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A STUDY OF KERATINOLYTIC FUNGI IN MOUNTAIN SEDIMENTS. I. HAIR-BAITING DATA

BADANIA GRZYBÓW KERATYNOLITYCZNYCH W OSADACH DENNYCH GÓRSKICH STRUMIENI. I. DANE UZYSKANE METODĄ PRZYNĘTY WŁOSOWEJ

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Sediments form mountain streams in Brenna (Beskid Śląski) were examined for keratinolytic fungi. A rare psychrophilic dermatophyte, Keratinophyton ceretanicus, occurred abundantly in the sediments. The qualitative and qualitative compositions of keratinolytic fungi depended on the water contamination with sewage and on the contents of plant organic material, small mineral particles and salts in the sediments.

INTRODUCTION

Keratinolytic fungi has been widely recorded form soil and other natural and manmade habitats. These microorganisms play a crucial role in the decomposition of keratin remnants of human and animal origin and form anthropogenic and/or zoogenic communities in the environment. As keratinolytic fungi have the potential to attack keratinized structures attached to human beings and other animals, they can also be causative agents of epidermal mycoses.

In previous papers [18, 19], the occurrence of keratinolytic fungi in marine and freshwater sediments in Spain and Poland was presented. However, no mountain sediments were examined for these microorganisms in Poland. In the present study, results on the occurrence of keratinolytic fungi in sediments of mountain streams in the Beskid Śląski mountains are presented.

MATERIAL AND METHODS

The study area was close to the experimental area of the Institute for the Ecology of Industrial Areas (IETU) in Brenna within the Beskid Śląski mountains (range of the Carpathian Mountains). On the IETU experimental area, there is a monitoring station (49°40' N; 19°56' E). The altitude of the site is 660 m a.s.l. The compact Norway spruce (*Picea excelsa*) cover with single dispersed beech trees (*Fagus silvatica*) characterizes both the study and IETU experimental area. The characteristics of these areas are presented in a separate paper [16].

On the study area, three shallow (up to 0.5 m of depth) streams were studied. Five locations of sediment sampling were established. They were the following:

1. small unnamed stream with one location (1) on the small experimental overflow which is an element of the IETU monitoring station; the small stream is a tributary of the Wilczy Potok stream;
2. Wilczy Potok stream with two locations (2 and 3); location 2 was on the large experimental overflow (another element of the IETU monitoring station) below the inflow of the unnamed stream and location 3 was at the inflow of the Wilczy Potok stream into the Leśnica stream;
3. Leśnica stream with two locations (4 and 5); the locations were above and below of the inflow of the Wilczy Potok stream into the Leśnica stream.

The sampling locations are illustrated in Fig.1. At locations 3, 4 and 5, sediments were sampled once, in August 1995, while at locations 1 and 2 sediment samples were collected four times, in October 1994, June and August 1995, and in August 1996.

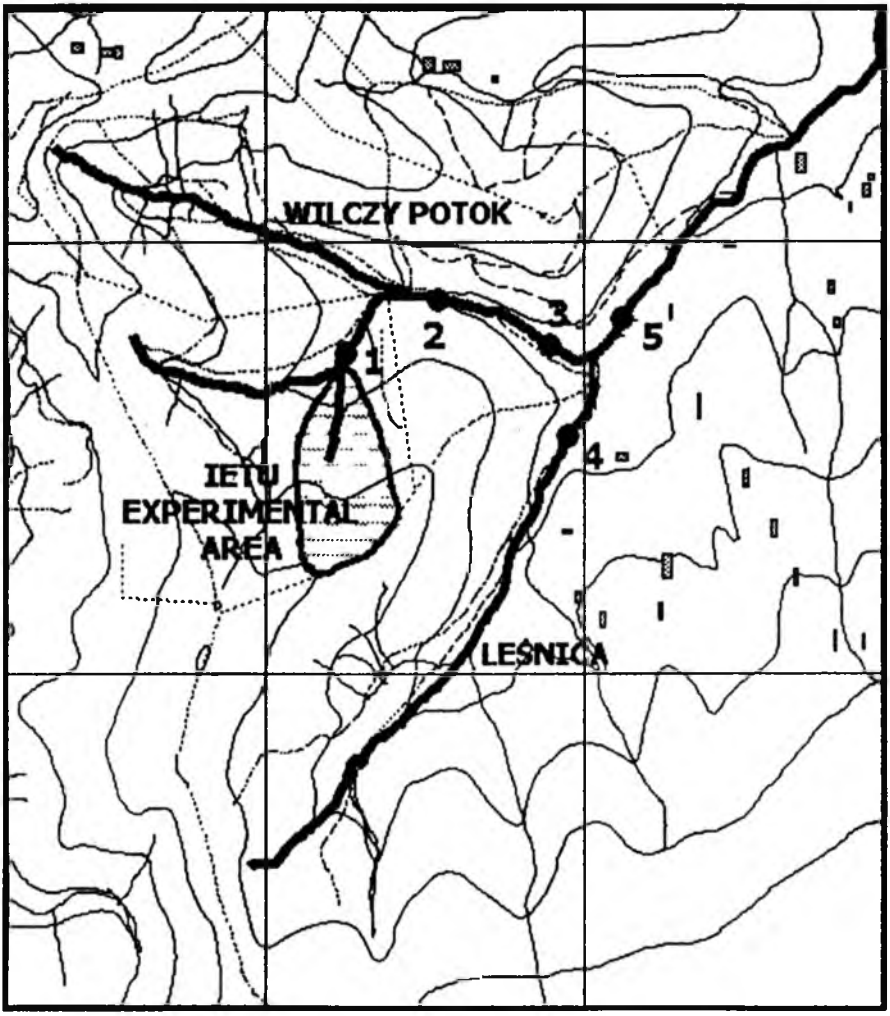

Fig. 1. The mountain streams with sampling locations in the study area in Brenna

Sediments were sampled from the superficial layer of stream bottoms (up to 5 cm of depth) to plastic bags disinfected with 75% ethyl alcohol and an UV lamp. At each location and sampling, ca. 3 kg of sediment was collected from 25 places along the stream banks. These subsamples were taken at the distance of roughly 0.5 m from one place to another. This method of sampling allowed covering both a high bottom surface and long riverbank for examination. Samples were delivered to the laboratory within 3–4 hours, first cleaned form fresh plant remnants and stones, then crumbled and thoroughly mixed. The mixed samples were examined for microbiological and physico-chemical properties.

Keratinolytic fungi were isolated using the children's hair baiting method [20]. For each mixed sample, 10–20 *Petri* dishes were set up. In each *Petri* dish, ca. 30 g of sediment were placed and covered with short pieces of detergent-washed and 3 times autoclaved child hair. The *Petri* dishes were incubated for 4–6 months in the dark and cool place (air temperature did not exceed 18°C). Sterilized distilled water was periodically added to the dishes to keep constant moisture. The developments of fungi on hair bait were examined macro-and microscopically at roughly 4-week intervals. In positive cases, fungal strains were isolated on *Sabouraud* glucose agar (SGA) with chloramphenicol (100 mg/l) and on *Wiegand* agar with chloramphenicol (100 mg/l) and actidione (=cycloheximide; 500 mg/l).

The isolated fungi were identified basing upon their macro- and microscopic characteristics. Selected taxonomic monographs were used [4, 13]. The keratinolytic abilities of the fungi were tested i vitro with the method described previously [19].

The "STATISICA" for Windows program (ANOVA/MANOVA test) was used for statistical analysis of the data obtained for dermatophyte macroconidia.

The occurrence of keratinolytic fungi in sediments of mountain streams was characterized by the following indices: frequency of isolation of keratinolytic fungi (FI; number of *Petri* dishes positive for keratinolytic fungi divided by the total number of Petri dishes set up x 100%), number of species isolated (NS), number of fungal strains (NA), frequency of isolation of predominating fungal species (FIPS; number of stains of a given species divided by the total number of fungal strains x 100%) and L index (number of strains divided by the number of *Petri* dishes set up). The abbreviations for the predominating species were TAJ, MGYP, KCER and AQ for *Trichophyton ajelloi, Microsporum gypseum, Keratinomyces ceretanicus* and *Arthroderma quadrifidum*, respectively.

The following physico-chemical parameters were determined for each sediment sample: particle size distribution (PSD) analysis by *Casagrande's* aerometric method in *Prószyński's* modification; pH in H_2O (pHH_2O) by potentiomeric method; conductivity (COND) by electrical method; ignition losses at 600 C (IGNL) by gravimetric method; organic carbon (C_{ORG}) by *Walklay-Black* method; total nitrogen (N_{TOT}) by *Kjeldahl* method; total sulphur (S_{TOT}) by *Eschka* method; sulphate sulphur (SSO_4) by *Bardsley* and *Lancaster's* method; available phosphorus (P_2O_5) and available potassium (K_2O) by *Egner-Riehm's* method; heavy metals (Zn, Cd, Pb, Cu, Ni and Hg) by atomic absorption method after aqua regia digestion. The C:N ratios were calculated.

The following fungal groups were quantitatively examined in sediment samples:

1. Total number of microscopic fungi (TNMF) on malt extract agar (MEA) with chloramphenicol (100 mg/l) at 25 C;
2. Number of actidione-resistant fungi (AF) on *Wiegand* agar with chloramphenicol (100 mg/l and actidione (500 mg/l) at 25 C;
3. Number of mesophilic (MF) and thermophilic fungi (TF) on YpSs agar with chloramphenicol (100 mg/l) at 37 and 45 C, respectively. The dilution technique was employed.

The following bacteriological analyses were performed: total number of bacteria (TNB), number of mesophilic bacteria (MB), *Colstridium perfringens* (CP), total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS). The FC:FS ratios were calculated. Results are

presented as the most probable number of bacterial cells in 1 or 100 g of sediment dry weight. The methods recommended by the Countryside Hygiene Institute in Lublin, Poland [7] were used to perform the analyses.

RESULTS

Of 190 sediment samples (*Petri* dishes) examined, 88 (46.31%) samples were found to be positive for keratinolytic fungi (Table I). Altogether, 132 fungal strains from 12 species were isolated from the sediments, with *Keratinophyton ceretanicus, Arthroderma quadrifidum* (with its anamorph *Trichophyton terrestre*), *Microsporum gypseum* and *Trichophyton ajelloi* as the predominating species. The community of keratinolytic fungi in mountain sediments showed high annual and seasonal variations at the locations examined. However, *T. ajelloi* with *K. ceretanicus* and *T. ajelloi* with *A. quadrifidum* (anamorph *T. terrestre*) were always present at locations 1/2 and 4/5, respectively.

The richest keratinolytic mycoflora occurred at location 2 (Table II), with *K. ceretanicus* and *M. gypseum* as the predominating species. At location 1, the mycoflora was qualitatively close but much poorer than at location 2, with lower mean frequencies of the above-mentioned species but with the highest frequency of *T. ajelloi*. No keratinolytic fungi were isolated from sediments at location 3. At location 4, the keratinolytic mycoflora was small, with *A. quadrifidum* (anamorph of *T. terrestre*) and *T. ajelloi* as the predominant species. The mycoflora at location 5 was richer, with the same predominant species as at location 4.

The highest mean numbers of total bacteria (TNB), *Clostridium perfringens* (CP), total and faecal coliforms (TC, FC) and the highest FC:FS ratio were noticed at location 2 whereas the highest number of faecal streptococci (FS) was at location 5 (Table 4). Subsequently, the highest MB value was at location 3. In comparison with locations 1 and 2, much lower level of bacterial contamination was observed at locations 3–5. The highest concentrations of fungal propagules (TNMF, AF, MF, and TF) were detected at location 2 while the lowest fungal contamination occurred at location 1 and 3. The TNMF value at location 1 was also high.

In comparison with sediments at locations 3–5, the considerably higher percent content of small particles characterized sediments at locations 1 and 2. The reaction (pH) of sediments at all locations was acidic and varied between 5.02–5.75. The ignition losses (IGNL), organic carbon (C_{ORG}), total nitrogen (N_{TOT}) and total sulphur (S_{TOT}) contents together with the C:N ratios were high at locations 1 and 2 and much lower at locations 3–5. Except for the available potassium content (K_2O), the cnductivety (COND) together with the concentrations of available phosphorus (P_2O_5) and sulphate sulphur was higher at locations 1 and 2 than at the other locations. The highest concentrations of all heavy metals (Zn, Cd, Pb, Cu, Ni and Hg) were observed at locations 1 and 2.

Due to the rare isolations of *K. ceretanicus* from the environment throughout the world, the description of our strains is presented below.

Keratinophyton ceretanicus Punsola et Guarro

Colonies on PYE at 18°C growing slowly, with a mean daily spread of 0.55–0.75 mm (average 0.63 mm/day), slightly elevated (up to 1.5–2 mm of height) and irregularly

Table I. The occurrence of keratinolytic fungi in sediments of mountain streams in Brenna

Keratinolytic species	Location/month/year										
	1 Oct 1994	1 June 1995	1 Aug 1995	1 Aug 1996	2 Oct 1994	2 June 1995	2 Aug 1995	2 Aug 1996	3 Aug 1995	4 Aug 1995	5 Aug 1995
Total no. of samples set up	10	15	15	20	10	20	20	20	20	20	20
No. of samples positive for keratinolytic fungi	10	1	4	8	10	16	10	11	0	6	12
Frequency of isolation (FI; %)	100	6,67	33,33	40	100	80	100	55	0	30	60
No. of samples positive for:											
K. ceretanicus	1	–	–	7	10	10	14	8	–	–	–
A. quadrifidum	–	–	–	–	–	–	–	–	–	2	3
T. terrestre complex	–	–	–	–	3	–	–	1	–	2	9
M. gypseum complex	6	–	–	–	5	4	4	–	–	–	–
T. ajelloi	4	1	1	1	1	3	3	–	–	1	2
Aph. durus	–	–	–	–	1	–	–	2	–	–	–
Chrysosporium an. of *Aph. durus*	–	–	–	–	1	–	–	2	–	–	–
Chrysosporium an. of *A. curreyi*	–	–	3	–	–	–	–	–	–	2	–
Aph. fulvescens	1	–	–	–	–	–	–	–	–	–	–
Chrysosporium an. of *Aph. fulvescens*	1	–	1	–	1	–	–	–	–	–	1
Ch. europae	–	–	–	–	–	–	–	1	–	–	3
Ch. tropicum	1	–	–	–	2	–	–	–	–	–	–
M. flava	1	–	–	–	–	–	–	–	–	–	–
Myceliophthora sp.	–	–	–	–	–	–	–	–	–	1	–
Ch. pannicola	–	–	–	–	–	–	–	–	–	–	1
No. of species isolated (NS)	6	1	3	2	7	3	3	4	0	4	5
No. of strains (NA)	15	1	5	8	24	17	21	14	0	8	19
L index	1,5	0,07	0,33	0,4	2,4	0,85	1,05	0,7	0	0,4	0,95

radial folded at the center, dense, cottony, yellow to yellow-orange; margin defined, slightly fimbriate, regular; reverse orange-brown to brown.

Colonies on SGA at 18°C growing more rapidly than on PYE, with a mean daily spread of 0.55–1 mm (average 0.97 mm/day), slightly elevated (up to 2 mm of height), irregularly folded at the center, cottony, dense, yellowish to orange; margin defined,

Table II. Mean values of frequencies and indices for growth of keratinolytic fungi in mountain sediments in Brenna

Indices and frequencies	Means for location no.				
	1	2	3	4	5
FI (%)	44,99	83,75	0	30	60
NS	3	4,25	0	4	5
L index	0,57	1,25	0	0,4	0,95
TAJ	39,79	9,02	0	12,5	10,52
MGYP	10	15,85	0	0	0
KCER	23,54	56,07	0	0	0
AQ	0	4,91	0	50	63,15
Other fungi	26,66	14,13	0	37,5	26,31

regular, fimbriate; reverse pink-orange to orange-red, with the pigment diffusing into the agar.

Hyphae hyaline, septate, branched, 2–4 μm thick. Raquet hyphae not observed. Numerous macroconidia, long and narrow, hyaline, narrowly fusiform to pencil-shaped, trucate at the base and apiculate at the apex, relatively thin-walled (0.7–1.1 μm), usually borne in dense clusters, 4–15-celled (mostly 8–10-celled), 33–86.1 x 2.7–5 μm (Fig. 2). Microconidia not observed in both hair and agar cultures.

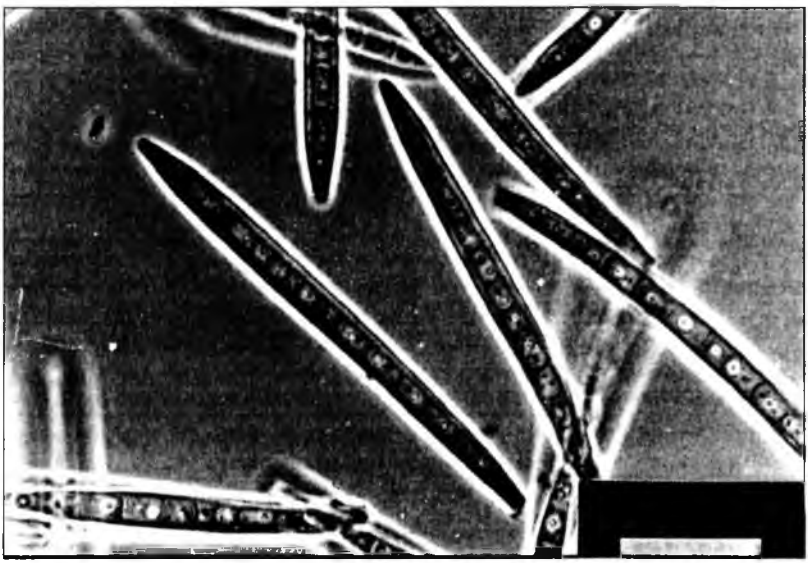

Fig. 2. Macroconidia of *Keratinomyces ceretanicus* from sediments of mountain streams in Brenna (bar = 20 μm)

No growth noticed at 25°C. Clearly keratinolytic *in vitro* but no perforation organs observed. Slow growth on hair bait, chiefly in *Petri* dishes without the growth of other keratinolytic fungi. Very restricted growth on *Wiegand* medium containing chloramphenicol (100 mg/l) and actidione (500 mg/l). Teleomorph not observed on the hair-baited sediment samples and when crossing the isolate strains.

Material examined: living strain IETU (Institute for the Ecology of Industrial Areas) 1164 and IETU 1165, numerous children's hair-baited strains.

The differences between *T. ajelloi* and *K. ceretanicus* in the length and width of macroconidia, the numbers of their cells and in the width of macroconidial walls were statistically important. The largest difference between these two species was observed in the width of macroconidia. The means and standard deviations for the macroconidia are presented in Table III.

Table III. Characteristics of macroconidia in *T. ajelloi* and *K. ceretanicus* (n = 150)

Fungal species	Length (μm)	Width (μm)	Wall width (μm)	No. of cells
T. ajelloi	51,15 ± 11,14[a]	9,5 ± 1,16	2,46 ± 0,72	7,86 ± 1,86
K. ceretanicus	61,12 ± 14,14	4,82 ± 0,7	0,86 ± 0,17	9,9 ± 2,33

[a] – mean ± standard deviation

DISCUSSION

In general, the frequency of keratinolytic fungi in the sediments of mountain streams in Brenna was found to be rather restricted as compared with the sediment of lowland and highland freshwater in Poland [19]. It can be explained by the lack of considerable amounts of keratin debris of human and animal origin in the forest environment [8], the poverty of nutrients in acidic coniferous mountain areas and by the severe mountain conditions in temperate climate (lower temperatures, shorter vegetation periods). On the other hand, however, the results indicate that these fungi also depended on the water contamination with sewage and on the supply of plant organic material (mostly spruce cones, needles and branches), small mineral particles and slats to the sediments. An usual community of keratinolytic fungi, included *Microsporum gypseum*, *Keratinophyton ceretanicus*, *Trichophyton ajelloi*, *Arhtroderma quadrifidum* (anamorph *T. terrestre*) and some other related species, was associated with those environmental conditions.

Some authors [3, 11] have emphasised the importance of pH in the distribution of keratinolytic fungi in the environment. The reaction of mountain sediments in Brenna was acidic and, therefore, favoured *T. ajelloi*, a world-wide acidophilic dermatophyte. The other predominating dermatophyte species, i.e., *M. gypseum* and *T. terrestre*, are, however, neutro- and alkalophilic but occurred in the sediments with relatively high frequencies. The explanation is that the division of the dermatophytes into the reaction groups is based on the fungal pH optima. In the environment, the fungi occur in much wider pH ranges. Nothing is known about the pH preferences of *K. ceretanicus*. On the basis of the present results, it is hypothesised that the species is acidophilic. Generally, because of the small differences in the pH values between locations, the reaction factor seems to have no or little effect on the distribution of keratinolytic fungi in the study area.

Table IV. Mean microbiological and physico-chemical parameters for mountain sediments in Brenna

Parameters	Unit	Means for locations				
		1	2	3	4	5
TNB	CFU/gdw	43520	185567	83659	149942	134325
MB	CFU/gdw	25645	23439	61704	12290	25502
CP	CFU/gdw	25	169	0	0	15
TC	MPN/100 gdw	78354	2451398	11772	35846	125473
FC	MPN/100 gdw	5041	895240	11772	6657	12547
FS	MPN/100 gdw	244	910	112	317	1202
FC:FS	–	12,2	5288,1	105,1	21	10,4
TNMF	CFU/gdw	8421	11967	412	1029	747
AF	CFU/gdw	599	3907	29	112	397
MF	CFU/gdw	23	170	49	82	94
TF	CFU/gdw	0	49	0	5	5
PSD 1–0,1 mm	%	42	22	62	78	77
PSD 0,1–0,05 mm	%	18	21	10	9	12
PSD 0,05–0,02 mm	%	28	34	10	2	7
PSD 0,02–0,005 mm	%	7	10	5	1	2
PSD 0,005–0,002 mm	%	1	6	3	1	2
PSD < 0,002 mm	%	4	7	10	9	0
pH in H_2O	–	5,69	5,58	5,02	5,66	5,75
COND	µS/cm	114,2	106,65	33,2	31,3	33,2
IGNL	% dw	20,15	15,18	4,13	2,58	2,77
C_{ORG}	% dw	9,89	8,46	0,98	0,61	0,84
N_{TOT}	% dw	0,34	0,30	0,07	0,03	0,04
C:N	–	28,9	27,7	12,7	17,4	20
S_{TOT}	% dw	0,067	0.055	0,008	0,003	0,006
SSO_4	mg/100 gdw	1,88	1,68	0,1	0,16	0,08
P_2O_5	mg/100 gdw	0,31	0,51	0,03	0,01	0,03
K_2O	mg/100 gdw	53,5	54,2	93	91,3	93,2
Zn	mg/kgdw	296,2	221,6	75,9	55,7	68,9
Cd	mg/kgdw	1,27	1,17	0,05	0,25	0,2
Pb	mg/kgdw	126,9	73,1	21,4	17	18,2
Cu	mg/kgdw	25,4	18,4	13,2	8,9	9,6
Ni	mg/kgdw	41,6	41,2	21,2	16,6	18,2
Cr	mg/kgdw	57,6	56	12,1	6,4	9,4

Abbreviations for units:
CFU – colony forming units
MPN – most probable number
dw – dry weight of sediment

However, the sediments at locations 1 and 2 contented much higher amounts of plant organic matter (including carbon, nitrogen and sulphur contents), mineral salts (except for the available potassium content) and small particles (clay and loam). Also, the concentrations of bacteria (including faecal bacteria) and microscopic fungi (actidione-resistant species in particular) were much higher at these locations. This faecal contamination mostly originated from farms and tourist facilities situated in the study area. The above-mentioned factors divide the locations into two groups: 1/2 and 4/5. Location 3 should be considered as intermediate between the two groups. The observed qualitative and quantitative compositions of keratinolytic fungi at the locations reflect the above division.

M. gypseum occurred at locations 1 and 2. On the one hand, the preferences of this species to habitats containing high amounts of organic matter have been demonstrated [6]. On the other hand, however, short vegetation periods with relatively low temperatures should not have favoured this mesophilic fungus in the mountain environment. The only explanation is that during the vegetation periods the temperatures in Brenna were sufficient to support the existence or even development of *M. gypseum* in sediments. This explanation fits the data obtained in other studies. For instance, *Dvořák* and *Hubálek* [9] demonstrated that *M. gypseum* shows slow growth at 4°C. *Meinhof* and *Grabowski* [12] regularly isolated the fungus from alpine soils. Finally, *Ulfig et al.* [17] observed the slow growth of *M. gypseum* in the hair placed in the superficial layer of mountain sediments and in that suspended in the overlying water.

K. ceretanicus was the second species clearly associated with locations 1 and 2. Since the species has only been recorded in two countries throughout the world (Spain and Chile), and since no physico-chemical data on its distribution are available [5, 15] our knowledge of the ecology and biology of *K. ceretanicus* is highly restricted and requires special attention.

On the basis of some strains isolated from alpine forest soil in Catalonian Pyrenees, Punsola and Guarro [15] described *K. ceretanicus* as a new species of psychrophilic dermatophyte. *Padhye et al.* [14] placed the fungus in synonymy with *T. ajelloi*. Since more strains of *K. ceretanicus* were available, *Cano* and *Sigler* [5] re-examined the collection and concluded that *K. ceretanicus* should be maintained as a separate species. This conclusion has been confirmed in the present study.

On the basis of a shared teleomorph, *Arhtroderma*, *Ajello* [1] considered *Keratinomyces* as a synonym of *Trichophyton*. However, *Vanbreuseghem* [21] and *Von Arx* [2] did not share this opinion. The last author included all species with fusiform, smooth- and thick-walled macroconidia in the genus *Keratinomyces* whereas all species with cylindrical or obovate thin-walled macroconidia in the genus *Keratinomyces* whereas all species with cylindrical or obovate thin-walled macroconidia were placed in the genus *Trichophyton*. *Punsola & Guarro* [15] followed this criterion. In opinion of the senior author, both the shape of macroconidia as well as the thickness of macroconidial wall are good taxonomic criteria in the distinction of many dermatophyte species but rather useless in the distinction of dermatophyte genera. On the basis of the present results, it is felt that *K. ceretanicus* should be placed in *Trichophyton*. However, genetic evidence is needed at the point. The present data have confirmed the psychrophilic nature of the fungus and indicated its association with acidic reaction as well as with

high concentrations of organic matter, small particles, salts, faecal bacteria and fungal propagules.

T. ajelloi occurred at all locations and was rather irrespective of organic matter content. This finding fits the conclusion of *Chmel et al.* [6]. Although *A. quadrifidum* (an. *T. terrestre*) occurrred at location 2, it's highest frequencies were observed at location 4 and 5. Since the organic matter contents at these locations were the lowest, and since *A. quadrifidum* has been reported as a species preferring habitats with low organic matter content [10], the above distribution of the fungus is quite understandable.

The dermatophytes and related fungi isolated form sediments of mountain streams in Brenna are geophilic species rarely causing epidermal mycoses in man and animals. Among them, only *M. gypseum* is more frequently encountered in medical laboratories [1]. The input of sewage to mountain streams associated with accumulation of plant organic matter supports spreading and development of the fungus and deteriorates the hygienic conditions in the mountain environment.

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BADANIA GRZYBÓW KERATYNOLITYCZNYCH W OSADACH DENNYCH GÓRSKICH STRUMIENI. I. DANE UZYSKANE METODĄ PRZYNĘTY WŁOSOWEJ

Streszczenie

Osady denne z kilku górskich strumieni w Brennej (Beskid Śląski) przebadano pod względem występowania grzybów keratynolitycznych. W porównaniu z osadami dennymi rzek nizinnych i wyżynnych południowej Polski częstość izolowania grzybów keratynolitycznych z osadów górskich była niższa. Można to wytłumaczyć stosunkowo małą ilością odpadów keratynowych w środowisku leśnym, ubóstwem składników odżywczych w kwaśnym siedlisku świerkowym oraz ostrością górskiego klimatu (niższe temperatury, krótszy sezon wegetacyjny). Na podstawie uzyskanych wyników można wnosić, że występowanie grzybów keratynolitycznych w osadach dennych uzależnione było również od zrzutu ścieków, dopływu materii organicznej pochodzenia roślinnego i soli mineralnych oraz od zawartości w osadach dennych cząstek pylastych i ilastych. w górskich osadach dennych gatunkami dominującymi były cztery dematofity: *Keratinophyton ceretanicus*, *Microsporum gypseum*, *Trichophyton ajelloi* i *T. terrestre* (forma doskonała *Arthroderma quadrifidum*). K. ceretanicus jest gatunkiem psychrofilnym, przystosowanym do górskich warunków klimatycznych. Wśród wyizolowanych grzybów tylko *M. gypseum* częściej spotykany jest w laboratoriach medycznych. Jego obfite występowanie w osadach dennych, bogatych w materię organiczną, cząstki pylaste i ilaste oraz w drobnoustroje pochodzenia kałowego (bakterie i grzyby), pogarsza warunki higieniczne środowiska leśnego i zwiększa ryzyko zakażenia mikroorganizmami chorobotwórczymi.

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