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Cultivable bacterial composition and BIOLOG catabolic diversity of biofilm communities developed on *Phragmites australis*

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Abstract

Biofilm samples formed on submerged young and old stems of reed, *Phragmites australis* (Cav.) Trin ex Steudel were taken during summer at different sites of Lake Velencei, Hungary. BIOLOG GN microplates were used to analyze the patterns of sole carbon source utilizations by microbial communities. From the carbon sources, carbohydrates and amino acids were preferred by all microbial communities. In the case of the old reed stem samples, higher number of carbohydrates, carboxylic acids and polymers were used than in young samples. Biofilm bacterial communities from the old reed samples of the nature conservation area of the lake used the highest number of ($\geq$50% of the available) substrates. In principal component analysis (PCA), the metabolic potential of the microbial communities from the middle open water region of the lake showed the smallest variability. The variability within metabolic potential of the reed stem microbial communities from a given sampling site was the largest in the case of samples originating from the western, reed-covered nature conservation area. A total of 251 bacterial isolates obtained after serial dilutions and plating onto different media were characterized by traditional phenotypic tests. The strains showed high activities mainly in the hydrolysis of certain biopolymers (gelatine and casein). PCA was used to evaluate the phenotypic variability of strain groups of different sampling sites. The two open water regions were similar to each other, and separated from the western reed covered part of the lake. Similarly to the BIOLOG community-level physiological profiles, strain groups of the young and old reed stem samples originating from the nature conservation area had the largest metabolic potential. On the basis of 16S rDNA sequence analysis, 23 representative strains with different ARDRA patterns were identified. The cultivation-based investigations of bacterial diversity showed characteristic differences in the number of identified taxa in connection with the sampling sites. No characteristic differences could be observed according to medium or sample type (young, first year and more than 1-year old stems) among the identified species. 16S rDNA sequence comparisons resulted in the identification of the genera *Aureobacterium*, *Arthrobacter*, *Kocuria*, *Microbacterium*, *Micrococcus*, *Rhodococcus*, *Bacillus*, *Marinibacillus*, *Rhodobacter*, *Defluvibacter*, *Pseudomonas*, *Klebsiella*, *Serratia* and *Aeromonas.* The results of the cultivation-based and BIOLOG investigations revealed characteristic differences in the bacterial community composition and activities of the open water region and the reed covered nature conservation part of the lake.

Keywords: *Phragmites australis*; Biofilm; BIOLOG; 16S rDNA sequence analysis

1. Introduction

The common reed, *Phragmites australis* (Cav.) Trin ex Steudel is a cosmopolitan, highly resilient and very productive emergent macrophyte (Den Hartog et al., 1989). Great shallow soda lakes (e.g. Lake Fertő/Neusiedlersee and Lake Velencei) show the largest *Phragmites* dominance in the Carpathian-basin (Hungary). In these natural aquatic ecosystems, large scale reed die-back has been observed during the past decades (Ágoston-Szabó et al., 2006). A number of potential direct (e.g. mechanical damage by harvesting machines, grazing, wind-wave actions, water and sediment quality, water table fluctuation, parasites) and indirect causes (e.g. eutrophication in combination with stagnant water tables) were studied in connection with reed die-back (Ostendorp, 1989). Healthy and declining stands of reed rhizospheres of Lake Velencei could unambiguously be separated according to the species composition of cultivable aerobic chemo-organotrophic bacterial communities, as well (Micsinai et al., 2003). The results of the Most Probable Number (MPN) investigations demonstrated

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that in the reed rhizosphere germ counts of the obligate fermentative clostridia and anaerobic sulphate-reducing bacteria were about one order higher than in the sediment (Borsodi et al., 2003). Studying the cultivable alkaliphilic and alkalitolerant bacterial communities involved in the aerobic mineralization of autochthonous organic materials and associated with decomposing rhizomes of *Phragmites australis* in Lake Fertő confirmed that soda lakes can harbor microbes adapted to the special environment (Borsodi et al., 2005).

Reeds provide extensive surface for aquatic biofilms. The degradation of autochthonous and allochthonous organic matter may be affected by the composition and activity of biofilm communities associated with submerged parts of aquatic plants (Pollard et al., 1995). Following an early spring sampling, a complex algological–bacteriological study of biofilms developed on the submerged reed stem surfaces in Lake Velencei revealed a wide-scale degrading potential of the most frequent members of cultivable bacterial species, which might be correlated with the presence of the large amount of algal exudates and lysis products (Ács et al., 2003). The composition and the architecture of the biofilm are influenced by a number of factors, including several physico-chemical attributes of the surrounding environment (Ács et al., in press). Although the decomposition and mineralization of different compounds in nature is mainly determined by complex interactions within microbial communities, our knowledge of the naturally existing catabolic potential has mostly been obtained from defined mixed cultures or pure strains of bacteria (Stoodley et al., 2002).

The aim of the present study was to ascertain the catabolic and the community-level metabolic potential, as well as the possibly existing spatial heterogeneities of species composition of reed periphyton bacterial communities in Lake Velencei. Community-level physiological profiles of microbial consortia were studied by statistical analysis of BIOLOG sole carbon source utilization patterns. Cultivation on different selective media along with molecular sequence analysis of the 16S rRNA gene was applied to reveal the phylogenetic diversity of the cultivable fraction of aerobic chemo-organotrophic biofilm bacterial communities.

2. Materials and methods

2.1. Sampling sites and sample preparation

Lake Velencei (47°10′N, 18°35′E) has a surface area of 24.9 km^2, 9.8 km^2 of which is covered by dense populations of common reed, *Phragmites australis* (Cav.) Trin ex Steudel. The average depth of the lake is 1.45 m. The dominant ions are sodium, magnesium, hydrocarbonate and sulfate. Total dissolved salt content ranges from 1000 to 3000 mg L^{-1} and pH values vary between 7.8 and 10.0. Selected physical and chemical parameters of the water of Lake Velencei at the time of sampling are presented in Table 1. Submerged reed stems were sampled in July 2001 at three sites (Lángi-tisztás, Gárdony-Hosszútisztás and Fürdető) of the lake (the location of the sampling sites are presented by Ács et al. (2003)). Five replicates of 10–10 cm long first-year young (LNE 1–5, GNE 1–5, FNE 1–5) and more than 1-year old (LNA 1–5, GNA 1–5, FNA 1–5) reed stems (taken from the direction of the open water) were collected from 20 to 30 cm below the water surface from each sampling site. Samples were stored in sterile saline solution (15.23 mM NaCl) at 8–10 °C for 2–3 h until laboratory processing took place. For bacterial community-level metabolic fingerprinting, biofilm samples were removed separately from the reed stems into saline solutions by using sterile brushes. Composite samples – collected from the same sampling sites – were prepared for cultivation-based bacteriological investigations.

2.2. Community-level metabolic fingerprinting

BIOLOG Gram-negative (GN2) microplates (Hayward, California, USA) were used to analyze substrate utilization patterns of biofilm microbial communities. BIOLOG GN2 plates contained 95 wells with different carbon sources and a blank well with no substrate. The majority of carbon sources on BIOLOG GN2 plates were carbohydrates (30), carboxylic acids (24) and amino acids (20). Polymers (5), amines/amides (6) and miscellaneous compounds (10) were represented in lower numbers (Preston-Mafham et al., 2002). Each well contained the redox dye tetrazolium which was reduced by NADH produced by microbial metabolic pathways. The rate of color development correlated with the rate of bacterial metabolism in the wells.

An initial 10^{-1} biofilm dilution was prepared by suspending wet biofilm – equivalent to 10 g of dried matter – in 100 mL of sterile saline solution. Serial dilutions were prepared up to a dilution factor of 10^{-3}. A 20 mL aliquot of each dilution was shaken for 10 min and left settling for 10 min, to avoid interference in the assay by co-extracted biofilm components, causing unspecific turbidity and absorbance. Aliquots (150 μL) of the supernatant were added to the wells of BIOLOG GN2 microplates. Following 24, 48, 72 and 96 h of incubation at 28 °C, absorbance data were recorded at 590 nm with an ELISA Reader (Labsystems Multiscan PLUS).

Comparison of the substrate oxidation results originating from the different samples was accomplished by principal component analysis (PCA), using the SYNTAX 2000 software package (Podani, 2001).

2.3. Isolation and phenotypic characterization of bacterial strains

Serial dilutions of the six composite samples were prepared and four different media were used for plating. King's B (Barrow and Feltham, 2003) and TSY (DSMZ Medium 92) were applied as media frequently used for the cultivation of plant-associated bacteria. The alkaline (pH 9.0) Horikoshi medium (DSMZ Medium 940) was chosen because of the moderately alkaline characteristics of Lake Velencei, and *Caulobacter* medium (DSMZ Medium 595) was used as an oligotrophic medium (http://www.dsmz.de/media/media.html). Random isolation was carried out following a 7 d incubation