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Investigation of epilithic biofilms in the River Danube

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INTRODUCTION

The assemblage of organisms which colonize and grow on gravels, pebbles and rocks in natural waters is defined as epilithic biofilm. The metabolic activity of attached populations continues to be an area of intense interest. The organisms in association with surfaces have an elevated metabolic activity compared to planktonic bacterial populations, for this reason these populations may be the most important in aquatic enviroments, responsible for nutrient cycling, corrosion processes and degradation of xenobiotics. The epilithon microbial communities that develop on gravels or pebbles in riverbeds significantly contribute to changes in the chemical-biological quality of water that penetrate through the sediment. The water quality of the bank wall filtered wells is influenced by this biological filter layer of the riverbed. Among algae the diatoms are found to be dominant groups of the epilition communities of the River Danube and in the summer-period in smaller quantities Chlorophyta and Cyanobacteria are also found (Ács & Kiss 1991, 1993). The algae as producer organisms play role in the cycling of elements and the energy flow in water ecosystems and one has to realize that the surfaces of them serve also as habitats for bacteria.

MATERIAL AND METHODS

Study site: The study site chosen was at the north-eastern part of Szentendre Island in the River Danube main arm, situated North of Budapest towards the Danube Bend. On the island there are rows of bank wall filtered wells, which supply with drinking water the capital of Hungary. The trophic level of the Danube at the reach of Szentendre Island is eutrophic - hypertrophic during the growing season (Kiss 1994).

Methods: Samplings were made on 30.09.94, 03.07.95 and 08.06.96. Water temperature and water discharge fluctuated between 17,2-19,9 ^{0} C and 1320-3958 $m^3 s^{-1}$. The gravels or pebbles were taken out from 80-100 cm depth with a grab sampler and collected with pincers into autoclaved tap water. The biofilm coat of appr. 10 cm^2 gravel surface was scrubbed with a sterilized fine brush, and collected in 25 ml of autoclaved algal nutrient broth. The samples were cooled and transported into the laboratory and used for further microscopic studies. At the third time the collected samples were plated onto three different diatom media (Beakes,

Canter and Jaworski diatom medium [B type], Allan's modification of Huges, Gorham and Zehnder's medium [N type], Schlösser's modification "Bacillariophyceen-medium" [C type]), (Thompson & Rhodes & Pettman 1988, Schlösser 1986, Droop 1969). After a month of incubation diatom colonies were isolated and also diatom cultures were used as samples for diatom-associated bacterial isolation. Strains were isolated and purified by the use of nutrient and Yeastrel agars (Cowan & Steel 1974). Representative strains were selected on the basis of cultural-morphological features, and were characterised subjected to detailed micromorphological and physiological investigations (e.g. Gram and spore staining, BIOLOG). The diatoms were examined by scanning electron microscope (SEM) and light microscope (LM) after H_2O_2 treatment. 500 biofilm and 100 cultivated diatom valves were counted under LM. The proportion of dead cells was calculated from fixed samples (3% formalin, 100 cells counted). The laboratory diatom cultures and original gravel samples from the River Danube were investigated by SEM, too (Makk & Ács 1996).

RESULTS AND DISCUSSION

The proportion of Centrales, dead cells and dead Centrales cells were highest in 1996 and lowest in 1994 (Fig. 1) in biofilms. The high number of dead cells indicate that the circumstances were harsh for the algae (e.g. low light intensity, rolling substrates).

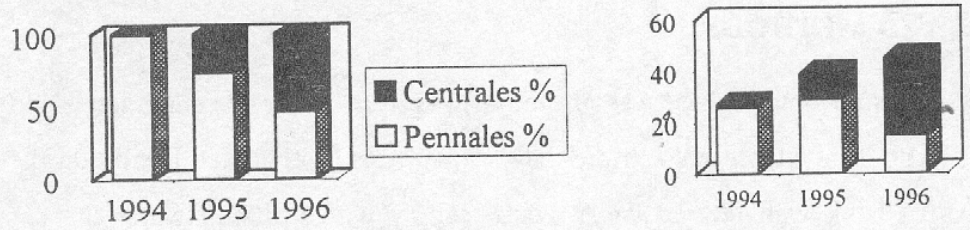

Fig. 1. The proportion of Centrales and Pennales species (left) and the proportion of dead cells (right) in biofilm samples

The diatom taxa of the epilithon biofilm of the Danube river indentified directly (1994, 1995, 1996) or after cultivation on diatom agars (B, C, N types) are listed in Table 1. The number of diatom species determined was 81 during this survey. C-and B type agars contained vitamins B_1 and B_{12}, too. On these substrates more diatom species can grow than on type N. It is not surprising that diatoms growing well in culture are not necessarily the dominant species as determined by microscopic investigations. For example *Amphora montana* growing well in

culture was found only in minor numbers in epilithon biofilms. This species is regarded as an aerophytic taxon (Krammer & Lange-Bertalot 1986).

Table 1. List of taxa found in three investigated years.

Achnanthes delicatula (Kütz.) Grun.*
A. lanceolata (Bréb.) Grun. **(B)**
A. minutissima Kütz.
A. ploenensis Hust.
Amphora inariensis Krammer
A. fogediana Krammer
A. libyca Ehr.
***A. montana* Krasske (N)**
A. ovalis (Kütz.) Kütz. **(C)**
A. pediculus (Kütz.) Grun. **(B,N)**
Asterionella formosa Hassall
Aulacoseira distans (Ehr.) Sim.
A. granulata (Ehr.) Sim.
A. granulata var. angustissima (O. Müller) Sim.
Caloneis bacillum (Grun.) Cleve
Cocconeis placentula Ehr. **(C)**
Cyclostephanos dubius (Fricke) Round
Cyclotella atomus Hust. **(B)**
C. cryptica Rein., Lew. et Guill **(B,C,N)**
C. glomerata Bachmann
C. meneghiniana Kütz. **(B,C,N)**
C. pseudostelligera Hust.
C. radiosa (Grun.) Lemmer.
Cymbella laevis Naegegeli
C. minuta Hilse
C. silesiaca Bleisch
C. sinuata Gregory
Diatoma tenuis Ag.
D. vulgaris Bory
Eunotia bilunaris (Ehr.) Mills
Fragilaria arcus (Ehr.) Cleve
F. brevistriata Grun.
F. capucina DeSmith **(C)**
F. capucina var. vaucheriae (Kütz.) Lange-Bert. **(B)**
F. pinnata Ehr.
F. ulna (Nitzsch) Lange-Bert. **(B,C)**
F. ulna var. acus (Kütz.) Lange-Bert.
Gomphonema olivaceum (Hornemann) Bréb.
G. parvulum (Kütz.) Kütz. **(B,C,N)**
Gyrosigma acuminatum (Kütz.) Raben.
Melosira varians Ag. **(B,C,N)**
Navicula accomoda Hust.* **(C,N)**
N. atomus var. permitis (Hust.) Lange-Bert. (B)
N. capitata var. hungarica (Grun.) Ross
N. capitatoradiata Germain
N. cryptocephala Kütz. **(B)**
N. lanceolata (Ag.) Kütz **(B)**
N. menisculus Schumann **(B)**
N. minima Grun. **(B,C,N)**
N. radiosa Kütz.
N. rhynchocephala Kütz. **(B,C)**
N. saprophila Lange-Bert. **(B,C,N)**
N. subminuscula Manguin **(B,C,N)**
N. tripunctata (O.F. Müller) Bory **(B)**
N. veneta Kütz. **(B,C,N)**
Nitzschia acicularis (Kütz.) W. Smith
N. communis Rabenhorst **(B)**
N. dissipata (Kütz.) Grun..
N. fonticola Grunow **(B,C)**
N. fruticosa Hust. **(B,C,N)**
N. gracilis Hantzsch **(C)**
N. inconspicua Grun. **(B,N)**
N. levidensis (W.Smith) Grunow*
N. palea (Kütz.) W. Smith **(B,C,N)**
N. palea var. debilis (Kütz.) Grun. **(B,C,N)**
N. paleacea Grun.
N. perminuta (Grun.) M. Peragallo
N. sociabilis Hust. **(B)**
N. umbonata (Ehr.) Lange-Bert. (C)
Rhoicosphenia abbreviata (Ag.) Lange-Bert.
Skeletonema potamos (Weber) Hasle
Stephanodiscus delicatus Genkal
S. hantzschii Grun. **(C)**
S. invisitatus Hohn et Heller.
S. minutulus Grun. **(B)**
S. tenuis Hust.
Surirella ovalis Bréb.
Tabellaria fenestrata (Lyngb.) Kütz.
Thalassiosira pseudonana Hasle et Heimdal **(C)**
T. weissflogii (Grun.) Fry.et Hasle

Abbrevations: * = new data with respect to the attached algae of Hungarian part of the River Danube, **(B, C, N)** = types of media (see in text), italic = dominant (relative abundance at least 5%) in culture, boldface= new data for occurence of algae in Hungary, underlined = dominant in biofilm

We attempted to isolate diatom surface associated bacteria by isolating the bacterial "contaminants" of diatom cultures, too. Morphologically different bacteria (rods, spirillum-like, budding filamentous, etc.) can be seen on surfaces of cultivated diatoms and in original

samples. *Caulobacter spp.* e.g. appeared on surfaces of cultivated *Amphora pediculus* (Foto 1.). *Caulobacter* depending on life cycle stage may be polarly flagellated rods or possess a stalk and holdfast, by which they attach to solid substrata (algae) and may absorb nutrients released by their hosts. Caulobacters were also isolated together with 61 other strains. The two-third of them were Gram negative rods. Among Gram positive coryneforms were dominant.

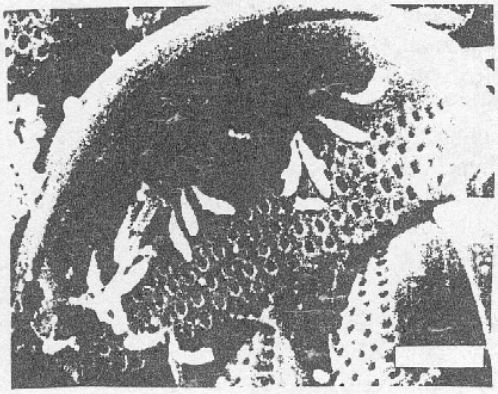

Foto 1. *Caulobacter spp.* are on the surfaces of cultivated *Amphora pediculus*. Bar=2 µm.

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