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The Contribution of Capsulated Bacteria to the Total Bacterial Community in the Water Column of the Northern Marmara Sea, Küçükçekmece Lagoon and Strait of Istanbul, Turkey

Kuzey Marmara Deniz'i, Küçükçekmece Lagünü ve İstanbul Boğazı Deniz Suyu Örneklerinde Kapsüllü Bakterinin Toplam Bakteri Sayısına Oranı

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Abstract

The contribution of capsule-bearing bacteria to the total number of bacterioplankton community was enumerated to assume the metabolically active number of bacterioplankton in the eight different water masses of the Marmara Sea, Kucukcekmece lagoon and Istanbul strait. Capsulated and non-capsulated bacteria were determined using modified the negative staining technique. Total bacteria abundance and capsulated bacteria number was compared according to sampling area. As a result capsulated bacteria number was found higher at the surface water of eutrophic lagoon to the total bacteria (25.8 %) as compared to the water column of the Northern Marmara Sea and Istanbul Strait. Capsulated bacteria 5-20 meter, was found less than lagoon samples. The surface water of lagoon percentage of capsulated bacteria was significantly higher ($P<0.001$) than all areas. It was observed that there was no statistically significant difference between the dates of sampling in respect to contribution of the

number of the samples, which were taken from water above the sandy sediments of the Marmara Sea and deep from capsulated bacteria of the bacterioplankton community.

Keywords: Marmara Sea, Istanbul Strait, Lagoon, Capsulated Bacteria, Bacterial Community

Introduction

In the aquatic environments, metabolically active bacteria number can be assumed with the studies related to the contribution of capsulated bacteria to the total bacterial community. Bacterioplankton are the central mediators of the transformation of dissolved organic carbon into biomass and carbon dioxide (Azam and Cho, 1987). Bacterioplankton abundance and productivity remain fairly constant over a wide range of aquatic systems (Cole et al., 1988; Ducklow and Carlson, 1992). Bacterioplankton represent the largest living surface in the world oceans and might exceed phytoplankton biomass even in the euphotic zone of oligotrophic regions (Cho and Azam 1990; Fuhrman and Noble 1995; Herndl, 1991).

There are basic methodological problems related to the determination of the number of bacteria actually active especially in oligotrophic waters (Zweifel and Hagström, 1995). Standard enumeration techniques for bacteria using epifluorescence microscopy and acridine orange or DAPI (4, 6- diamino-2-phenylindole) staining (Hobbie et al., 1977; Porter and Feig, 1980) however, do not distinguish between metabolically active and dormant (Heisenberger et al., 1996) or even "ghost" cells, which lack DNA completely (Zweifel and Hagström 1995., Choi et al.,Vosjan and Noort, 1998). According to the most frequently applied techniques to enumerate the metabolically active bacteria, there is some consensus that, at a given time, a large fraction (up to 80 %) of the bacterial community is inactive (Hoppe 1978; Tabor and Neihof 1982; Grosssman 1994; Karner and Fuhrman, 1997).

Research on the bacterial capsule is almost exclusively centered around pathogenic bacteria because it has been well recognized that the capsule plays an important role in protecting the cell from a variety of potentially harmful substances. From studies from

medical microbiology, we know that the capsules of bacteria, such as Esherichia coli or Salmonella species are mostly composed of highly hydrated polysaccharides (Bayer and Bayer, 1981).

Heissenberger et al., (1996) hypothesized that only bacteria with intact internal structures exhibit a pronounced capsular envelope while obvious internal destruction, indicative for a nonactive state of the cell is accompanied by a less developed or even a missing capsular envelope. Almost all intact or active bacterioplankton are surrounded by a more or less dense polysaccharide capsule fixed to the outer cell wall of bacteria that is constantly renewed (Heissenberger et al., 1996). Potentially active bacteria have a well-developed capsule whereas inactive bacteria rapidly release the capsule. Active bacteria are constantly renewing their capsular envelope and releasing a significant fraction of the polysaccharide layer into the ambient water. These layers were found to be remarkably resistant to further bacterial utilization (Stoderegger and Herndl, 1998).

In the study we investigated visualize capsulated bacteria ("capsulated bacteria" term is used as "capsule bearing bacteria") to assume the level of metabolically active bacteria in the water column. We enumerated the contribution capsulated bacteria to the total bacterial community during four months (February, May, August, November, 2002), for the first time in the water column of the Northern Marmara Sea, Kucukcekmece Lagoon and Istanbul Strait with an aim to assume the potentially active bacteria number.

Material and Methods

Study area and sampling: Water samples were taken eight stations from different parts of the Northern Marmara Sea and Istanbul Strait during the four months of February, May, August, and November (2002). The samples were collected from Ambarli Harbour, Kucukcekmece Lagoon, Menekse seashore, Yesilkoy seashore, Zeytinburnu fishermen shelter and Istanbul strait.

Water samples were taken with Nansen bottles close the surface and at 5-20 meters to determine the contribution of capsulated bacteria to the bacterioplankton community. 20 ml water samples

were fixed during cruise in the Marmara Sea with % 2 glutaraldehyde (Final conc.)

Discrimination of capsulated and non-capsulated bacteria: To discriminate capsulated from non-capsulated bacteria used principally the same method as Stoderegger and Herndl (2001) modified of Plante & Shriver (1998) staining method.

1. Gelatin coated slides (0.1% gelatin solution and 0.01% CrK(SO4)2. 12H2O) were prepared and stored frozen until use.

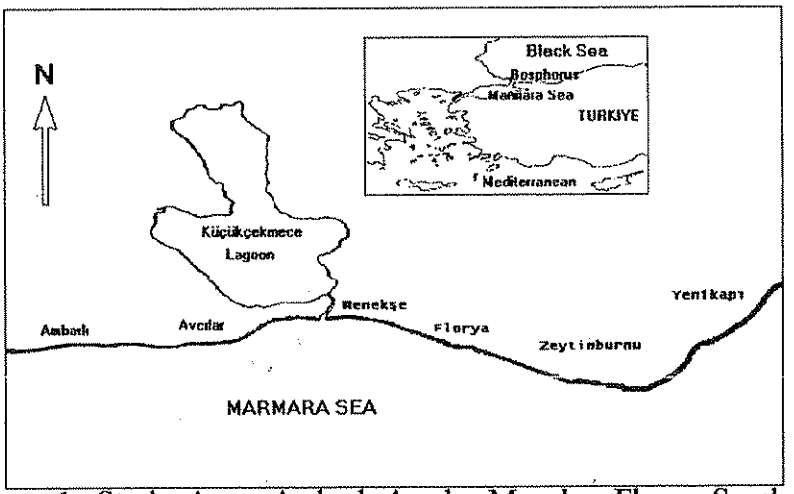

Figure 1. Study Area, Ambarlı-Avcılar-Menekse-Florya Seashore, (Northen Marmara Sea, Turkey)

2. Each sample was fixed with 2% glutaraldehyde (final conc.) and filtered onto 0.2 µm polycarbonate filter.

3. The filter was transferred on the gelatin-coated slide and frozen in a horizontal position. It was stored at -20 °C until analysis.

4. Before enumeration, the filter was thawed. The filter area was coated firstly with 0.25 % Congo red (3 to 5 drops) and later with Maneval's stain (three to four drops about 1 min.).

5. The slide was examined under a phase contrast microscope (Stoderegger and Herndl, 2001).

Enumeration of total bacteria: To enumerate the total bacterial abundance on 5 ml samples they were stained with acridine orange (Hobbie et al., 1977) and filtered onto black polycarbonate filters (0.2μm pore size, Millipore) and examined with an epifluorescence microscope at 1250x magnification. At least 300 bacteria per filter were counted. The Kruskal- Wallis non-parametric Anova test was used for statistic evaluations.

Results and Discussion

Contribution of capsulated bacteria to the total number of bacteria in the different water body, which were taken from eight stations, (Ambarlı Harbour, Kucukcekmece Lagoon, Menekse Seashore, Yesilkoy Seashore, Zeytinburnu Fishermen Shelter and Istanbul Strait) was summarized within Table 1.

Table 1. Percentage of capsulated bacteria in different water body averaged four months (February, May, August, and November 2002) and depths layers

Station	Sample Type	Capsulated Bacteria	Number of samples
1.	Water above sandy sediment (10 m)	11.5±2.3	14
2.	Surface (0-2 m)	24.4±1.4	13
3.	Water above sandy sediment (10 m)	14.7±2.7	18
4.	Surface (0-2 m)	13.8±1.7	21
5.	Surface (0-2 m)	20.4±2.4	24
6.	5 m	12.2±3.1	12
7.	5 m	14.2±2.3	10
8.	20 m	10.4±1.9	11

Stations: 1.Ambarlı Harbour, 2.Lagoon, 3.Menekse, 4.Yesilkoy 5.Fishermen shelter 6.Yenikapı Harbour 7.Bosphorus (I) 8.Bosphorus (II)

The contribution of capsule-bearing bacteria to the total number of bacteria was determined to be the lowest in the Harbour (I) from water above the sandy sediment (10 meters) as a minimum of 12.2 %, and highest in the lagoon samples from surface (0-2 meter) with a maximum of 25.8 %. The percentage of capsulated bacteria was found higher in the samples that were taken from the surface (0-2 meters) than other samples that were taken deeper. The samples which were taken from 5 meters Bosphorus (I) the percentage of capsulated bacteria number was detected as maximum 16.5 %.

However, the samples that were taken from 20 meters in the Bosphorus (II) the percentage of capsulated bacteria number was detected as a maximum at 12.3.

In the surface water of the lagoon the percentage of capsulated bacteria number was detected significantly higher ($P<0.001$) than all areas. There have no significant differences ($P>0.05$) in contribution of capsulated bacteria to the bacterioplankton community among the various sampling periods.

In our study we investigated, the contribution of capsule-bearing bacteria to the total bacterioplankton community to assume the level of metabolically active bacteria in the water column of the Northern Marmara Sea, Kucukcekmece lagoon and Istanbul Strait.

Production of the capsular envelope is related to specific environmental conditions. Although some specific reactions for the biosynthesis of specific exopolymers are well known and frequently used in biotechnology, the knowledge on the factors regulating capsule exopolysaccharide formation is limited (Heissenberger et al., 1996).

The bacterial capsule has many functions like adsorption of nutrients or protection against predators. Furthermore, it plays an important role in the biofilm formation on surfaces (Costerton et al., 1987; Decho, 1990). Almost all-intact or metabolically active bacterioplankton are surrounded by polysaccharide capsule fixed to the outer cell wall of bacteria that is constantly renewed (Heissenberger et al., 1996; Stoderegger and Herndl, 1998). Stoderegger and Herndl (2001) reported that a higher percentage of capsulated bacteria was found in areas with generally higher nutrient concentrations.

The Sea of Marmara, an inner sea, is under the influence of biological pollution due to the fact that the inland is heavily populated with respect to dwelling, industrial activity, and marine transportation. In this area, there are limited local bacteriological studies, related to the only bacterial pollution (Altuğ and Güler, 2002).

The Kucukcekmece lagoon, which is connected to the Marmara Sea via a 2 kilometre long strait, is in a state of eutrification due to the decrease of sources of fresh water as well as increase of the deposits of waste products of dwellings and industrial

establishments. The Istanbul Strait is under the influence of the waste products of dwellings and naval transportation.

It was observed that there was no statistically significant difference ($P>0.05$) between the date of sampling in respect to the contribution of capsulated bacteria to the bacterioplankton community. However, in the surface water of the lagoon the percentage of capsulated bacteria was significantly higher ($P<0.001$) than all areas. Except from lagoon, no specific trends in the distribution of capsulated bacteria in the water column were detectable for the other stations. This situation can be explained as different effects of environmental factors. It is known that there are many environmental factors which affect microorganisms' density and activities (Gaman and Sherrington, 1981; Jay, 1986; Jacob, 1989). Nutrient concentrations were found related to percentage of capsulated bacteria in aquatic environments (Stoderegger and Herndl, 1998). Due to this situation it can be considered that it is probable that there are higher nutrient concentrations in the surface water of Kucukcekmece lagoon. These areas must be observed and protected for ecological purpose. Analytical controls will be able to lead to rational and reasonable utilization of natural resources.

In the summary the contribution of capsule-bearing bacteria to the total number of bacteria was determined in the water column of the Northern Marmara Sea, Kucukcekmece lagoon and Istanbul Strait. The contribution of capsulated bacteria to the total bacterioplankton was shown as a higher value in the surface water of the Kucukcekmece lagoon. But no specific trends in the distribution of capsulated bacteria in the water above sandy sediment and water body was detectable for the Northern Marmara Sea and Istanbul strait

Özet

Kuzey Marmara Denizi kıyısal alanı, Küçükçekmece Lagün'ü ve İstanbul Boğazı'nda seçilen sekiz ayrı istasyonda, deniz suyu örneklerinde, metabolik olarak aktif bakteri sayısını belirlemek amacı ile, modifiye negatif boyama tekniği kullanılarak, kapsüllü bakteri miktarının toplam bakteri sayısına oranı araştırıldı. Sonuç olarak, ötrofikasyonda olan Küçükçekmece lagün'ü yüzey suyu örneklerinde, kapsüllü bakteri sayısının toplam bakteri sayısına oranı İstanbul Boğaz'ı ve Marmara denizinden alınan örneklere göre daha yüksek (% 25.8) olarak bulundu. Marmara denizi kumlu sediment üstünden ve 5-20 metre derinlikten

alınan deniz suyu örneklerinde kapsüllü bakteri sayısı Küçükçekmece lagün'ü yüzey suyu örneklerinden daha az bulundu. Lagün yüzey suyu örneklerinde bulunan kapsüllü bakteri oranı diğer örnekleme alanlarına göre önemli derecede ($P<0.001$) yüksek bulunurken, örnekleme tarihlerine göre kapsüllü bakteri oranı dağılımında önemli bir farklılık görülmedi.

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