100% acetone, and dried in air prior to addition.

Isolation procedure. The strain was isolated by micromanipulation into liquid medium FPG.

Purification. The numbers of contaminants in cultures used for purification experiments were counted microscopically and never exceeded the number of filaments of *Prochlorothrix hollandica*. In general, the ratio was <0.5 . Several techniques were used to obtain axenic cultures.

(i) **Plating onto agar.** A 0.3-ml portion of an actively growing cell suspension was dispersed on agar, and the preparation was incubated at 20°C and 20 μ mol of photons per m^2 per s. Within 2 to 4 weeks a single-layered film of trichomes was formed. Trichomes were transferred two times to fresh plates, and this was followed by inoculation into medium FPG. Subsequently, the cultures were tested for heterotrophic bacteria by using glucose (5 mg/liter) as a carbon source.

(ii) **Washing.** The filaments were washed and then serially diluted in medium FPG (5).

(iii) **Treatment with antibiotics in the dark.** Preparations were treated with antibiotics in the dark by using the method described by Vaara et al. (27). Actively growing cells of *Prochlorothrix hollandica* were transferred to medium FPG and incubated in the dark for 40 h; this was followed by the addition of glucose (5 mg/liter) and either cephalosporin (30 mg/liter) or chloramphenicol (2 mg/liter). In blanks glucose was omitted from the medium. After incubation in the dark for 24, 48, and 72 h, cells were collected by centrifugation at 2,500 rpm, washed at least three times, and suspended in medium FPG. After incubation at 20 μ mol of photons per m^2 per s for 2 to 4 weeks, the cultures were tested for chemoheterotrophic bacteria as described above.

Maintenance. Batch cultures were incubated at 10 μ mol of photons per m^2 per s and 10°C and were transferred to fresh medium every 4 to 6 weeks. Cell suspensions in mineral medium supplemented with 5% dimethyl sulfoxide could be stored at -70°C. After defrosting, suspensions had to be inoculated onto solid mineral medium (0.5% agar) before transfer to liquid medium.

Microscopy. Light microscopy, transmission electron microscopy (see reference 2 for the method used for sample preparation), and scanning electron microscopy were used for morphological studies. Samples for scanning electron microscopy were fixed overnight in 4% glutaraldehyde in phosphate buffer (pH 7.2), and this was followed by dehydration in an ethanol series (10 to 100% ethanol). Subsequently, the samples were critical point dried, gold sputtered, and analyzed by using a Cambridge Instruments model S180 scanning electron microscope.

Gram staining and general microbiological staining techniques for the detection of lipids, polysaccharides, polyphos-

phate, and poly- β -hydroxybutyric acid were performed as described by Norris and Swain (21).

Epifluorescence was studied by using a Dialux model 20 EB/Ploemopak 2.3 combination apparatus equipped with type BP 515-530 (excitation light), RKP 580 (short-wavelength reflection), and LP 580 (short-wavelength blocking) filters, which allowed detection of emission wavelengths of 580 nm or longer (Leitz).

Morphological studies were carried out by using samples from cultures grown under light, phosphorus, or nitrogen limitation or under conditions of light and nutrient sufficiency. In addition, microscopic checks were carried out regularly during all experiments.

Growth conditions and physiological tests. The ability to fix dinitrogen was tested in batch cultures grown in medium FPG or BG-11 (25) under continuous light or with light/dark cycles (16h/8h) of a growth-saturating photon flux density (40 to 100 μ mol/ m^2 per s). In order to maintain a constant light and aeration climate, the batch cultures were suspended by gentle shaking on an orbital shaker at 80 rpm. The cultures were transferred to fresh nitrate-free medium FPG at least three times. Nitrogenase activity was measured by acetylene reduction (26). Assays were carried out with samples taken during both the light period and the dark period and were run in the light, in the presence or absence of 10^{-5} M 3-(3,4-dichlorophenyl)-1,1-dimethyl urea, or in the dark. Subsequently, the strain was tested for its ability to synthesize nitrogenase under anaerobic conditions (22). The cells were maintained in the absence of combined nitrogen for 48 h and then incubated anaerobically in the light in the presence of 10^{-5} M 3-(3,4-dichlorophenyl)-1,1-dimethyl urea. Anaerobiosis was obtained by gassing with oxygen-free helium. Acetylene was added to a partial pressure of 15%. Acetylene and ethylene were determined by gas chromatography.

Unless stated otherwise, all other experiments were carried out by using continuous cultures grown at pH 8.4 ± 0.1 and $20 \pm 1^\circ C$. Warm white fluorescent light was provided continuously at a photon flux density of 60 μ mol/ m^2 per s. The cultures were aerated at a rate of 50 liters/h. The pH was controlled by adding either 1 M NaOH or 1 M HCl using a pH stat.

Photosynthetic activity was measured by using the method of Dubinsky et al. (3).

Analyses. The in vivo absorption spectrum was recorded by using a Uvikon model 860 UV/VIS spectrophotometer (Kontron).

Deoxyribonucleic acid (DNA) was extracted and purified by the hydroxyapatite method (12). The DNA base ratio was calculated from the thermal denaturation temperature derived from the spectrophotometrically obtained melting curve of DNA (model 2600 spectrophotometer; Gilford Instrument Laboratories, Inc.) (20).

Ribonucleic acid (RNA) was isolated by phenol extraction (14). The crude RNA extract was separated by polyacrylamide gel electrophoresis [ratio of acrylamide to bisacrylamide, 20:1; 8% polyacrylamide in the presence of 8 M urea, using the tris(hydroxymethyl)aminomethane-borate buffer system] (14). RNA extracted from *Pseudomonas putida* was used as a control.

Carbohydrate was determined with lyophilized material by the anthrone method (10), using glucose as a standard.

Growth rate was calculated by using the following equation: $\mu = D + (\ln X_t - \ln X_0) \cdot 1/t$, where μ is the specific growth rate (per hour), D is the dilution rate (per hour), t is the sampling interval (in hours), and X_t and X_0 are biomass concentrations (in milligrams per liter) at time t and zero

FIG. 1. Phase-contrast light micrographs showing the general morphology of the cylindrical cells that form the trichomes of *Prochlorothrix hollandica*. Note the many refractile cell inclusions. Cells were grown in a continuous culture at a photon flux density of $25 \mu\text{mol}/\text{m}^2$ per s. Bars = $5 \mu\text{m}$.

time, respectively. In all experiments biomass was measured as protein (19).

RESULTS AND DISCUSSION

Morphology. Individual cells of *Prochlorothrix hollandica*, forming trichomes of variable length, were readily observed by phase-contrast microscopy (Fig. 1). Neither selective staining techniques (see above) nor the electron microscopic techniques used thus far resolved the nature of the refractile cellular inclusions. The cell size depended on the growth conditions and on the phase of cell division (Fig. 2). Under conditions of severe nutrient limitation or increased salt concentration ($\geq 25 \text{ mM NaCl}$), cell length and diameter may increase to 15 and $3 \mu\text{m}$, respectively. Increased concentrations of salt or extreme pH values of the growth medium (see below) resulted occasionally in the formation of cells of irregular shape. This phenomenon has also been described for *Oscillatoria redekei* by Gibson and Fitzsimons (8). In contrast to the results of these authors, we never observed true branching, and the irregular cell shape disappeared upon transfer to medium FPG. Therefore, it is likely that

extreme growth conditions induce irregularities in the cell wall structure.

The lack of specific morphological characteristics of *Prochlorothrix hollandica* probably accounts for the fact that this organism has been overlooked in natural phytoplankton samples. This organism is often mistaken for *Oscillatoria limnetica*. Thus far, no successful techniques are available to detect *Prochlorothrix hollandica* in natural phytoplankton samples. Epifluorescence microscopy has proven to be an acceptable although not very reliable, method. This method is based on fluorescence by cyanobacterial pigments. Therefore, it only gives a negative indication; i.e., the nonfluorescing trichomes might be oxychlorobacteria.

Gas vacuoles have been observed at the cell poles of *Prochlorothrix hollandica* by transmission electron microscopy (U. J. Jürgens, personal communication). The presence of these vacuoles was confirmed by pressure nephelometry that showed that the constituent gas vesicles had a mean critical pressure of approximately 9 bars, which is within the range encountered in phytoplanktonic cyanobacteria (A. E. Walsby, personal communication).

FIG. 2. Scanning electron micrograph of trichomes of *Prochlorothrix hollandica* showing the variability in cell length due to stage of cell development. Bar = 5 μm. Micrograph by S. Seuffer, University of Oldenburg, Oldenburg, Federal Republic of Germany.

For other morphological characteristics, see the species description below.

Motility. The trichomes of *Prochlorothrix hollandica* showed no motility on solid medium (0.5% agar in medium FPG) under conditions of high light intensity and low light intensity (80 and 10 μmol of photons per m² per s, respectively) at 20 or 23°C. Efforts to induce phototactic motility or photophobic motility by partial irradiance of the plates were not successful.

Pigments. The in vivo absorption spectrum of *Prochlorothrix hollandica* is shown in Fig. 3. The peaks at 438 and 676 nm are attributable to chlorophyll *a*. Because of the low content of chlorophyll *b* in *Prochlorothrix hollandica* (the ratio of chlorophyll *a* to chlorophyll *b* ranges from 7:1 to 19:1; T. Burger-Wiersma and A. F. Post, submitted for publication), no specific peak or shoulder corresponding to this pigment is observed in the in vivo spectrum. The presence of chlorophyll *b* is only apparent as a slight broadening of the chlorophyll *a* peak at 655 to 665 nm. The lack of phycobilin pigments is obvious since no absorption peaks are found at 564 and 618 nm, the absorption maxima of phycoerythrin and phycocyanin, respectively. This observation is consistent with the negative results obtained in

FIG. 3. In vivo absorption spectrum of *Prochlorothrix hollandica* cultivated in a continuous culture at 25 μmol of photons per m² per s.

FIG. 4. Specific growth rates of *Prochlorothrix hollandica* in relation to temperature (A) and pH (B). Cultures were grown at 80 μmol of photons per m^2 per s and pH 8.0 (A) or 20°C (B). Averages of five replicates. The standard deviation was less than 5%.

experiments in which phycobilin-specific extraction techniques were used (2).

Purification. Cultures of *Prochlorothrix hollandica* were contaminated with low numbers of bacteria. Given the composition of the medium and the growth conditions, we concluded that the contaminants were chemoheterotrophic bacteria. This was confirmed by the instantaneous increase in the growth rate of the contaminants after the addition of glucose (5 mg/liter). By staining the DNA with 4',6-diamidino-2-phenylindole, we found that the contaminating bacteria were not attached to the cells of *Prochlorothrix hollandica*. Nevertheless, and despite the different purification methods used, axenic cultures have not yet been produced. The most plausible explanation for the failure of the plating technique is the property of *Prochlorothrix hollandica* to form a film instead of discrete colonies. The trichomes are incapable of gliding motility, a property that greatly facilitates purification of filamentous cyanobacteria. The second technique which we used was sterile washing followed by serial dilution. With this method dilution rather than washing appeared to reduce the viability of the cells since growth occurred only if the number of trichomes per culture exceeded four. However, tests proved the presence of heterotrophic bacteria in all growing cultures. This might indicate that the presence of contaminants is essential for the growth of *Prochlorothrix hollandica*. Treatment of the cultures with antibiotics reduced the viability of *Prochlorothrix hollandica*. This phenomenon was observed in cultures with and without glucose.

Genetics. The DNA base composition of *Prochlorothrix hollandica* was 53 mol% guanine plus cytosine (G+C). This value differs markedly from the values found for different samples of *Prochloron* sp. (range, 31 to 41 mol% G+C) (11, 18). It is also in marked contrast to the value of 37.5 mol% G+C usually found for DNA isolated from chloroplasts of green algae (4). However, the value of 53 mol% is within the range (40 to 67 mol% G+C) reported for filamentous, non-heterocystous cyanobacteria (12).

Gel electrophoresis of extracted RNA showed distinct bands of 5S, 16S, and 23S rRNAs, confirming that the rRNA of *Prochlorothrix hollandica* conforms to that of procaryotes, as indicated previously by growth inhibition experiments in which antibiotics were used (2).

Growth characteristics. Since its isolation, the strain has been maintained in liquid cultures in medium FPG. *Prochlo-*

rothrix hollandica can also be grown in medium BG-11 (25). However, the maximum specific growth rate was lower in medium BG-11 (0.007 h⁻¹) than in medium FPG (0.020 h⁻¹) when the organism was cultivated under identical growth conditions in batch cultures. The maximum specific growth rate of 0.02 h⁻¹ at 20°C is low compared with the maximum specific growth rates of most cyanobacteria and green algae. Under nutrient-sufficient conditions and at 20°C, the maximum specific growth rate was reached at an average photon flux density of 40 $\mu\text{mol}/\text{m}^2$ per s.

We found an inverse relationship between the agar content of the solid media and the growth of the strain. No growth occurred in media containing 1% or more agar. Growth was optimal when 0.4% agar was used, but the medium was semisolid. The best results were obtained with 0.5% agar.

Prochlorothrix hollandica is capable of using either ammonium or nitrate as a nitrogen source. A concentration of 3 mM NH_4^+ proved to be inhibitory for growth, whereas 0.75 mM NH_4^+ allowed the maximal growth rate. No growth occurred in medium FPG if nitrate was replaced by urea (1.5 mM). Attempts to grow *Prochlorothrix hollandica* in medium FPG or BG-11 devoid of combined nitrogen failed, indicating the inability of this organism to fix dinitrogen under aerobic conditions. This was confirmed by the absence of any nitrogenase activity during the light period as well as during the dark period. All efforts to induce nitrogenase activity were unsuccessful. Incubation of nitrogen-starved cultures under completely anaerobic conditions did not result in the development of nitrogenase activity. Under these conditions the high endogenous carbohydrate pool (40 to 50% based on dry weight) could provide sufficient reducing power to allow nitrogenase activity.

Because of the contaminating chemoheterotrophic bacteria, all experiments to study the ability of the strain to use various carbon sources for growth were hampered. All purification attempts have failed thus far.

Prochlorothrix hollandica was sensitive to NaCl; an NaCl concentration of 25 mM inhibited growth severely ($\mu = 0.0005 \pm 0.0004$ h⁻¹). At 100 mM NaCl or the equivalent seawater concentration (20% seawater) growth ceased completely. It appeared that *Prochlorothrix hollandica* produced no organic osmotica when it was subjected to these osmotic upshocks (R. H. Reed, personal communication).

The optimum temperature for growth ranged from 20 to 30°C (Fig. 4A). At temperatures below 19°C the growth rate

FIG. 5. Influence of the photon flux density on the oxygen consumption and evolution of *Prochlorothrix hollandica*. Cells were grown at 20°C and 80 μmol of photons per m² per s. Triplicate cultures. The bars indicate standard deviations. chl *a*, Chlorophyll *a*.

decreased drastically. However, tests revealed that *Prochlorothrix hollandica* remained viable at low temperatures. In these experiments *Prochlorothrix hollandica* was grown in batch cultures in medium FPG at 60 μmol of photons per m² per s and 20°C. The actively growing cells were diluted with fresh medium, transferred to 10°C, allowed to adapt to this temperature for 1 week, and then transferred to 4°C. Experiments were carried out in the light and in the dark. After 7, 14, 28, 56, and 84 days samples were tested for viability by transferring them to fresh medium at 20°C. After 7 days at 4°C cultures kept in the light showed reduced growth capacity. However, cultures kept in the dark at 4°C maintained their growth potential even after 84 days.

Optimum growth occurred at pH 8.4 (Fig. 4B). This was the pH of turbidostat cultures without external pH adjustment.

Prochlorothrix hollandica grows photoautotrophically by using water as an electron donor. Exposing *Prochlorothrix hollandica* to an increasing photon flux density resulted in increasing production of oxygen, which leveled off at high photon flux densities (Fig. 5). Respiratory oxygen uptake took place in the dark.

The growth characteristics indicate that the habitat of *Prochlorothrix hollandica* is restricted to slightly alkaline freshwater systems.

Habitat. *Prochlorothrix hollandica* has been isolated only from the shallow, highly eutrophic Loosdrecht Lakes system in The Netherlands. There is now evidence of significant numbers of this species in several comparable lakes in The Netherlands. The Loosdrecht Lakes system originated from peat excavation and consists of several interconnected sub-systems. The lake surface area is approximately 18 km², and the average depth is 2 m (28). The average Secchi disk depth ranges from 0.2 m in summer to 0.8 m in winter. Because of the shallowness of the lake, no thermal stratification develops, and the phytoplankton is homogeneously mixed over the entire water column. Therefore, it is unlikely that the phytoplankton encounters anaerobic conditions in nature.

In the summer *Prochlorothrix hollandica* is one of the dominating species along with several filamentous cyanobacterial species. This organism is most abundant in those parts of the lake where the availability of phosphorus is relatively more limiting for phytoplankton growth. During *Prochlorothrix hollandica* blooms, the water temperature is 15 to 25°C, and the pH varies between 8 and 10.

The most plausible explanation for the abundance of *Prochlorothrix hollandica* during the summer season is the relatively high water temperature (20 ± 5°C). In the winter only low numbers have been observed. However, experiments have revealed that the organism can tolerate low temperatures for long periods without losing its viability. The need for elevated temperatures to exhibit substantial growth might restrict the habitat of *Prochlorothrix hollandica* to shallow systems where complete mixing of the entire water column takes place throughout the year. This allows inoculation from the sediments when the temperature increases, a situation almost never encountered in deeper lakes. Although temperature might explain the abundance in the summer compared with the winter, it does not give us any clues concerning the competitive strength of the organism. A study on the significance of light climate and nutrient status in determining the abundance of *Prochlorothrix hollandica* is in progress.

Taxonomy. Based on similarities in pigment composition and ultrastructure between *Prochloron* spp. (31) and *Prochlorothrix hollandica* (2), the latter species should be placed in the order *Prochlorales* Florenzano, Balloni, and Materassi 1986 (6). However, although Florenzano et al. aimed at a broad definition of the ordinal rank, their description would exclude *Prochlorothrix hollandica* because this organism contains gas vacuoles and its DNA is located in the center of the cells. Therefore, we propose emendation of the ordinal description.

The proposal of a new family for the genus *Prochlorothrix* is based on the overall morphology of the organism. The cells of *Prochlorothrix hollandica* are arranged in trichomes. Therefore, the genus cannot be assigned to the family *Prochloraceae* Florenzano, Balloni, and Materassi 1986, which is restricted to unicellular organisms. The considerable difference in G+C contents further supports placing these two oxychlorobacteria in separate families.

Prochlorales Florenzano, Balloni, and Materassi 1986 (emend. Burger-Wiersma, Stal, and Mur 1989). *Prochlorales* (Pro.chlo.ra'les. M.L. fem. n. *Prochloraceae*, type family of the order; -ales, ending to denote an order; M.L. fem. pl. n. *Prochlorales*, the *Prochloraceae* order).

Unicellular or filamentous procaryotes that perform oxygenic photosynthesis. The cell wall combines structural features of both gram-positive and gram-negative cell wall types (13). Chlorophylls *a* and *b* are the primary photosynthetic pigments; phycobiliproteins are absent. Free living or associated with invertebrate hosts.

Prochlorotrichaceae fam. nov. *Prochlorotrichaceae* (Pro.chlo.ro.tri.cha'ce.ae. M.L. fem. n. *Prochlorotrich*, type genus of the family; -aceae, ending to denote family; M.L. fem. pl. n. *Prochlorotrichaceae*, the *Prochlorotrich* family). Description as for the order *Prochlorales*, but restricted to filamentous forms. The type genus is *Prochlorotrich*.

Prochlorotrix gen. nov. *Prochlorotrix* (Pro.chlo'ro.thrix. Gr. pref. *pro*, before [primordial]; Gr. adj. *chloros*, green; Gr. n. *thrix*, hair; M.L. fem. n. *Prochlorotrix*, primordial green hair). Cells cylindrical. The cells divide by binary transverse fission in a single plane and are held together by the outer layer of the cell wall to form trichomes of varying lengths. The cell wall has both gram-positive and gram-negative characteristics. The trichomes lack well-defined structured sheaths, do not possess differentiated end cells, and are nonmotile. Oxygenic photoautotrophs containing chlorophylls *a* and *b* as their primary photosynthetic pigments; phycobiliproteins are absent. The G+C content of

FIG. 6. Longitudinal section of a *Prochlorothrix hollandica* trichome showing details of cell wall constriction and cross wall septum (glutaraldehyde-osmium tetroxide fixation). (A) Magnification, $\times 82,000$. (B) Magnification, $\times 38,000$. Micrographs by M. Veenhuis, University of Groningen, Groningen, The Netherlands. Note that the thylakoid membranes were lost due to the fixation method (2).

the DNA is 53 mol%, as determined by the thermal denaturation method (one species, one strain).

The type species is *Prochlorothrix hollandica* sp. nov.

Prochlorothrix hollandica sp. nov. *Prochlorothrix hollandica* (hol. lan' di. ca. L. adj. *hollandica*, pertaining to Holland, part of The Netherlands).

Filamentous organism composed of cylindrical cells that are 0.5 to 1.5 μm wide and 3 to 10 μm long. Unfavorable growth conditions may increase both the cell length and the cell diameter. The straight undifferentiated trichomes are composed of 2 to more than 100 cells, depending on the degree of shearing that occurs as a result of stirring liquid cultures. The trichomes lack sheaths, do not possess differentiated end cells, and are nonmotile. The cell wall is characterized by both gram-positive and gram-negative features. Periodic constrictions along the trichomes mark the locations of cross walls between adjacent cells. The cross walls are formed by invagination of the cytoplasmic membrane and the peptidoglycan layer of the cell wall. The outer layer of the cell wall does not participate in cross wall

formation (Fig. 6). Reproduction occurs by trichome fragmentation; motile hormogonia are not produced.

The thylakoid membranes are arranged in parallel layers at the periphery of the cytoplasm around a thylakoid-free central body (2). The thylakoid membranes show lateral heterogeneity (Miller et al., in press). Refractile cell inclusions of indefinite nature may be present. Gas vacuoles are present at the polar ends of the cells. Carboxysomes seem to be restricted to the center of the cells in the vicinity of the thylakoid membranes. The nuclear region is located in the center of the cells (2).

The primary photosynthetic pigments, chlorophylls *a* and *b*, have a ratio of chlorophyll *a* to chlorophyll *b* ranging from 7:1 to 19:1. Zeaxanthin and β -carotene are the major carotenoids; α -carotene, lutein, and echinenon are absent. Dinitrogen fixation does not occur in media devoid of combined nitrogen, nor can nitrogenase activity be induced under anaerobic conditions. Salt sensitive; 25 mM NaCl inhibits growth, and no growth occurs at 100 mM NaCl. The optimum growth temperature is between 20 and 30°C, and the optimum pH is 8.4.

Isolated from a shallow, highly eutrophic freshwater lake in The Netherlands.

The G+C content of the DNA is 53 mol%, as determined by the thermal denaturation method (one strain).

The type strain has been deposited in the Culture Collection of Algae and Protozoa (Cumbria, United Kingdom) as strain CCAP 1490/1.

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