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Interaction between diatoms and bacteria in the biofilm of the River Danube

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Introduction

The microorganisms play a decisive role in the self-purification, i.e. the decomposition of organic materials and detoxification of water of rivers and lakes. The epilithon microbial communities that develop on gravels in riverbeds are metabolically highly active, and significantly contribute to changes in the chemical-biological quality of water that penetrate through the sediment. Accordingly the water quality of the bank wall filtered wells is influenced by this biological filter layer of the riverbed. The biofilm consists of bacteria, algae (where light levels permit), protozoa and fungi (Lock 1993). The bacteria of the biofilm are known to play important role in degradation of organic materials and transformation of inorganic components, and through this play a role in the trophic relation of the river ecosystem. The bacteria attached to surfaces are metabolically more active than planktonic bacteria (Lappin-Scott et al. 1992, Morikawa 1988). Among algae the diatoms are found to be dominant groups of the epilition communities of the Danube river and in the summer-period in smaller quantities Chlorophyta and Cyanobacteria are also found (Ács & Kiss 1991a, b, 1993a). 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 as habitats for bacteria. Adhesion by cell surfaces plays a considerable role in many biological processes: interaction between pathogenic bacteria and various target cells, rhizobia on plant root cells, *Azospirillum spp.* on corn roots. These involves highly specialized mechanisms of recognition mediated by adhesins (carbohydrate-binding protein) and specific receptors on the cell surfaces (Old 1988, Fatton & Shilo 1984). This adhesion is also important when an empty substratum is colonized by the algae (Ács & Kiss 1993b). Earlier studies of have shown bacteria associated with algae: specific bacterial attachment sites have been found on the chain-forming diatom *Navicula confervacea* Kütz. (Rosowski 1992) and on heterocysts of *Anabeana spp.* (Marshall 1989).

Material and methods

Study site: The study site chosen was at the North-Eastern part of Szentendre Island in the Danube river main arm. Szentendre Island is situated in the North of Budapest towards The Danube Bend. On the island there are rows of the bank wall filtered wells, which supply with drinking water the capital. Water temperature and water discharge fluctuated between 16.0-17.2 °C and 3880-5234 m^3 sec^{-1}. The trophic level of the Danube at the reach of Szentendre Island is eutrophic - hypertrophic during the growing season (Bartalis et al. 1984, 1985, 1987a, b, Dvihally et al. 1984, Kiss 1984, 1994).

Methods: Artificial substrata were submerged in the river water on June 6, June 21, 1995. Metal frames containing glass microscope slides were used as artificial substrata in five replicates. The slides were roughened with sandpaper, because microorganisms

can attach to the rough surfaces more easily. The frames were fixed with nails at 60-100 cm water depth in the riverbed. The artificial substrata were taken out of the river after 14-15 days of incubation. The biofilm from the surface of slides was scrubbed with a sterilized fine brush, and collected in 25 ml of autoclaved algal nutrient broth. Gravels were also collected with a sediment grab sampler and placed into autoclaved water till sample preparation. The samples were transferred to laboratory in cooling bag. The collected samples were plated on two different diatom media (Beakes, Canter and Jaworski diatom medium, Allan's modification of Huges, Gorham and Zehnder's medium, in Droop 1968). After 2-4 weeks of growth brown colonies were isolated and purified. Bacterial strains were isolated from surfaces of the cultivated diatoms by the use of nutrient agar. The biofilm diatoms were examined by electron microscopy after shattering with 30% H_2O_2 solution (in water bath, 100 °C, 2 hours). The laboratory cultures and original gravel samples from the Danube river were investigated by scanning electron microscope (SEM) too. The samples for the SEM investigations were prepared by a chemical procedure and by liofilization method. Samples were usually immediately fixed in 5% glutaraldehyde, postfixed in 1,5% OsO_4 in phosphate buffer, dehydrated in acetone or ethanol dilution series (30, 50, 70, 80, 90% once, and 100% twice). They were critical point dried with liquid CO_2 after being infiltrated with amyl-acetate, coated with approximately 15 nm of gold and viewed with SEM. In other case samples were pre-frozen (-90 °C) in fridge, then freeze-dried (2x10-2 mbar, -60 °C). Bacteria isolated from surfaces of cultivated diatoms were investigated for their haemagglutination capacity. Serial two fold-dilutions of bacteria suspensions were made in PBS (25µl) in the wells of a round-bottomed microtitre plate. An equal volume of an erythrocyte suspension (human, guinea-pig, chicken (0.5-0.75%) was added and after mixing, the plate was incubated for 1 to 2 hours (Patrisk & Larkin 1993, Ainouz & Sampaio 1991). Agglutination has been scored.

Results and discussion

In Table 1. the taxa of algae are listed which were detectable, and were indentified from the biofilm of the River Danube by SEM and TEM (D), which were cultivated in algal nutrient broth (B) and on algal nutrient agar (A). Boldface typed taxa are new data with respect to the attached algae of the hungarian part of the River Danube.
SEM plays an integral and important role in studies of adherence, and exopolymer production of microorganisms inoculated on media or growing on surfaces. The results show that glutaraldehyde fixation and ethanol or acetone dehydratiflg stages of the sample preparation procedure can be responsible for the appearance of attachment "fibrils" and "fibrillar polymer" (Foto 1), which are considered to be artefacts due to shrinkage during dehydration (Gilmour et al. 1993). The liofilization can see considerable degree of collapse as compared with chemically fixed, dehydrated and critical point dried specimens, but the samples are exposed to a lower level of washing out (Foto 2, 3). On surfaces of the cultivated diatoms different bacteria can be seen with coccus, rod and filamental morphology (Foto 1, 2, 3). The haemagglutination test is the first step of investigation of specific interactions between different cells which is one of the simplest methods for determination of the adherence of algae or bacteria to carboydrates of erythrocytes. The 7 strains from the 11 ones were isolated from the surface of diatoms showed haemagglutinating activity with guinea-pig erythrocytes, but chicken and human 0 group erythrocytes were not agglutinated by bacteria strains. We suppose these fore, that polysaccharides and polysaccharide-containing molecules an important role in the microbial adhesion of bacteria onto diatoms.

Table 1. The list of identified taxa. Abbrevatoins: D=growth on artificial substrata, B=growth on algal nutrient broth, C=growth on algal nutrient agar

taxa	D	B	A
Achnanthes minutissima Kütz.	x		
Achnanthes ploenensis Hust.	x		
Amphora pediculus (Kütz.) Grun.	x	x	
Cocconeis placentula Ehr.	x	x	
Cyclotella menenchiniana Kütz.	x	x	x
Cyclotella pseudostelligera Hust.	x	x	
Cymatopleura solea (Bréb.) W. Smith	x		
Diatoma vulgaris Bory	x		
Flagilaria capucina var.vaucheriae (Kütz).Cange-Bert.	x		
Flagilaria ulna (Nitzsch) Lange-Bert.	x	x	
Gomphonema angustatum (Kütz.) Raben.	x		
Gomphonema angustum Ag.	x		
Gomphonema olivaceum (Hornemann) Bréb.	x		
Gomphonema parvulum (Kütz.) Kütz.	x		
Melosira varians Ag.	x	x	x
Navicula atomus (Kützing) Grunow. ?	x	x	x
Navicula capitata var. hungarica (Grun.) Ross	x		
Navicula capitoradiata Germain	x	x	
Navicula lanceolata (Ag.) Kütz.	x		
Navicula menisculus Schumann	x	x	x
Navicula minima Grunow	x	x	x
***Navicula seminulum* Grunow**	x		
Navicula subminuscula Manguin	x	x	x
***Navicula tenelloides* Hustedt. ?**	x	x	x
Navicula tripunctata (O.F. Müller) Bory	x	x	
Navicula veneta Kütz.	x	x	x
Nitzschia dissipata (Kütz.) Grun.	x	x	
Nitzschia fonticola Grun	x	x	
Nitzschia frustulum (Kütz.) Grun.	x	x	x
Nitzschia inconspicua Grun.	x	x	x
Nitzschia linearis (Ag) W. Smith.	x	x	x
Nitzschia palea (Kütz.) W. Smith	x	x	x
***Nitzschia sociabilis* Hust.**	x		
***Nitzschia vitrea* Norman**	x	x	x
Rhoicosphenia abbreviata (Ag.) Lange-Bert.	x		
***Stephanodiscus delicatus* Genkal**	x		
Stephanodiscus hantzschii Grun. *f. hantzschii*	x		
Stephanodiscus hantzschii Grun. *f. tenuis* (Hust.) Hak. at Stoer.	x		
Stephanodiscus macarove Genkal	x		
Stephanodiscus minutulus (Kütz.) Cleve & Möller	x		
Suriella ovalis Bréb.	x		

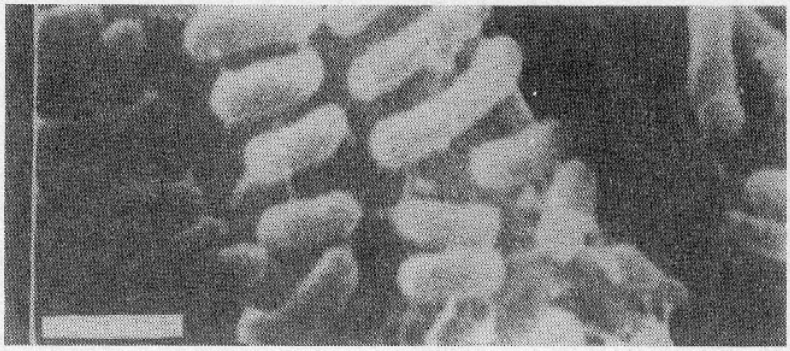

Foto. 1:Rods with extracellular fibers are attached to the surfaces of *Nitzschia spp.* , and ones also connected extracellular to each other. Glutaraldehyde fixation, OsO_4 post-fixation, acetone dehydration followed by critical point drying. Bar = 1,3µm, (magnification x 30.000)

Foto 2. Various types of bacteria associated with the mucous blanket of *Navicula atomus*. Freeze-dryed preparation. Bar = 2 µm. (magnification x 3000)

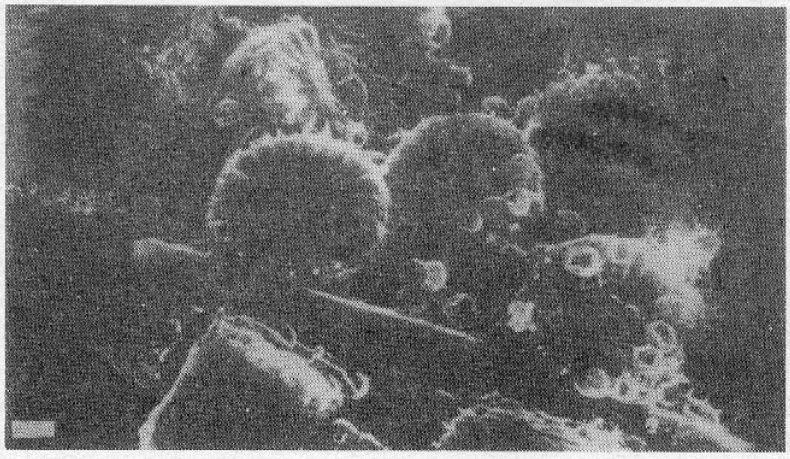

Foto 3: Various bacterial forms are on surfaces of *Cyclotella menenghiniana* and between *Nitzschia linearis* and *C. menenghiniana* in slime. Freeze-dryed preparation. Bar = 2µm. (magnification x 2000)

Summary

The biofilms were investigated on surfaces of artificial substrata (glass microscope slides) submerged into the River Danube at the NE part of Szentendre Island. The collected biofilm samples were plated on two different diatom media. Diatoms were isolated and identified by scanning electron microscope from biofilm samples. Bacterial strains were isolated from surfaces of cultivated diatoms. The laboratory cultures and original gravel samples of the River Danube were similary examined by scanning electron microscope in order to determine the characteristic partners in diatom-bacterial interactions. The studies have shown that bacteria with extracellular fibers attached to each other and to the diatoms. Specific (molecular level) interactions between bacteria and diatoms were also investigated.

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References

ÁCS, É., KISS, K. T. (1991a): Investigation of periphytic algae in the Danube at Göd. (1669 river km, Hungary) - Arch. Hydrobiol. Suppl. 89 - Algological Studies 62, 5-19. - ÁCS, É., KISS, K.T. (1991b): Neuere Methode zu den Untersuchungen des Donauperiphytons. - 29. Arbeitstagung der IAD, Kiew, september 1991. p. 37-40. - ÁCS, É., KISS, K.T. (1993a): Effects of the water discharge on periphyton abundance and diversity in a large river (River Danube, Hungary). - Hydrobiologia 249, 125-133. - ÁCS, É., KISS, K.T. (1993b): Colonization process of diatoms on artificial substrate in the River Danube near Budapest (Hungary). - Hydrobiologia 269/270, 307-315. - AINOUZ, I.L., SAMPAIO, A.H. (1991): Screening of brazilian marine algae for haemagglutinins. - Botanica Marina. 34, 211-214. - BARTALIS, É., DVIHALLY, ZS.T, KISS, K.T., SCHMIDT, A. (1984): Mit dem Sauerstoffgehalt zusammenhängende Untersuchungen in der mittleren Donau III. -24. Arbeitstagung der IAD, Szentendre/Ungarn 1984. p, 1-4. - BARTALIS, É.T., DVIHALLY, ZS.T., ERTL, M., KISS, K.T., SCHMIDT, A.(1985): Mit dem Sauerstoffgehalt zusammenhängende Untersuchungen in der mittleren Donau IV. - 25. Arbeitstagung der IAD. Bratislava. 1985. p, 117-120. - BARTALIS, É.T., DVIHALLY, ZS.T., ERTL, M., KISS, K.T., SCHMIDT, A., TOMAJKA, J. (1987a): Mit dem Sauerstoffgehalt zusammenhängende Untersuchungen in der Mittleren Donau V. /1985/. - 26. Arbeitstagung der IAD. Passau/Deutschland. 1987. p, 326-330. - BARTALIS, É.T., DVIHALLY, ZS.T., KISS, K.T., SCHMIDT, A., TOMAJKA, J. (1987b): Mit dem Sauerstoffgehalt zusammenhängende Untersuchungen in der mittleren Donau VI. /1986/. - 26. Arbeitstagung der IAD. Passau/Deutschland. 1987. p, 330-334. -DROOP,M.R. (1969): Algae. -In: NORRIS,J.R., RIBBONS,D.W. (eds): Methods in microbiology.-Academic Press. London and New York. pp. 269-308.- DVIHALLY, ZS.T., ERTL, M., KISS, K.T., SCHMIDT, A. (1984): Mit dem Sauerstoffgehalt zusammenhängende Untersuchungen in der mittleren Donau II. -24. Arbeitstagung der IAD, Szentendre/Ungarn. 1984. p, 9-12. - FATTON, A., SHILO, M. (1984): Hydrophobicity as an adhesion mechanism of benthic cyanobacteria. - Appl. Environ. Microbiol. pp. 153-143. - GILMOUR, A., WILSON, A.B., FRASER,

T.W. (1993): Microbial adherencia to food contact surfaces. - In: DENYER, S.,P., GORMAN, S.,P., SUSSMAN, M. (eds.): Microbial biofilms: formation and controll. - Blackwell Scientific Publications. LTD.Oxford. pp.109-131. - KISS, K.T. (1984): Phytoplanktonuntersuchungen in den Donauabschnitten oberhalb und unterhalb von Budapest im Jahre 1983. 24. - Arebeitstagung der IAD. Szentendre/Ungarn. 1984. p, 105-108. - KISS, K.T. (1994): Trophic level and eutrophication of the River Danube in Hungary. Verh.Internat.Verein.Limnol. 25, 1688-1691. - LAPPIN-SCOTT, H.M., COSTERTON, J.W., MARRIE, T.J. (1992): Biofilms and Biofouling. - Encyclopedia of Microbiology, 1, 277-284. - LOCK, M.A. (1993): Attached microbial communities in river. - In: FORD, T.E. (ed.): Aquatic Microbiology. - Blackwell Scientific Publications, I.n.c. Boston, pp. 113-138. - MARSHALL, K.C. (1989): Cyanobacterial heterotrophic bacterial interaction. - In: COHEN, Y., ROSENBERG, E. (eds.): Microbial mats. Physiological ecology of benthic microbial communities. - American Society For Microbiology, Washington pp. 239-245. - MORIKAWA, K. (1988): Differences in plating efficiency of bacteria from river epilition sampled from from upper and lower surfaces of artificial substrata. - Microb. ecol. 15, 217-228. - OLD, C.D. (1988): Bacterial cell envilopes in adhesion. - In: HANCOCK, I.C., POXTON, I.R. (eds.): Bacterial cell surfaces techniques. John Wiley Sons, Inc., New York pp. 227-240. - PATRISK, S., LARKIN, M.J. (1993): Attachment in disease. - In: DENYER, S.P., GORMAN, S.P., SUSSMAN, M. (eds.): Microbial biofilms: formation and controll. Blackwell Scientific Publications. LTD. Oxford pp. 109-131. - ROSOWSKI, J.R. (1992): Specificity of bacterial attachment sites on the filamentous diatom Navicula confervacea (Bacillariophyceae). - Can. J. Microbiol. 38, 676-686. - SUTHERLAND, I.W. (1983): Microbial exopolysaccharids their role in microbial adhesion in aqueos systems. - Crit. Rev. Microbiol. 10, 173-201.

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