CHARACTERIZATION OF CYANOBACTERIAL PICOPLANKTON IN LAKE ONTARIO BY TRANSMISSION ELECTRON MICROSCOPY

by
GARY G. LEPPARD and DINA URCIUOLI
and F. R. PICK

National Water Research Institute
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RÉSUMÉ ADMINISTRATIF

Composé de bactéries et d'algues de dimensions infimes, le picoplancton (0,2 à 2 µm de diamètre) se retrouve en très grande quantité dans le lac Ontario (jusqu'à 5 x 10^6 bactéries/ml et jusqu'à 2 x 10^5 cyanobactéries/ml). Le picoplancton peut contribuer grandement à l'activité biologique des eaux de surface et ses caractéristiques physiologiques sont importantes pour le lac. Le présent document décrit le principal microbe photosynthétique des proliférations de fin d'été. Il s'agit d'une cyanobactérie du type chroococcus possédant une ultrastructure de type I, soit le principal type dans les eaux de mer. Le microbe le plus fréquent associé à cette cyanobactérie est une bactérie gram-négative non photosynthétique, dont la taille est de beaucoup inférieure au diamètre des pores des filtres qui sont utilisés pour séparer les bactéries du COD.

RÉSUMÉ

Des cyanobactéries du type chroococcus provenant du lac Ontario ont été étudiées au moyen d'épifluorescence et de microscopie électronique par transmission. Ces études ont permis de découvrir qu'un grand nombre des cellules des cyanobactéries possèdent une ultrastructure de type I, soit le principal type dans les eaux de mer. Ces cellules ont une enveloppe composée de plusieurs épaisseurs et leurs structures polyèdres sont réparties dans le noyau central. Tous les thylakoids se trouvent en périphérie, concentriquement, et ne pénètrent pas dans la région du noyau. D'autres organismes du type chroococcus ont été observés, mais en beaucoup moins grande quantité. Les cellules présentes en plus grand nombre dans tous les échantillons recueillis étaient celles d'une petite bactérie gram-négative en forme de baguette. Un grand nombre de ces baguettes étaient suffisamment petites pour traverser un filtre dont les pores mesuraient 0,45 µm, mais aucune d'elles n'avait un diamètre inférieur à 0,2 µm. Les essais qui ont été faits pour isoler et décrire le picoplancton composé de cyanobactéries présentaient certaines difficultés particulières, qui pourraient nuire aux analyses limnologiques. Ces difficultés sont décrites et, dans une certaine mesure, résolues dans le présent article.

key words: cyanobacteria, electron microscopy, epifluorescence, picoplankton

Characterization of Cyanobacterial Picoplankton in Lake Ontario by Transmission Electron Microscopy

Gary G. Leppard, Dina Urciuoli

Aquatic Ecology Division, National Water Research Institute, Box 5050, Burlington, Ont. L7R 4A6

and F.R. Pick

Department of Biology, Trent University, Peterborough, Ont. K9J 7B8

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Many chroococcoid cyanobacteria from Lake Ontario, characterized by epifluorescence in concert with transmission electron microscopy, had a Type I ultrastructure, the major type in oceanic waters. Such cells had a multilayered cell envelope and their polyhedral bodies were interspersed with the central nuclear material. All thylakoids were peripheral and were

arranged concentrically, with no intrusion into the nuclear region. Related chroococcoid types were also seen but these were much less abundant. The most numerous cell type in all water samples was a small, Gram-negative, rod-like bacterium. Many of these rods were sufficiently small to pass a filter of 0.45 µm pore size but none had a diameter less than 0.2 µm. Attempts to isolate and describe the cyanobacterial picoplankton presented some unusual difficulties having a potential to mislead limnological analyses. These are described and, to some extent, they are resolved here.

NOTE INTRODUCTION

For many years, microscopical investigations have been revealing that microbes of unusually small dimensions, both bacterial (Bae et al. 1972; Sieburth et al. 1978) and algal (Manton 1959; Johnson and Sieburth 1982), may be common in nature despite their rarity in laboratory cultures. This is true of some soils where up to 72% of microbial cells can have diameters less than 0.3 µm (Bae et al. 1972). This is also true of surface waters where the phenomenon is documented for estuaries (Johnson and Sieburth 1982) and oceanic waters of tropical (Waterbury et al. 1979; Johnson and Sieburth 1979; Li et al. 1983), temperate (Manton 1977; Johnson and Sieburth 1979, 1982) and arctic types (Johnson and Sieburth 1982). As a consequence of these findings, attention is now being focussed on the picoplankton of lakes (Sicko-Goad and Stoermer 1984; Caron et al. 1985). The application of transmission (TEM) electron microscopy to picoplankton studies and to the study of natural microbial populations in general continues to reveal the

ubiquitousness of unusually small microbes (0.2 to 0.4 µm), including some as small as 0.1 µm in diameter (von Hofsten et al. 1971).

Physiological studies on lake community metabolism have shown that microbes with diameters approaching the lower limit for both prokaryotic and eukaryotic cells make a disproportionately great contribution to primary production (Munawar et al. 1978; Lean et al. 1985). These findings have been complemented by oceanographic investigations of the physiological importance of aquatic microbes in the smallest size category for cells, the picoplankton (0.2 to 2.0 µm). For example, Li et al. (1983) found that up to 90% of the phytoplankton biomass and up to 80% of the inorganic carbon dioxide fixation in one tropical oceanic water was found in a particle fraction which passed through a screen having a pore diameter of 1 µm.

This paper addresses itself to the characterization of lacustrine cyanobacterial picoplankton in Lake Ontario and to the technical problems of fractionating lake water so as to obtain representative picoplankton populations, while minimizing any artifactual selective losses of cell types.

Methods

Water samples were taken from epilimnetic water of Lake Ontario at a mid-lake station (403, 43°35'52" and 78°13'51") on 28 August, 1984, during a period when microbial biomass is typically at a maximum (Pick and Caron 1985). Fresh and preserved samples (final 1% glutaraldehyde solution

buffered with cacodylate) were examined by epifluorescence microscopy as outlined in Caron et al. (1985). Whole lake water and lake water filtered through 8 µm, 5 µm and 1 µm Nuclepore polycarbonate membranes were enumerated for chroococcoid cyanobacteria, photosynthetic nanoplankton and heterotrophic nanoplankton by autofluorescence. Bacteria were enumerated using the fluorescent stain DAPI (Porter and Feig 1980).

The initial stages of the preparation of microbiota for examination by TEM were done as follows: a fresh water sample was poured through an 8 µm membrane (see above) and the fresh filtrate was mixed gently with a buffered aldehyde fixative (at the same temperature as the water sample) to give a final concentration of 3.4% glutaraldehyde and 0.08 mol/L cacodylate-HCl buffer at pH 7. The filtrate and the fixative were mixed in approximately equal portions to optimize the rate of fixative penetration. In the laboratory, all subsequent chemical preparation for TEM was carried out according to Burnison and Leppard (1983) with a second filtration step interposed as follows: the glutaraldehyde-fixed cells were collected on a 0.4 µm filter (Nuclepore N40 polycarbonate membrane) using an added filtration pressure of 70 kPa. While still moist, the filter's upper surface and its adhering microbiota were coated with a thin layer of warm (60°C) liquid agar (0.6% Agar Technical #3 from Oxoid); when the agar had gelled, a strip of the filter-microbe-agar continuum was processed chemically as a unit, starting at the secondary fixation stage. The counterstained sections of 50 - 70 nm thickness were observed and photographed with a Philips 300 TEM.

The overall preparatory scheme above was derived by integrating a series of water fractionation schemes (involving centrifugation and/or cascade filtration) with several chemical fixation procedures. Preliminary testing conducted on samples collected in 1982 from Lake Ontario gave insight into some anomalous behaviour of the chroococcoid cyanobacteria with regard to fractionation schemes used previously for concentrating them.

Results and Discussion

Concentrations of microbes, estimated by epifluorescence counts, were of the same order of magnitude (Table 1) as in previous years for August (Caron et al. 1985; Pick and Caron 1985). Chroococcoid cyanobacteria were of the phycoerythrin-containing type that autofluoresce yellow-orange in epifluorescence microscopy. Both fresh and preserved samples gave similar estimates for these populations (1.75×10^5/mL). 100% of these cells passed the 8 µm filter whereas only 87% passed the 5 µm one. For this reason, the 8 µm filter was used in TEM preparations. In other lakes where chroococcoid cyanobacteria are as abundant and of similar size (0.7 to 1.2 µm in diameter), less than 50% may pass a 5 µm filter, suggesting that colony formation or aggregation may be extremely variable (Pick, unpublished results).

The most common chroococcoid cyanobacteria found, using TEM criteria, were spherical to oval cells of Type I ultrastructure as characterized by Johnson and Sieburth (1979) and illustrated by the dividing cell of Fig. 1. This cell type, previously found to be ubiquitous in oceanic surface waters

(Johnson and Sieburth 1979), has the following general characteristics in epilimnetic Lake Ontario water.

(1) It has a multilayered cell envelope which sometimes displays an outermost extra layer of electron-opaque material, after counterstaining. This extra layer can be highly structured in the manner described earlier by Kursar et al. (1981).

(2) It has a peripheral thylakoid, or peripheral thylakoids arranged concentrically, with no intrusion into the nuclear region. The thylakoids number as many as three but not all of them encircle completely the cytoplasm.

(3) The thylakoids have a membrane structure which is difficult to resolve as one sees by comparing the thylakoid ultrastructure of the cyanobacterium in Fig. 2 with that of the large eukaryotic alga also present in Fig. 2. The difficulty resides partly in the extreme curvature of the thylakoids relative to section thickness (Figs. 1 and 2) and partly in the extreme compression of thylakoid structure as revealed by the high magnification micrograph of Fig. 3.

(4) In cells containing either two or three peripheral thylakoids, the thylakoids are widely interspaced by densely-packed, electron-opaque, cytoplasmic material (Fig. 1).

(5) The central nuclear material is interspersed with several polyhedral bodies of varying electron density relative to the cytoplasm (Fig. 1).

(6) The diameter is more variable than the documented range for Type I cells in oceanic waters (0.8 to 1.0 μm). Discounting cells undergoing division, the range of diameters for Lake Ontario cells extended

downwards into the ranges previously established for Type II (0.7 to 0.9 μm - see Fig. 5) and Type III cells (0.4 to 0.5 μm - see Fig. 6) as documented by Johnson and Sieburth (1979).

It is possible to split the lacustrine Type I cells arbitrarily into several subtypes. This can be done with respect to growth habit (e.g., the unicell of Fig. 1 vs. the four-celled colony of Fig. 4), the number of thylakoids and the structure of the outermost wall layer (e.g., the fibrous layer revealed by Fig. 5 vs. the layer of repeating subunits shown in Fig. 6. To establish a subtype classification scheme and to ascertain the relative proportion of each subtype is, however, an endeavor beyond the scope of this initial study. The variability of Type I cells was great enough that cells could be found with characteristics which blurred the distinctions between the accepted types as they were originally documented in the marine literature. Fig. 7 shows a cell whose size is in the Type I range and whose outer wall layer resembles that of many Type I cells. However, its thylakoid arrangement is atypical for Types I, II and III as described by Johnson and Sieburth (1979), and the spatial relationship between its thylakoid and its nuclear region is more like Type III than Type I. This cell (Fig. 7) has characteristics indicating a poor condition at the time of fixation. Had such a cell been seen as a rarity in a laboratory culture, it would have been ignored. However, such cells occur in varying abundance in natural populations (Leppard, unpublished results) and they may contribute to the physiological activities measured by limnologists; for this latter reason, more knowledge of their impact on water quality is needed now.

The unicellular cyanobacteria were associated with much greater numbers of small bacteria having a Gram-negative wall structure. Many of these bacteria were much smaller than 0.4 µm in diameter, with some well-preserved specimens showing least diameters (as measured in median cross-sections from the outermost tripartite layer of the cell envelope) approaching 0.25 µm (Fig. 8). These probably would not be enumerated accurately using epifluorescence techniques. It is possible that small bacteria which didn't pass the 0.4 µm filter were part of loose aggregates. Cross-connecting fibrils of the type described in Burnison and Leppard (1983) could be seen rarely, but the use of the fibrillar agar overlay precluded an analysis of fibril-mediated aggregation.

Among the other cell types seen in the 0.4 to 8 µm fraction were unicellular eukaryotic algae and some protozoa, but these were not commonly encountered. They were several orders of magnitude less abundant (Table 1) than the Gram-negative rods and the cyanobacteria. While these latter cells were studied intensively only at the sample site and date given above, they were seen in all Lake Ontario water samples taken for preliminary investigations in 1982. At this time, experiments on the fractionation of lake water were carried out to devise an optimal approach to concentrate picoplankton for TEM analyses.

Standard approaches to concentrating the Type I cells led to great losses of cells, including selective losses of cell types as judged by TEM monitoring (Burnison and Leppard 1983). Some particular problems were identified and these are summarized as follows:

(1) the many fluid changes and washes employed in the cytological processing required for TEM were undesirably effective in detaching Type I cells from fine filters used to trap natural microbial populations, thus leading to disproportionate losses of Type I cells;

(2) low speed centrifugation (up to 1400 g) was ineffective in concentrating chroococcoid cells;

(3) the distribution of chroococcoid cells on the surface of filters with pore sizes near 0.5 μm was always patchy to an extreme degree, even on the filter of choice (Nuclepore N40 polycarbonate membrane), thus suggesting that these cells approach a filter surface as aggregates.

The first problem was solved using an agar overlay to glue all particles (0.4 to 8 μm) onto the filter surface at an early stage of cytological processing and the second was solved by abandoning the use of low speed centrifugation. The patchiness problem was solved by taking an inordinately great number of sections to obtain the required number of images for a morphological study. This third problem suggested that unicellular picoplankton can aggregate (or are aggregated by physical effects during concentration by cascade filtration). Through the use of an 8 μm screen in conjunction with epifluorescence microscopy, we determined that any aggregates present have a major diameter less than 8 μm. The largest colonial chroococcoid seen (the four-celled colony of Fig. 4) had a major diameter of 3 μm, so such colonies would not be excluded at the screening stage.

Once we could demonstrate that our TEM analyses included an entire population of chroococcoid cyanobacteria in a water sample, an observation was made which has a bearing on future attempts to concentrate and/or isolate other members of the picoplankton. The Gram-negative nonphotosynthetic bacteria with a diameter less than the pore diameter of the trapping filter were more numerous on the filter surface than were the Type I cells. Some of these bacteria had a diameter 40% smaller than that of the filter pores. How were they trapped and what proportion of such small bacteria do they represent? Some of the small bacteria could be found trapped deep within the filter pore structure, an observation which complements that of Burnison and Leppard (1983) who used a filter of 0.45 µm pore size to make separations. It is clear that a straightforward consideration of cell size vs. pore size is not sufficient as a sole basis for the design of cascade filtration schemes employed to isolate sedimenting units which grade into the colloidal size range.

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Table 1. Microbial populations estimated by epifluorescence microscopy in Lake Ontario, 28 August, 1984.

	Whole lake water (#/mL)	8 µm	5 µm	1 µm
		(as a % of whole lake water)		
Bacteria	5.23×10^6	100	100	89
Chroococcoid cyanobacteria	1.75×10^5	100	87	23
Photosynthetic nanoplankton	3.23×10^3	90	80	0.9
Heterotrophic nanoplankton	1.52×10^3	96	83	4

FIGURE LEGENDS

Fig. 1 A dividing cell of a Type I chroococcoid cyanobacterium, with two thylakoids and an extra layer of wall structure projecting into the external milieu. The bar and all subsequent bars represent 0.5 µm.

Fig. 2 The effect of a tightly imposed curvature on the resolution of thylakoids. The small size of a chroococcoid cell, relative to section thickness, means that most views of its thylakoids will be equivalent to a glancing section through the thylakoids of a large cell. Compare the image of the thylakoids in the chroococcoid cell (small circle) with the image presented by the large eukaryotic cell (large circle).

Fig. 3 A median longisection view through a relatively large chroococcoid cell at high magnification. In views such as this, thylakoid structure is more evident, as shown within the large circle.

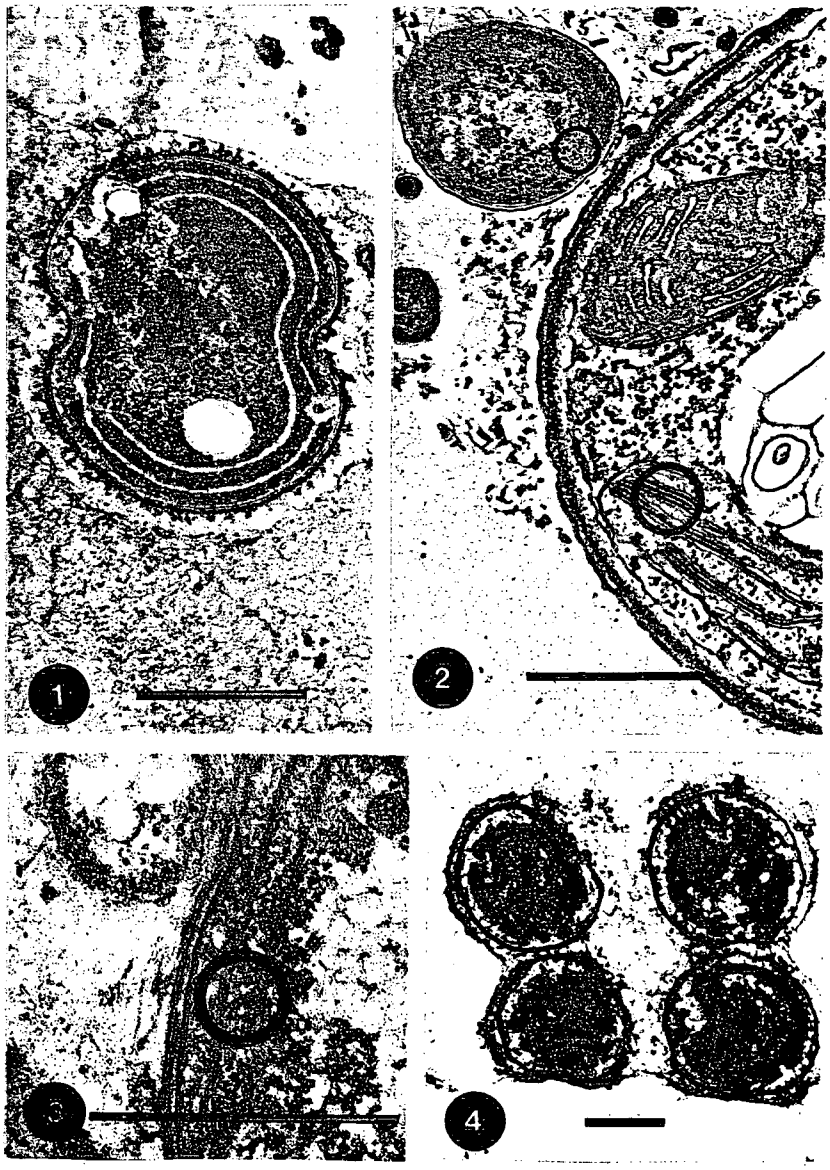
Fig. 4 A colony of four chroococcoid cells encased in a sheath.

Fig. 5 A Type I cell in the Type II size range. Compare with Figs. 1 and 6.

Fig. 6 A Type I cell in the Type III size range. Note the geometrical array of structural units representing the outermost wall layer. Compare with Figs. 1 and 5.

Fig. 7 A cyanobacterium with intermediate features.

Fig. 8 An unusually small nonphotosynthetic bacterium common to Lake Ontario picoplankton.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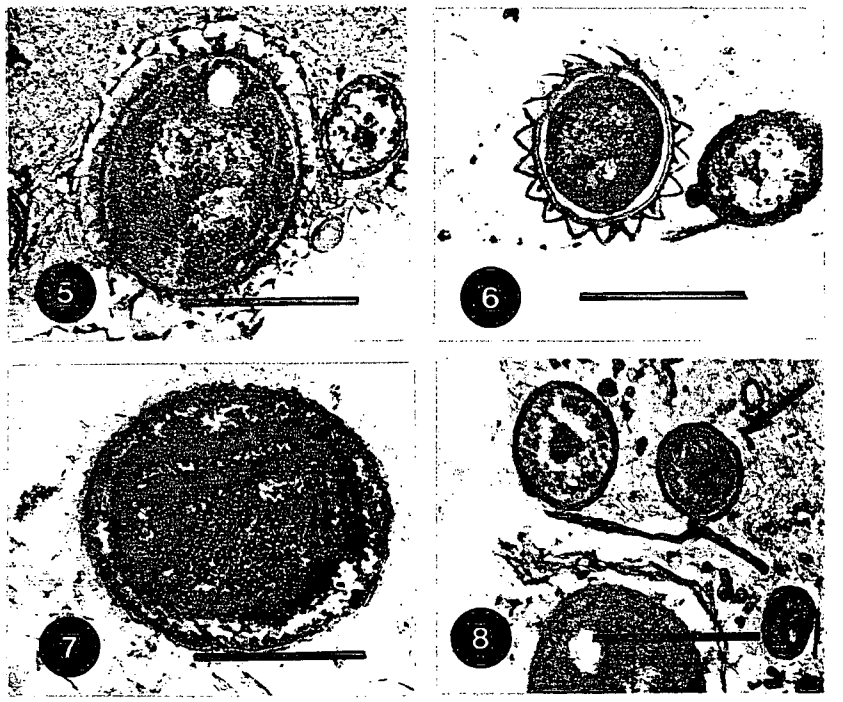
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