Bull. Environ. Contam. Toxicol. (1997) 59:35-43
©1997 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

Blue-Green Alga *Microcystis aeruginosa* Kütz. in Natural Medium

B. H. Kim,[1] M. K. Choi,[1] Y. T. Chung,[1] J. B. Lee,[2] I. S. Wui[2]

[1]Institute for Environmental Science, Wonkwang University, Iksan 570-749, Korea
[2]Department of Biology, Chonnam National University, Kwangju 500-757, Korea

Received: 4 October 1995/Accepted: 5 March 1997

Cyanobacteria, blue-green algae, has a large number of different granules and inclusions, or storage bodies, of various structures and shapes, although their distribution is, in respect to that of bacteria, only partly Gram-negative (Wolks, 1973; Healy, 1982;Golecki et al.,1990). The chemical nature of some of these inclusion bodies has been identified with light microscopic staining methods or by chemical analyses (Hascoet et al.,l985;Streichan et al.,1990). The characteristics of these bodies under light and electron microscopy has been well known for the many kinds of blue-green algae used by most researchers doing culture experiments. Colonial Microcystis aeruginosa has been associated with numerous livestock deaths and implicated epidemiologically in human illness. They are known as hepato- and neurotoxins, and are more toxic in tests done on domestic animals than any other algal group (Aune and Berg, 1986;Galey et al,1987;Utkilen and Gjolme,1992). These studies have focused more on the toxicity to experimental cells and tissues due to algal products, such microcystins (Wicks and Thie1,1990), than on the morphological changes rendered by treatment with foreign materials. While there are a number of reviews on the blue-green algae (Wolk,l973;Carr and Whitton,1982), reports on the fine structures of M. aeruginosa were very few. Even so, the ultrastructural characteristics of M. aeruginosa have been examined mainly under culture conditions. The purpose of this work was to examine the fine structures of M. aeruginosa taken from different natural habitats and to find a useful tool for classifying blue-green algae by genus.

MATERIALS AND METHODS

Lake Chuam (35°20'-34°43'N, 127°17'-127°05'E) is an artificial multipurpose, and dendrite type lake with many tributaries, surrounded by the provinces of Whasungun, Sungjugun and Bosunggun, located in the southwestern Korea peninsula. The water body represents 1,144km^3 of drainage basin and has 700 million tons of reserve capacity. Sample collection was carried out at the mouths of Tongbok and Bosung streams at Lake Chuam on 13 September, 1994, which showed the highest counts. To collect M. aeruginosa colonies, a plankton net was used (Müller gauze No. 2.5, 50mL). To analyze the habitats, water samples were taken from the surface to a one meter depth using a Vandom's type water sampler (2L). Water temperature and pH values were measured directly at the time. Chemical oxygen demends, suspended solids, total nitrogen, nitrate, nitrite, total phosphorus, phosphorus and Chlorophyll'a were measured by the standard methods (APHA, 1985) at the laboratory.

Correspondence to: B .H. Kim

For light microscopy, all samples were fixed with formalin acetic acid (FAA) solution or 5.7% neutral formalin solution. Before analysis, all samples were dispersed by ultrasonic disintegration (20kHz, 60 sec). To count the total number of cells, a Sedgwick-Rafter Chamber was used under the light microscope with a camera system (Leitz, X 1,000).
For electron microscopy, colonial M. aeruginosa were collected, and 10 mL were fixed with 1% glutaraldehyde overnight. The samples were post-fixed with 1% buffered osmium tetroxide for 1.5 hrs (0.1M phosphate buffer, pH 7.0 was used for both fixatives). The fixed colonies were dehydrated in ascending grades of ethanol and embedded in epoxy resin. Thin sections were cut with a glass knife on an LKB microtome. The sections were stained with uranyl acetate, saturated in a solution of 50% ethanol followed by lead citrate, and examined with a Hitachi-600 electron microscope.

RESULTS AND DISCUSSION

There were remarkable differences in the physicochemical factors between both branches, except for water temperature and pH value. In particular, the concentration of suspended solids and chlorophyll's in Tongbok stream were three times those of Bosung. Also, the concentration of chemical oxygen demends (COD) and total phosphorus in Tongbok branch were nearly twice those of Bosung. From our examinations, it is clear that both branches have considerable differences in environmental habitat, especially in terms of organic loading or of nutrient level, assuming of course, due to differences in their upstream feeders. We could not distinguish any remarkable differences in blue-green algal bloom by naked-eye between the two branches. M. aeruginosa averaged 1.2 X 10^5cells/mL accounting for 87.5% of total organism, and the number in the Tongbok branch was nearly twice that of Bosung. Thirty one phytoplankton assemblages were classified, among them: Melosira granulata and Synedra ulna in diatoms; Scenedesmus quadricauda, Coelastrum microporum, Eudorina elegans and Pediastrum duplex var. reticulatum in green alga; and Peridinium willei and Ceratium hirundinella dominated in dinoflagellates, but their numbers were below 1 X 10^3cells/mL.

Table 1. Habitat comparison of M. aeruginosa Kütz.

collection conditions	Tongbok branch $34^0$80'N - $127^0$08'E	Bosung branch $34^0$60'N - $127^0$12'E
Water temp. (^{0}C)	26	26
pH	7.8	7.4
COD (mg/L)	5.9	3.1
Suspended solids (mg/L)	22.1	6.9
NO_2-N (mg/L)	0.04	0.01
NO_3-N (mg/L)	0.41	0.20
NH_3-N (mg/L)	0.15	0.09
Total phosphorus (mg/L)	0.05	0.03
Chl. *a* (mg/m^3)	8.6	2.7
Total counts (cells/mL)	7.3 X 10^4	4.7 X 10^4

Light microscopy was used mainly for counting M. aeruginosa cells, distinguishing cell aggregates from mixed colonies, and for photographing the cells. Natural M. aeruginosa colonies obtained from Lake Chuam, formed irregularly shaped non-directional arrays with distinct hyaline mucilage with individual cells either ovoid or spherical 3-5μm in diameter. Many whitish granules or organelles, possibly gas vacuoles, were visible mainly on the outside of the cells, and sometimes, both the nucleoplasm and cytoplasm were divided into several parts by them (Fig. 1).

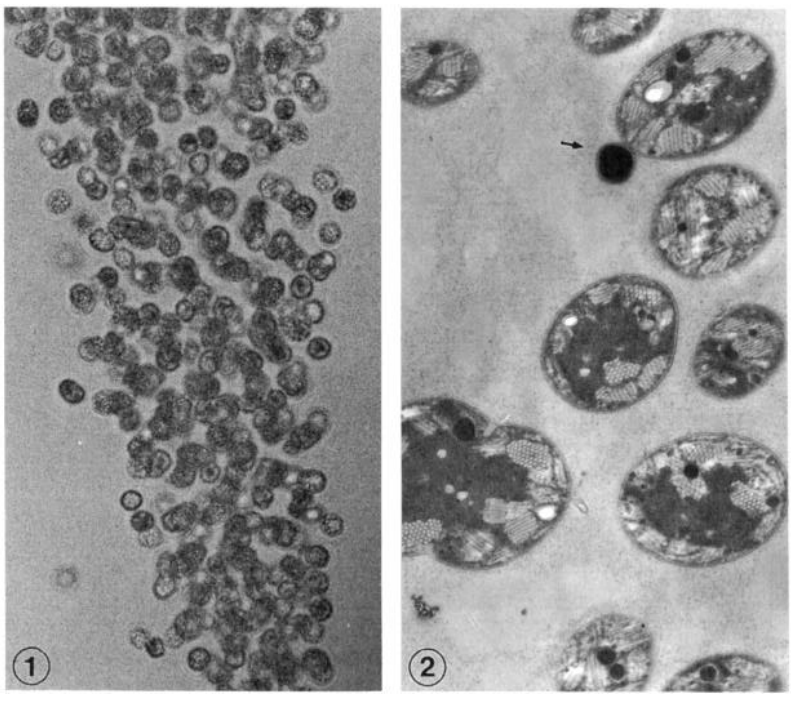

Figure 1. Light micrograph of blue-green alga M. aeruginosa Kütz. X 1,100

Figure 2. Electron micrograph of M. aeruginosa. These colonies has many kinds of granules and bundles of gas vacuoles, widely distributed in cytoplasm. It accidently shows unknown membranous granules whether uptake from outside or discharge from cytoplasm, or phosphate granules(arrow). X 7,500

Under the electron microscope, M. aeruginosa colonies consisted of many kinds of cells, young and old, in various sizes and stages of division. Cells ranged from 2.61 to 5.40μm in diameter, averaged 3.54-±0.19μm (n=3 l,p<0.05), and were either ovoid or spherical in shape (Fig.2-4). In the cytoplasm, there were a number of inclusions of various sizes, shapes and appearances. Among inclusions, polyhedral bodies or carboxysomes, large or small structured and dense granules, photosynthetic lamellae or thylakoids, and gas vacuoles were prominent and easy to recognize (Fig.3-7). Incidentally, there were large structured granules adhering to the cell surface (Fig. 2). We could not ascertain whether they were uptake of electron dense materials from extracellular medium, discharge from the cytoplasm, or phosphate sewage bacteria (Golecki and Heinrich, 1990). There were many granules diverse in shape and electron density in the cytoplasm of M aeruginosa, which are known to be widely distributed in a number of blue-green algae (Fig.4-7). These granules were categorized as follows; cyanophycin granules or structured granules, ployphosphate granules, polyhedral bodies, glycogen granules, and ribosomes. Among them, the polyhedral bodies (PB) we observed (Fig.6), often called crystalline bodies or carboxysomes, are known as vital for cell activities and have many functions (Mehta and Hawby,1979). In ultrathin section, they varied in size and in the density of their electron fields, were normally seen in the center of the cell or near nuclear materials, and were

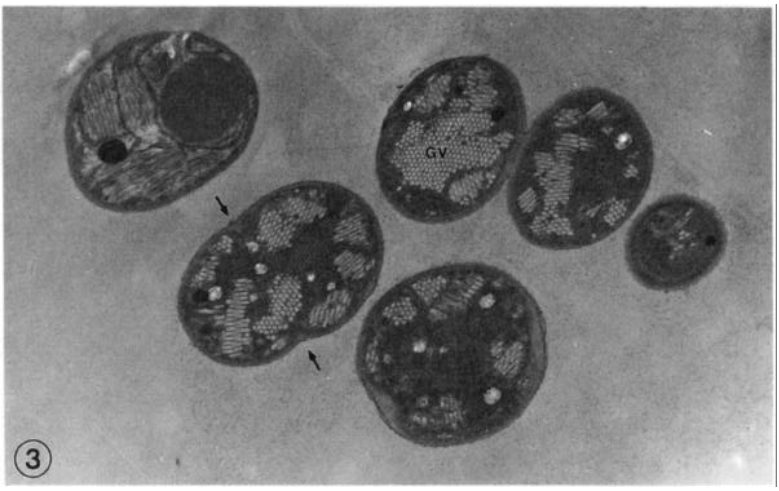

Figure 3. Large arrow indicates that cytoplasmic invagination or meristemic constraction of M. aeruginosa, thereby divided in two future daughter cells. Large cyanophycin granule(small arrow), bundle of gas vacuoles(GV). X 11,500

hexagonal in shape.

There were no limiting or membranous structures around the PB's, but sometimes a definable interface covered them. The number and length of the PB's varied from 1 to 8, and 120-750mn, respectively (Table 2, Fig. 4 and 6). These bodies were a ubiquitous characteristic of the blue-green algae, often not only showing angular or irregular shapes, but also having a new membrane formation around the PB in many species (Mehta and Hawby,1979). It is clear that the PB's always present in M. aeruginosa, have a morphological diversity of length, shape and number. Although more detailed experiments are need, we conclude for the time being that the PB's are a useful characteritic for blue-green algal classification of the genus level.

Table 2. Numerical characteristics of polyhedral bodies.

	Mehta and Hawby (1979)			present study
	young cells	old cells	with Simazine	
numbers	1-6 (1-3)	2-4 (2)	?	1-8 (5)
diameter	66-267 nm	80-360 nm	94-426 nm	120-750 nm
cells		A. nidulans		M. aeruginosa

( ): numbers in common or average

Until now, the ultrastructure of the outer membranes or outside cell wall of M. aeruginosa has been less understood than any other group, such as the unicellular cyanobacterium Anacystis nidulans (Golecki, 1974) or most Gram-negative bacteria (Weise et al.,1970). The extracellular covering of M. aeruginosa was divided into several layers; the cytoplasmic membrane or plasmalemma, the peptidoglycan layer (Fig.5c), and the multilayered structure of the cell wall (Fig5b).

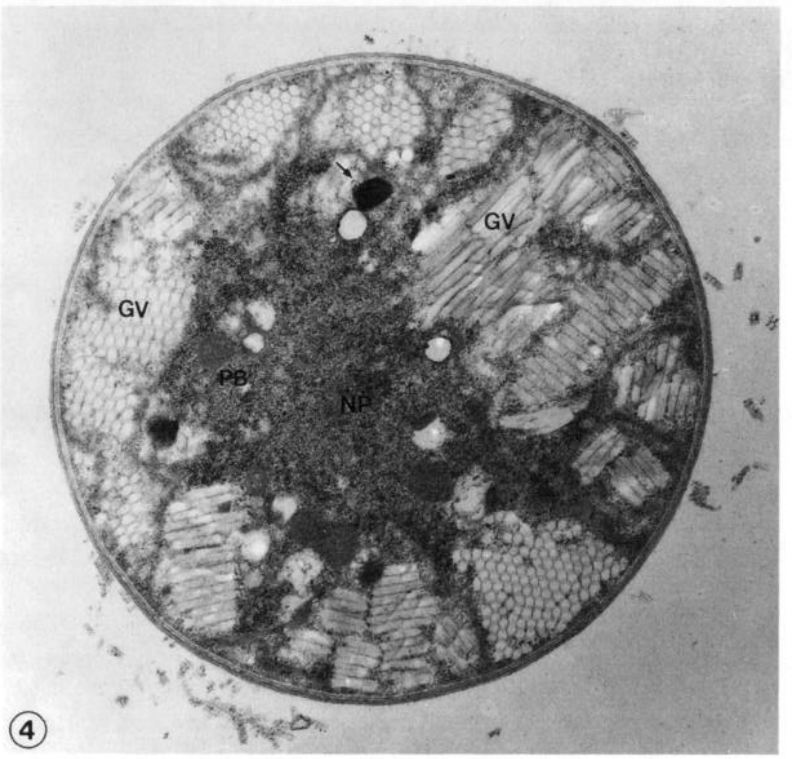

Figure 4. Blue-green algae M aeruginosa of Lake Chuam. It show spherical form, bundle of gas vacuoles(GV) more constricted to periphery, polyhedral bodies(PB), nucleoplasm(NP), and cyanophycin granule(arrow). X 21,000

There were numerous filaments or filamentous projections, consisting of two parts, with the proximal portion of each appearing thicker than the distal one. Also, the filaments were approximately 136.4Å in length and 56.5Å in diameter (Fig.5a), similar to those of cyanobacterium A. nidulans (Golecki,1977). These filaments are morphologically similar to pili previously known to exist among algal and bacterial groups such as Svnechocystis and Anabaena. As table 3, shows there are considerable differences in the microscopic morphometry of the genus blue-green algae. This indicates that the outer layer of the cell wall may also be a useful characteristic for blue-green algal taxonomy of the genus level. As shown by the arrows in figure 3, natural M. aeruginosa shows various cell shapes and developmental stages of cell division or invagination. The majority of these cells observed in our electron microscopic findings were "two cells about-to-become-four", as do rod-shaped bacteria. In general cell division is symmetrical, and is activated by the septum forming a median constriction, or invagination of the cell membrane. Also, cell wall layers LI and LII participate equally in the pattern of Gleocapsa alpicola and A. nidulans (Allen,1968). Although the amounts of walls LI and LII were not quantitatively measured, in fact, layer LII is much greater than that of LI in terms of relative distribution.

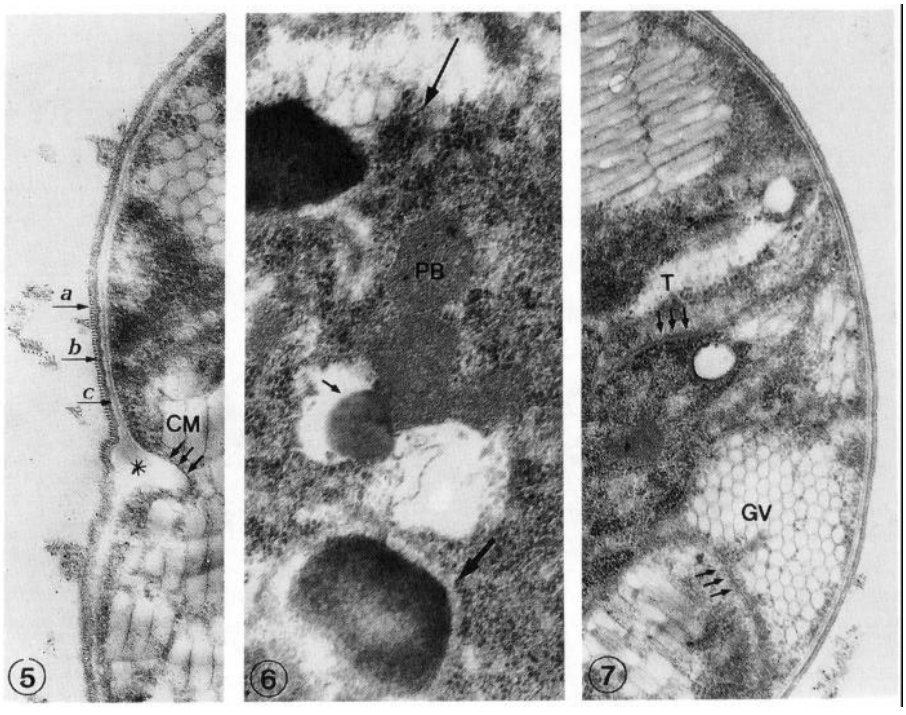

Figure 5. Cell coverings of M. aeruginosa are divided into four layers, filamentous projections(a), multi-layers of cell wall(b), peptidoglycan layer(c), and cell membrane(CM), It show meristemic constraction or definable interface (*) in early division. X 43,000

Figure 6. Various granular bodies in cytoplasm. Polyhedral bodies(PB), dense cyanophycin granules (thick arrow), polyribosomes(long arrow) and undefinable granule(smal1 arrow). X 43,000

Figure 7. Thylakoid lamellae(T), glycogen granules(thick arrow) and gas vacuoles (GV) of M. aeruginosa. X 37,000

Table 3. Comparison of filamentous projections in the outer wall

	Lounatmaa et al.(1980)	Leak(1967)	Golecki(1977)	present study
length	1 μm	0.5 μm	150 Å	136.4 Å
diameter	600Å	60-80 Å	60-75 Å	56.5 Å
cells	Synechocytis	Anabaena	Anacystis	Microcystis

Blue-green algae was the first major group of phototrophs to arise with a two-stage photosynthetic pathway capable of oxdizing water to produce molecular oxygen (Giovannoni et al,1988). The photosynthetic lamellae or thylakoids in the cytoplasm of M. aeruginosa, unlike those of other chlorophyllous plants, are not enclosed in membrane-bounded groups to form chloroplasts, nor are they grouped or associated with each other.

They are widely distributed, are more restricted to the periphery, and sometimes, the bundle of gas vacuoles are divided into two parts, across the proximal to the distal portion of the cytoplasm (Fig.7). Phycobilisomes were multiprotein complexes and were observed by electron microscopy in the Rhodo- and Cyanophyceae, as discrete structures attached to the stroma surfaces of the lamellae(Gray et al, 1973). But our figures were either solitary or parallel to each other. Especially, their parallel arrangement between phycobilisomes and lamellae was essentially not face-to-face in the same attached line, but lied between clusters of phycobilisomes and lamellae. Also, they were difficult to distinguish from other subunits dispersed in the nucleoplasm such as ribosomes, electron dense granules or particles. From our observations, it is clear that phycobilisomes are present in M. aeruginosa, as accessory pigments without light intensity, while in some they are absent (Gantt and Conti,1969), but they are difficult to find in free living samples (Lefort,l965). Gantt and Conti(1969) have claimed that the reason why phycobiliproteins have not been. more commonly observed is evaluated to be due to the difficulty in preserving them for electron microscopy, and disorders or chaos in free living studies. The gas vacuole of M. aeruginosa is one of the most well-documented cell organelles, assuming because of interest in their ability to regulate algal buoyancy according to physical conditions, light intensity, temperature, pH and pressure (Weathers et al, 1977). They were visible as reddish granules in many planktonic blue-green algae and possibly aided in shading the photosynthetic pigments (Porter and Jost,1976). The vacuoles in our findings were cylindrical or hexagonal with a round termination. They were mainly distributed in the distal portion, as clusters, or bundles, in the cytoplasm, but sometimes were located proximally (Fig.3). They ranged from 0.3 to 0.5µm in length and were about 0.1µm in diameter (Fig.4 and 7), similar to those documented by Jone and Jost(1970). The fine structure of other granules or inclusions such as cyanophycin granules, phosphate granules, glycogen granules, ribosomes, lipid deposits and poly-ß-hydroxybutyrate granules in M. aeruginosa were not seen to a significant degree. However, the ultrastructure of cyanophycin granules and phosphate granules were observed indistinctly after fixation with OsO_4. Cyanophycin granules have an irregular spheroidal form up to about 0.6 to 1.5µm in diameter, similar to Anabaena variabilis in vegetative cells (Leak, 1967). Phosphate granules also have a more irregularly structured shape, approximately 0.5pm in diameter. These electron-dense bodies are seen as large and slight staining structures, or metachromatic granules (Fig.4), similar to the bacterial findings of Jensen (1968). But our results, taken from a different environment, as regards the concentration of total phosphate, shows hardly any morphological differences between the two samples. After being cultured in the absence of phosphate, few filmentous alga have these granules, whereas, after the same period in the presence of phosphate, such granules are abundant (Talpasayi,1963). Without much replication, our results indicate that it is difficult to find morphological differences in these granules between natural and culture conditions. In general, a large number of small and dense particles were distributed in the nucleoplasm or centroplasm and near thylakoid lamellae of M. aeruginosa. Among them, ribosomes and glycogen granules were seen little, in fact, glycogen granules were more easily stained than the ribosomal particles (Fig. 6 and 7). Glycogen granules were distributed mainly along with

thylakoid membranes in the distal portion of cytoplasm (Fig.7), while both granules were mixed in the proximal region. Although it is difficult to evaluate the exact locale of these granules, it seems to be a fact that the ribosomal particles are more likely to be distributed near the nucleoplasm than the glycogen granules, and close to thylakoid lamellae. In conclusion, there were no remarkable morphological differences in natural M. aeruginosa have two different environmental habitats. M. aeruginosa have a number of inclusions, similar to those of cyanobacterial cells and Gram-negative bacteria, although their morphological characteristics have some difference. We conclude that the characteristics of the cell wall outer layer, cell division, polyhedral bodies and gas vacuoles may be a useful keys for blue-green algal taxonomy above genus level.

Acknowledgments. We acknowledge the critical reading of Dr. BS Kil, Wonkwang Univ., and Dr. J Chung, Kyoungpook Nat. Univ.

REFERENCES

Allen MM(1968) Ultrastructure of the cell wall and cell division of unicellular blue-green algae. J Batceriol 96:842-852

American Public Health Association(APHA)(1985) Standard Methods for the Examination of Water and Wastewater, 16th ed. Washington, D.C.1268pp.

Aune T,Berg K(1986) Use of freshly prepared rat hepatocytes to study toxicity of blooms of the blue-green algae Microcystis aeruginosa and Oscillatoria agardhii. J Toxicol Environ Health 19:325-336

Carr NG,Whitton BA(1982) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, 688p

Cohen-Bazire G,Pfemrig N,Kunisawa R(1965) The fine structure of green bacteria. J Cell Biol 22:207-225

Galey FD,Beasley VR,Carmicheal WW,Kleppe G,Hooser SB,Haschek WM(1987) Blue-green algae(Microcystis aeruginosa) hepatotoxicosis in dairy cows. Am J Vet Res 48:1415-1420

Gantt E,Conti SF(1969) Ultrastructure of blue-green algae. J Bacterial 97:1486-1493

Gantt E, Lipschultz CA(1972) Phycobilisomes of Porphyridium cruentum. I. Isolation. J Cell Biol 54:3 13-324

Golecki JR(1974) Zur feinstruktur der zellwand bei einigen cyanophyceen. Zbl Bakt., I Abt Orig A 228,189-192

Golecki JR(1977) Studies on ultrastructure and composition of cell walls of the cyano-bacterium Anacystis nidulans. Arch Microbial 114:35-41

Golecki JR,Heimich UR(1990) Ultrastructural and elcetron spectroscopic analyses of cyanobacteria and bacteria. J Micrscopy 162:147-154

Giovannoni SJ, Turner S,Olsen GJ,Barns S, Lane DJ, Pace NR(1988) Evolutionary relationships among cyanobacteria and green chloroplasts. J Bacteriol 170:3584-3592

Gray BH,Lipscgultz CA, Gantt E(1974) Phycobilisomes from a blue-green alga Nostoc species. J. Bacterial 116:471-478

Hascoet MC, Florentz M, Granger(1985) Biochemical aspects of enhanced biological phosphorus removal from wastewater. Wat Sci Tech 17:23-41

Healy FP(1982) Phosphate. The biology of cyanobacteria(ed. by Carr and Whitton). pp. 105-124. Blackwell Scientific Publiactions, Oxford

Jensen TE(1968) Electron microscopy of polyphosphate bodies in a blue-green alga, Nostoc pruniforme. Arch Mikrobiol 62:144-152

Jensen TE(1969) Fine structure of developing polyphosphate bodies in a blue-green alga, Plectonema boryanum. Arch Mikrobiol 67:328-338

Jones DD, Haug A, Jost M, Graber DR(1969) Ultrastructural and conformational changes in gas vacuole membranes isolated from Microcystis aeruginosa. Arch Biochem Biophys 135:296-303

Jones DD,Jost M(1970) Isolation and chemical characterization of gas vacuole membranes from Microcystis aeruginosa Kütz. emend. Elenkin. Arch Mikrobiol 70:43-64

Leak LV(1967) Fine structure of the mucilaginous sheath of Anabaena sp. J Ultrastr Res 21:61-74

Lounatmaa K, Vaara T, Osterlund, Vaara M(1980) Ultrastructure of the cell wall of a Synechocystis strain. Can J Microbial. 26:204-208

Mehta RS, Hawby KW(1979) Effects of Simazine on the blue-green alga Anacystis nidulans. Buli Environ Contam Toxicol 23:319-326

Murray RGE,Steed P,Elson HE(1965) The localization of mucopeptide in sectiones of the cell wall of Escherichia coli and other gram negative bacteria. Can J Microbial 11:547-560

Porter J,Jost M(1976) Physiological effects of the presence and absence of gas vacuoles in the blue-green alga, Microcystis aeruginosa Kütz. emend. Elenkin. Arch Microbial 110:225-231

Streichan M, Golecki JR, Schon G(1990) Polyphosphate-acculmulating bacteria from sewage plants with different processes for biological phosphorus removal. FEMS Microbial Ecol 73:113-124

Talpasayi ERS(1963) Polyphosphate containing particles of blue-green algae. Cytologia 28:76-80

Utkilen H,Gjolme N(1992) Toxin production by Microcystis aeruginosa as a function of light in continous cultures and its ecological significance. Appl Environ Micobiol 58:1321-1325

Weathers PJ,Jost M,Lamport DTA(1977) The gas vacuole membrane of Microcystis aeruginosa - A partial amino acid sequence. Arch Biochem Biophys 178:226-244

Weise G,Drews G,Jann B,Jann K(1970) Identification and analysis of a lipopolysaccharide in cell walls of the blue-green alga, Anacystis nidulans. Arch Mikrobiol 71:89-98

Wicks K,Thiel PG(1990) Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium Microcystis aeruginosa in a hypertrophic African reservoir. Environ Sci Tech 24:1413-1418

Wildon DC, Mercer FV(1963) The ultrastructure of heterocysts and akinete of the blue-green algae. Arch Mikrobiol 47: 19-31

Wolks CP(1973) Physiology and cytological chemistry of blue-green algae. Bacterial Rev 37:32-101, 1973