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Investigation on rhizoplane actinomycete communities of cattail (*Typha angustifolia*) from a Hungarian wetland

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Wetlands play a special role in diverting plant materials towards fossilisation. These habitats are sinks for the atmospheric carbon: mineralisation of organic materials is inhibited by specific effects of the local N and S cycles, anaerobiosis, low pH, etc. It is not surprising therefore that microbial activity was detected to be highest in the rhizosphere of wetland plants. Cattail (*Typha angustifolia* L.) withstands extremely low soil redox values. Cattail root samples were collected in a floating mat at the Soroksár Arm of the River Danube. The root-tip regions were used for bacteriological studies. Using the plate-count technique an average of 3.3×10^4 CFU/g actinomycete rhizoplane count was detected. All actinomycete colonies were isolated, purified, and strains subjected to differential diagnostic analysis (phenotypical and chemotaxonomical tests). The dominant rhizoplane actinomycetes were: *Streptomyces anulatus*, and *Str. albidoflavus, Micromonospora chalcea* and *M. carbonacea, Micrococcus luteus, Brevibacterium sp.* and *Gordona sp.* Streptomycetes were characteristic for autumn sample, whereas the other actinomycetes dominated the rhizoplane microbiota early in the vegetation period. The ecological tolerance abilities of the members of dominant groups indicate that these bacteria might be active in the rhizosphere and can be present there in their vegetative forms.

Key words: actinomycete, cattail, Hungary, rhizoplane, streptomycetes, wetland.

INTRODUCTION

Early investigators of wetland microbiology focussed on questions of peat formation characteristic for many wetlands. They concluded that chemical processes dominate and peatlands were sterile (Waksman & Stevens 1929). Based on our present knowledge a variety of microbes and physiological activities associated with microbes can be detected in peat, and microbes are present in peatlands in high numbers probably (Gilbert et al. 1998, Richard & Ronald 1983). Peatland actinomycetes include *Streptomyces*, *Actinomyces* (Given & Dickinson 1975), *Micromonospora* and *Nocardia* (Cross 1981, Goodfellow & Williams 1983, Rheims et al. 1998). Actinomycetes are widely distributed in nature, where decaying plant residues are present. They play a decisive role in the decomposition of resistant biopolymers (like lignified cellulose, hemicellulose, pectin, etc.), and they are known as prime components of humification processes, thus they are active even in the mineralisation of recalcitrant humic substances. Their presence and activities in natural environments are often neglected, since on many media they grow very slow (visible colonies develop of-

ten after 2 weeks of incubation).

Peaty environments therefore seem to favour actinomycetes, though environmental extremities, like acidic pH, anaerobiosis will focus their presence to root environments e. g. Unfortunately, there is scanty information on microbiology of rhizoplane of helophytes, and especially on actinomycetes there, though the biogeochemical processes in peat are based on microbial metabolism of the root environment mainly. This work focussed on the characterisation of the quantitative and qualitative aspects of the *Typha* rhizoplane actinomycete communities, as possible close microbial partners of one of the dominant wetland plant species.

MATERIALS AND METHODS

Typha angustifolia root samples were taken in October 1995 and May 1997 in a floating mat (25 × 100 m) at the Csupics Island in Soroksár Danube Arm, 30 km South of Budapest (Fig. 1). In the middle of the mat a plant root and peaty pseudosoil block of 20 × 20 × 30 cm was cut and transferred to the laboratory. Basic pseudosoil pore-water physico-chemical properties were determined on spot (Table 1) by using a Hach DR 2000 portable water laboratory and adequate EPA approved methods. The rooting rhizomes of *Typha* were freed mechanically in original wetland water and then root tips with a diameter of 1–3 mm were cut off in a maximum of 5 cm length. The roots were vigorously washed in sterilised distilled water for six times. Washed roots were aseptically homogenised in a mortar and the macerate was serially diluted (10^0–10^{-6}) and plated (20 plates/dilution/medium). Three "selective" media were used: Starch-casein agar with cycloheximide (SC; Küster & Williams 1964), Malt-yeast extract agar with cycloheximide (MY; Pridham et al. 1956–57), and Difco-Actinomycete isolation agar (DA). Following incubation at 28° C for 2–3 weeks total and actinomycete counts were recorded, supposed actinomycetes were isolated (see e.g. Küster & Williams 1964) and subjected to purification. According to their macro- and micromorphological characteristics the strains were divided into three morphotype groups: "streptomycetes" (polysporic actinomycetes, forming characteristic aerial and substrate hyphae), "micromonosporae" (mostly monosporic actinomycetes, forming coloured spore mass over round "deep sitting" colonies), and "other actinobacteria" (nocardioform actinomycetes and strains with elementary branching or coryneform micromorphology). Adequate phenotypical test set and chemotaxonomical investigations were used for the identification of strains (colony- and micromorphological characteristics, pigment production tests, whole-cell sugar pattern, cell-wall chemotype, oxidase, catalase, aminopeptidase, benzidine test, OF glucose, lecithinase, lipolysis, hydrolysis of pectin, chitin, hippurate, casein, esculin, gelatine, soluble starch, degradation of xanthine, elastin, arbutin, growth with 2, 3, 4, 5, 6, 7 and 7.5% NaCl, utilisation of sucrose, m-inositol, mannitol, L-rhamnose, raffinose, D-melezitose, adonitol, D-melibiose, glycerol, D-ribose, salicin, glucose, arabinose, fructose, xylose, galactose, NO_3^- reduction, H_2S production; Williams et al. 1983, Holt et al. 1994). In case of other actinobacteria the BIOLOG metabolic fingerprint was similarly performed.

The SPSS for Windows release 6.0 statistical software has been used for generation of dendrograms, similarity calculations were based on simple matching coefficient (S_{SM}; Sokal and Michener 1958). At tree generation the UPGMA algorithm was used.

RESULTS

The results of germ counting are summarised in Fig. 2. Total germ counts ranged from a maximum of 5.1×10^5 to a minimum of 3.1×10^4 CFU/g. The range of actinomycete count was between 1×10^3 CFU/g and 1.8×10^5 CFU/g. 47 "autumn" and 21 "spring" isolates were obtained and characterised. The distribution of strains into different actinomycete morphotypes is shown in Fig. 3. Surprisingly only one streptomycete could be isolated from the spring sample, while in autumn they dominate. The ratio of *Micromonospora* species to other actinobacteria is in both samples practically equal.

The frequency of different *Streptomyces* species among the autumn and spring cattail rhizoplane strains is depicted in Fig. 4. The 32 strains fell into 7 species, six of them originating from

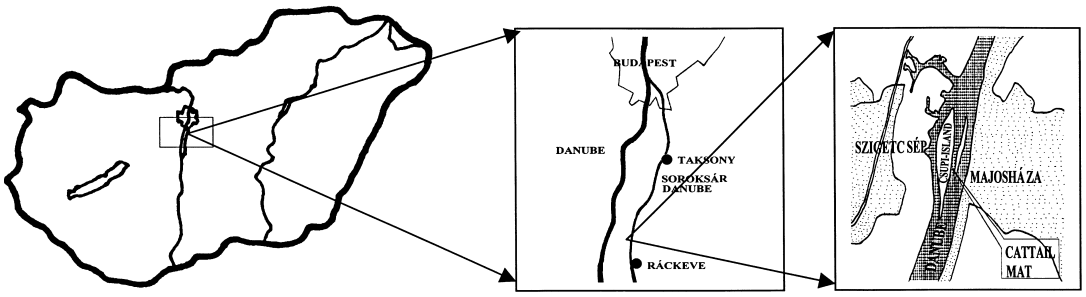

Fig. 1. The cattail (*Typha angustifolia*) mat sampling place at Soroksár Danube Arm, 30 km south of Budapest, the capital of Hungary.

the autumn sample. The dominant group with 24 strains could be identified as *Streptomyces anulatus*. Three strains are members of *Str. albidoflavus,* and 4 strains were identified only at genus level. The only spring streptomycete strain was delineated as *Str. rochei*.

The dendrogram of non-streptomycete actinomycetes isolated from autumn sample is shown in Fig. 5. Approximately half of the strains belong to the genus *Micromonospora.* They clustered into 3 phena: four strains form a relatively tight cluster of *M. chalcea;* a single member phenon represents *M. coerulea*; and three strains are close relatives of *M. carbonacea*. Other phena comprise members of the genus *Brevibacterium* and *Promicromonospora*. Three single member phena were not determined.

The dendrogram of spring season non-streptomycete actinomycetes is shown in Fig. 6. As in case of autumn strains here again half of the strains formed 5 clusters belonging to the genus *Micromonospora*. The first cluster could not be identified, at species level. Two tight clusters, both containing two strains were identified as *M. chalcea*, and *M. carbonacea*. Moreover members of genus *Micrococcus* (*Micrococcus luteus* and *Micrococcus diversus*), *Rhodococcus* sp. (*R. maris*), and *Gordona* sp. could be determined.

DISCUSSION

In the spring sample, excepting on SC agar, the total count values decreased in comparison to the figures obtained from the autumn sample, however the differences are not significant. The actinomycete counts with all the three media used, increased in spring sample compared with the autumn sample. The most important change in actinomycete count was observed on SC medium.

Analysing the possible causes behind, one has to be very careful (due to lack of repeated investigations), but there can be a link to the period of vegetation. The autumn sample was taken from a yellow, frostbite leafed stand, where the root metabolism was possibly retarded, and even decaying root parts could be numerous. On contrary to this, in spring freshly grown young roots were sampled. It is supposed that at autumn bacteria invade inter- and intracellular root spaces as a result of ageing. This is reflected by our opinion in the higher total CFU values on monomer containing media (MY, and DA agars). Similar differences in total counts were observed in other years using other media (Halbritter 1996, Kovács et al. 1997).

Table 1. Basic physicochemical parameters of the pseudosoil pore-water in the floating cattail (*Typha angustifolia*) mat at Csupi Island, Soroksár Danube Arm, south of Budapest, Hungary.

Parameter	Spring	Autumn
Temperature (°C)	12.7	8.8
Redox potential (mV)	+ 13.0	+ 2.0
pH	7.28	6.99
Conductivity (μS cm^{-1})	656	528
Total dissolved solids (g l^{-1})	0.329	0.267
NH_3-N (mg l^{-1})	0.69	0.95
NO_2^--N (mg l^{-1})	0.004	0.008
NO_3^--N (mg l^{-1})	0.3	0.0
S^{2-} (mg l^{-1})	0.016	0.023
SO_4^{2-} (mg l^{-1})	18	12

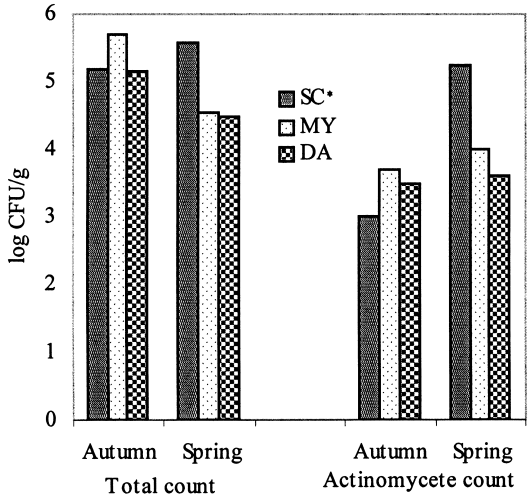

Fig. 2. The results of total and actinomycete counts in cattail rhizoplane of autumn and spring samples collected in a cattail floating mat at Soroksár Danube arm near Budapest, Hungary.
*Abbreviations: SC, Starch-casein agar with cycloheximide; MY, Malt-yeast extract agar with cycloheximide, and DA, Difco-Actinomycete Isolation agar.

Concerning the actinomycete counts we suppose that NO_3^- as N source in the medium could favour actinomycetes. It is well known, that Danube water has a typical yearly cycle in NO_3^- levels due to changes in nitrification activity: spring NO_3^- peaks are characteristic (Zalmum 1997). A relative spring NO_3^- abundance is highly probable in the wetland too, since aerobic conditions, a prerequisite of effective nitrification are favoured by the active spring root respiration processes. The pore-water chemical parameters (Table 1.) support our opinion.

It is interesting to compare the ratios of different actinomycete isolates (Fig. 3). Most of the elements in the low autumn actinomycete counts were streptomycetes whereas the high spring actinomycete counts are represented mostly by other actinobacteria. On contrary the relatively bed O_2 supply in the autumn roots there are high streptomycete numbers. The effects of rapid decomposition activities at the autumn sampling time can be traced among the pore-water chemical data too (lower pH, higher NH_3, NO_2^- and S^{2-} levels and lower NO_3^- and SO_4^{2-} levels). The streptomycetes probably prefer the easily decomposable polysaccharide (hemicellulose, starch, etc.) rich environment and most of our strains are capable of NO_3^- respiration too. The result stress the importance of the O_2 transfer through the root aerenchyma, which is most possibly far better at spring, than at the end of the vegetation period.

The dominant streptomycete (Fig. 4) could be identified as *Str. anulatus.* This variable species is widespread in nature where decaying organic matter is present. Interestingly the closest phenotypic relative of *Str. anulatus, Str. albidoflavus* could also be detected. The two clusters are connected at 79.2% S_{SM}. According to the investigations of Williams and co-workers (1983) this level was 77.5% S_{SM}. Our result is in very good accordance with this value.

The only spring isolate is identified as *Str. rochei*. Members of the species have a wide activity in degradation of polychlorinated phenols (Golovleva et al. 1992). The capability of an organism to degrade phenolic compounds is a selective advantage rather in a peaty environment.

Half of the strains of non-streptomycete actinomycetes (Fig. 5) belong to the genus *Micromonospora*. The first cluster comprises *M. chalcea* strains showing strong cellulose, starch, gelatine decomposition activities. The violet-blue soluble pigment formation helped the delineation of *M. coerulea. M. carbonacea* strains have orange yellow colonies, which rapidly turn into a black spore mass. *Micromonospora* and especially *M. chalcea* and *M. carbonacea* species are normal inhabitants of soil and sediments of aquatic environments. Members of this cluster utilise a wide variety of biopolymers, sugars and other carbon sources. Both *M. chalcea* and *M. carbonacea* accept NO_3^- as terminal electron acceptor while reducing it to NO_2^-.

Three strains are members of genus *Brevibacterium.* They were collected in the dendrogram at 84% average similarity. Two other strains – however they are treated as separate clusters – resemble this coryneform group too. Brevibacteria are found in soil or water/sediment and were isolated from epiphytic communities too. The next two strains have a morphology close to *Promicromonospora* sp. Actinomycetes belonging to the genus *Promicromonospora* are characterised by the formation of branched substrate hyphae which

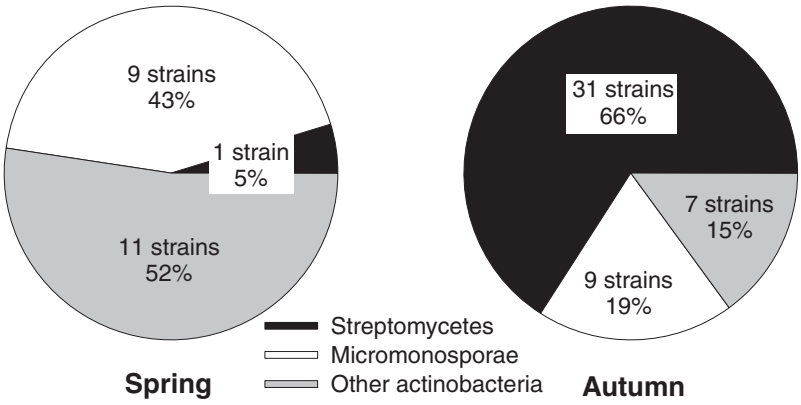

Fig. 3. Distribution of actinobacterial strains isolated from cattail (*Typha angustifolia*) rhizoplane spring (21 strains) and autumn (47 strains) samples (Csupi Island, Soroksár Danube arm near Budapest, Hungary) into three actinomycete morphotypes.

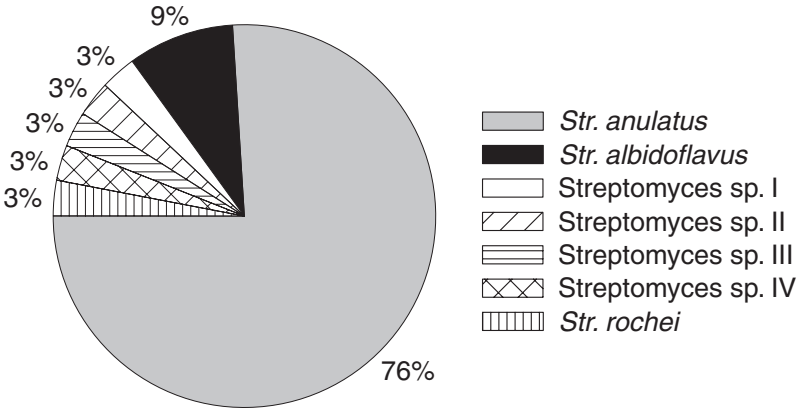

Fig. 4. Frequency of different *Streptomyces* species isolated from cattail (*Typha angustifolia*) rhizoplane autumn and spring samples taken at a floating mat of Csupi Island, Soroksár Danube arm, near Budapest, Hungary.

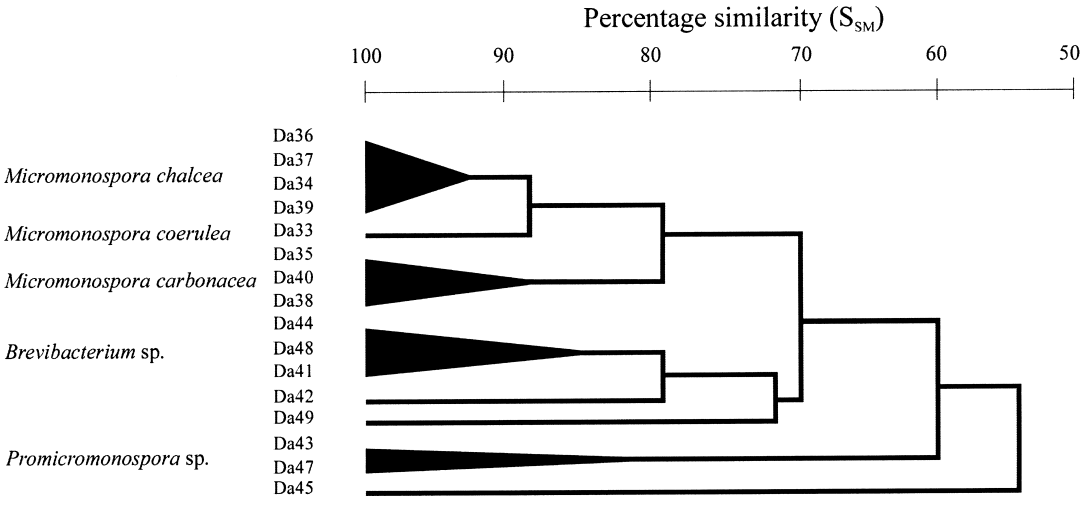

Fig. 5. Dendrogram of cattail (*Typha angustifolia*) rhizoplane non-streptomycete actinomycetes isolated from autumn sample taken from a floating mat at Csupi Island, Soroksár Danube arm, near Budapest, Hungary. The dendrogram was generated using simple matching similarity calculation and UPGMA treeing algorithm.

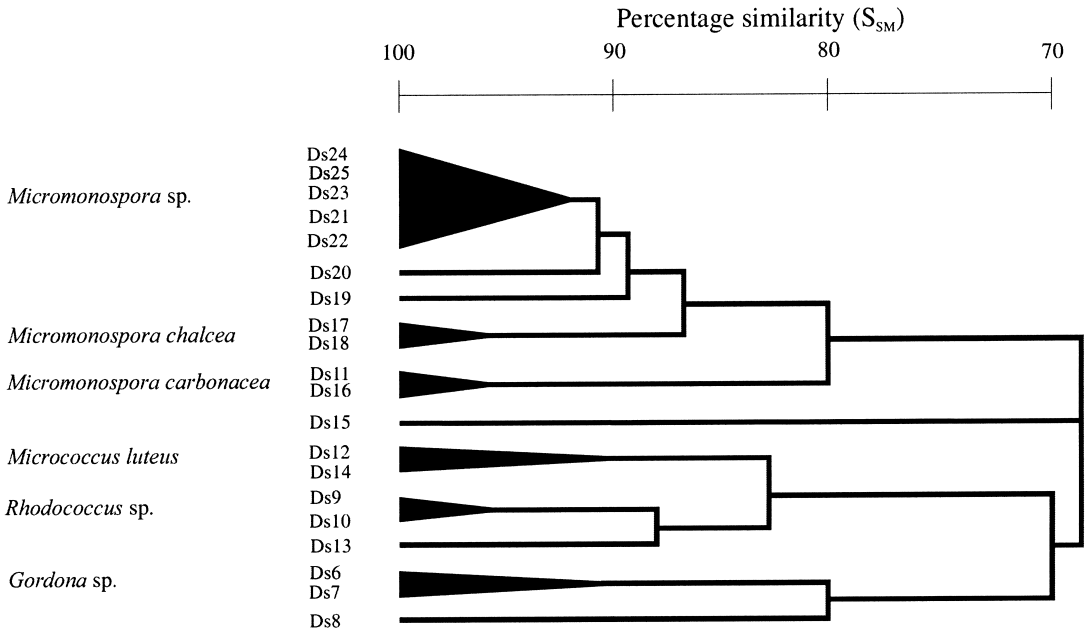

Fig. 6. Dendrogram of cattail rhizoplane non-streptomycete actinomycetes of spring sample taken at a floating mat of Csupi Island, Soroksár Danube arm, near Budapest, Hungary. The dendrogram was generated using simple matching similarity calculation and UPGMA treeing algorithm.

break up into non motile bacillary elements, and usually lack aerial mycelium. The taxa being phylogenetically close to *Promicromonospora* are *Oerskovia* and *Cellulomonas.* The members of this complex seem to be common in polysaccharide rich habitats, whether on surface of senescing leaves, or in habitats like soil fermentation horizons. We have to mention that in Danube bottom sediment biofilms, bacteria of this group are common (Zalmum 1997). The presence of these organisms in root environment is highly probable.

In the dendrogram of spring season sample of non-streptomycete actinomycetes (Fig. 6.) the first cluster was identified at genus level only (*Micromonospora* sp.). The strains show the typical morphological traits characteristic for the genus but do not resemble any species described yet. The second cluster was identified as *M. chalcea*, and the third one as *M. carbonacea.* The dominant presence of micromonosporae in cattail rhizoplane seems to be corroborated. Our results confirm the previous results of Kovács and co-workers (1997) who recorded the presence of *Micromonospora* sp. in cattail rhizoplane based on a total DNA isolation/r DNA cloning study. The next cluster comprises *Micrococcus* strains. The earlier results of Kovács and co-workers (1997) and Halbritter (1996) on cattail rhizoplane proved the constant, however not numerous presence of these organisms in this special environment. With help of the BIOLOG system members of the next phenon were identified as *Rhodococcus maris*. *Rhodococci* may take part in decomposition of compounds resisting biodegradation, may even utilise xenobiotics. Recent studies on the bacterial communities of several aquatic and terrestrial habitats recorded that *Gordona* spp. are widely distributed in the environment (Goodfellow 1992 and Zalmum 1997). Thus their presence in the cattail rhizoplane is not surprising.

CONCLUSIONS

It can be concluded that *Streptomyces* species seem to be active members of the microbial community in the cattail rhizoplane environment, being characteristic at autumn. They can find particular microhabitats satisfying their ecological requirement. It is suggested that *Micromonospora*

take constantly part of the indigenous microbiota of the cattail rhizoplane. It seems to be corroborated that these organisms are well adapted to survive under the drastically changing conditions found in wetland plant root surface/peat interface. Various taxonomic groups of other actinobacteria differ in the degree of adaptation to this environment. The species composition of other actinobacteria strongly changes from autumn to spring. It is supposed, that this phenomenon can be bound to the anaerobiosis tolerance of the communities. Actinomycetes could have a decisive role in recycling a broad range of resistant substances present in rhizoplane of wetland plant species.

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