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## Isolation and Abundance of Unicellular Cyanobacteria From Mosquito Development Sites

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**Abstract:**The genus composition of cyanobacteria (particularly *Chroococcus* sp.) and their abundance in mosquito breeding sites at Lake Mogan (Ankara, Turkey) increased from August to September. Six unicellular cyanobacteria that had different morphological properties were isolated and successfully cultivated. According to the mode of division, these six strain were classified as the representatives of subgroup 1 (order Choroococcales). One of the isolates was identified as *Synechococcus* sp. and one as *Gloeocapsa* sp., and four were identified as *Synechocystis* sp. One of the four *Synechocystis* spp. was shown to be capable of heterotrophic growth.

**Key Words:** Cyanobacteria, Isolation, Identification, Mosquito Breeding Sites.

### Sivrisinek Beslenme Alanlarındaki Tek Hücreli Cyanobacter'lerin Yoğunluğu ve İzolasyonları

**Özet:** Mogan Gölü (Ankara-Türkiye) sivrisinek beslenme alanlarındaki Cyanobacter cinsleri ve yoğunluklarının Ağustos-Eylül döneminde (özellikle *Chroococcus* sp. miktarının) arttığı belirlenmiştir. Alınan su örneklerinden altı adet tek hücreli Cyanobacter izole edilerek bunların kültürleri hazırlanmıştır. İzole edilen Cyanobacter'lerin bölünme şekillerine göre grup 1'e (Choroococcales), ve bunlardan birinin *Synechococcus*, birinin *Gloeocapsa* sp., ve dördünün de *Synechocystis* sp. oldukları saptanmıştır. İzole edilen *Synechocystis* suşlarından sadece birisinin heterotrofik gelişme özelliği ile diğerlerinden ayrıldığı da yapılan çalışmalar sonucunda belirlenmiştir.

**Anahtar Sözcükler:** Cyanobacteria, İzolasyon, Teşhis, Sivrisinek Beslenme Alanı.

### Introduction

Cyanobacteria serve as food for mosquito larvae, vectors of major tropical diseases. These photosynthetic prokaryotes are widely distributed in mosquito habitats and have been found in the guts of mosquito larvae. To determine species composition, abundance and distribution, cyanobacterial genera have been studied in various mosquito breeding sources (1, 2).

Lake Mogan is an important source for mosquito larval growth. Cyanobacteria abundance and distribution in this larval feeding area play an important role in the biological control of mosquito larvae.

This study reports the isolation of unicellular cyanobacteria from mosquito breeding sites at Mogan Lake (Ankara, TURKEY). Furthermore, the genus composition and abundance of cyanobacteria in this habitat were observed over the course of several months. To the best of our knowledge there are no previous reports on the isolation and cultivation of Cyanobacteria in the lakes of Turkey.

### Materials and Methods

*Sampling.* For isolation of cyanobacteria, samples were collected from three different mosquito breeding sites located along the Lake Mogan coasts. Water samples (ca. 1.5 l) were collected in April, May, June, July, August and September, 1994. The samples were stored in sterile plastic flasks and kept in the dark for about 24 h at ambient temperature.

*Cyanobacterial counting and identification.* Cyanobacteria in each sample were counted as described by Lund et al. (3). In addition, the samples (1000 ml) were concentrated by filtration through Whatman filter paper (GF/A). Identification of the cyanobacteria observed was performed according to the keys of Geitler (4) and Prescott (5).

*Isolation and cultivation of cyanobacteria.* The samples collected from the filter paper were diluted and plated onto plates of BG 11 (6), B 12, CB (7) and BG 13 (8) media solidified with 1% Bacto-Agar (Difco). In addition, each sample was inoculated to BG 11, B 12, CB and BG 13 liquid media. All cultures were incubated under continuous light (500 lux) at room temperature. Two weeks later, following the growth of colonies on the agar media, the colonies were removed with Pasteur micropipettes (6). The contents of the Pasteur micropipettes were gently blown into liquid medium. These liquid media were incubated under 500 lux for 7 to 15 days. After the colonies had grown in these liquid media, cultures were diluted and poured onto the agar media. One day later, single cells were removed with a Pasteur micropipette using a light microscope and in a laminar airflow. The single cells were again grown in liquid media. Liquid cultures that were prepared from the filter papers were diluted and the cyanobacteria were isolated using the same protocol. The unicellular cyanobacteria brought into culture were identified as described by Rippka (9) and Holt et al. (10). The efforts to obtain axenic cyanobacteria were carried out by solid media with agarose (7), and the addition of antibiotics (polimiksin B sulfate (2 mg/ml), rifampisin (1 mg/ml), streptomycin (2 mg/ml), chloromphenicol (2 mg/ml), ampicillin (2 mg/ml) to culture medium. As a control of purity, BG 11 liquid medium supplemented with 0.5% (w/v) glucose and 0.02% (w/v) casamino acids and nutrient broth were used (6). The presence of contaminating bacteria was detected by phase contrast microscopic examination, particularly of old cultures.

### Results and Discussion

The genera observed during the study period and their respective numbers are reported in table 1. Cyanobacteria were not observed in April and May. These months are thus not included

in table 1. Cyanobacterial abundance increased during August and September, *Chroococcus* sp. being the most numerous. It was also observed that the abundance of mosquito larvae was higher in the samples of the latter two months.

Representatives of the cyanobacterial genera, *Chroococcus*, *Merismopedia*, *Gomphosphaeria* and *Microcystis* were found in all of the samples (except April and May) that were concentrated by filtering. Their lack in some samples can be attributed to insufficient numbers in the unconcentrated samples used for the counting procedure (Table 1). For the isolation and cultivation of the cyanobacteria from these samples, four different media were tested. It was observed that isolated colonies from each medium grew best in BG 11 medium, supplemented with 1% soil extract. Consequently, six different unicellular cyanobacteria strains were isolated on the latter medium. They were purified from bacteria and fungi contamination in the lake water. Despite several trials by means of mechanical and antibiotic treatments, some parasite bacteria could not be removed from the cultures. These bacteria seem to live on compounds excreted from the cyanobacterial cells, since they did not grow in complex media, but grow well in developing cyanobacterial cultures.

Table 1. Cyanobacterial genera and their abundance in Lake Mogan (cells or filaments/cm<sup>3</sup>)

Months	6.1994			7.1994			8.1994			9.1994		
	I	II	III	I	II	III	I	II	III	I	II	III
<i>Chroococcus</i> sp.	-	-	-	-	-	-	158,4	211,2	-	88,9	12,0	-
<i>Merismopedia</i> sp.	-	-	-	-	-	-	28,8	206,4	-	2,4	-	-
<i>Gomphosphaeria</i> sp.	-	-	-	-	-	-	16,8	55,2	2,4	-	-	-
<i>Microcystis</i> sp.	-	-	-	-	-	-	7,2	-	2,4	2,4	4,8	-
<i>Oscillatoria</i> sp.	-	19,2	9,6	-	-	2,4	-	9,6	33,6	21,6	96,1	2,4
<i>Lyngbya</i> sp.	-	-	-	-	-	-	2,4	2,4	7,2	-	-	-
<i>Phormidium</i> sp.	-	-	-	-	-	-	4,8	33,6	2,4	-	-	-
Total number	-	19,2	9,6	-	-	2,4	218,4	518,4	48,0	115,3	112,9	2,4

Side cultures of these isolates were prepared according to Allen and Stanier (11). Growing cells were observed in the light microscope with phase contrast objectives and photographed. The mode of division of the six isolates examined in slide cultures showed that all reproduced by binary fission, typical of subgroup 1 (=order Chroococcales) as described by Rippka (9) and Holt et al. (10).

One of the unicellular cyanobacteria, identified as *Synechococcus* sp. (No.1), showed the following properties: division in only one plane; cells rod shaped; sheath layers absent and cells

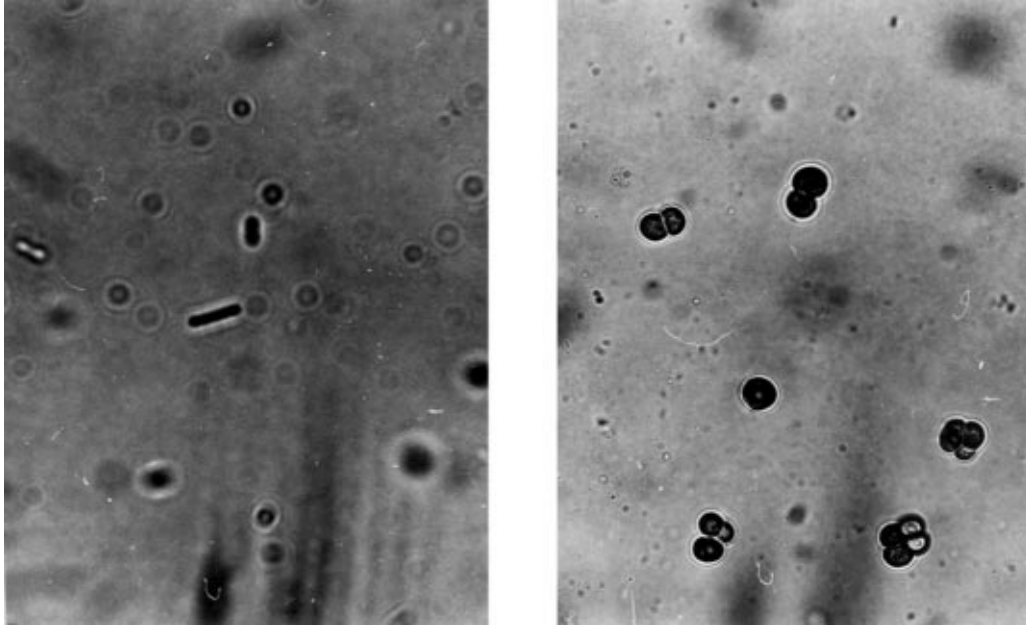


Figure 1. (a) *Synechococcus* sp. x500, (b) *Gloeocapsa* sp. x200.

3-15x1.5  $\mu\text{m}$  in diameter (Fig. 1a), while another isolate, identified as *Gloeocapsa* sp. (No.2), revealed the corresponding properties; division in 1-2 planes; cell shape spherical to hemispherical; sheath layers present; cells 3.7-11.2  $\mu\text{m}$  in diameter (Fig. 1b). The remaining isolates, identified as *Synechocystis* sp. (nos. 3, 4, 5 and 6), showed the following properties; division in 1-2-3 planes; cells coccoid or spherical; sheath layers absent; cell diameter 3.7-15  $\mu\text{m}$ , 1.7-3.5  $\mu\text{m}$ , 2.8-7.5  $\mu\text{m}$ , 2.8-7.5  $\mu\text{m}$  respectively. Division in 1, 2 and 3 planes of *Synechocystis* sp. no. 3 is shown in figure 2.

Although *Synechocystis* nos. 5 and 6 are similar in morphology, only strain no. 6 grew heterotrophically in the dark in BG 11 medium supplemented with glucose and casamino acid. Astier et al. (12) reported heterotrophic growth of mutant *Synechocystis* strain 6803 in liquid cultures. It has been shown to be capable of heterotrophic growth dependent solely on exogenous reduced organic compounds (2% glucose) as a source of both carbon and energy. In our study, it was shown that heterotrophic *Synechocystis* strain No 6 is found naturally in aquatic ecosystems.

The heterotrophic bacteria *Bacillus thuringiensis* var. *israelensis*, *Bacillus sphaericus*, which possess specific toxicity to larval mosquitoes, were used commercially in the combat against mosquitos in aquatic environments (13). However, the high larvicidal activity of these bacteria was only effective over a short period under field conditions. Much effort is being expended

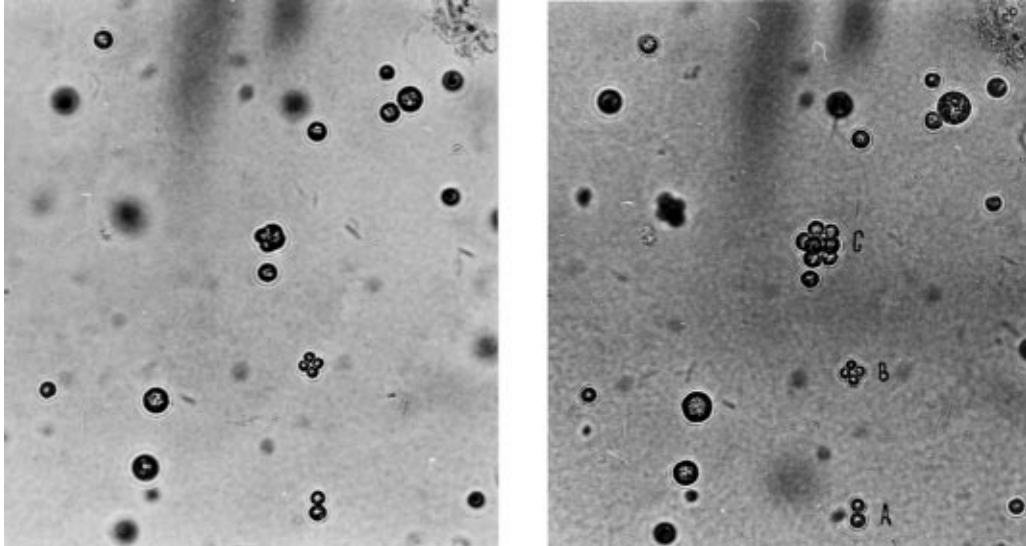


Figure 2.a. *Synechococcus* sp. No 3 in slide culture (a) time zero; (b) after 24 hours division of incubation. (A: Division in one plane. B: Division in two plans. C: Division in three plans)x200.

around the world to increase persistence of these bacteria in habitats of mosquito larvae (14). One approach is to express its cloned genes into unicellular cyanobacteria which could multiply in nature (15, 16). The study reported here was initiated to identify such species having an ecological potential for carrying larvicidal genes.

### Acknowledgements

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