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FIRST ISOLATION OF *MICROSPORUM RACEMOSUM* BORELLI IN POLAND

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During a study of keratinolytic fungi in the ash heap ground, considerably influenced by the municipal landfill site, a rare potentially pathogenic dermatophyte, Microsporum racemosum *Borelli, was recognized. This is the first report on its isolation in Poland. Morphological and ecological features of the fungus are discussed.*

INTRODUCTION

During a study of keratinolytic fungi (KF) in soils contaminated by municipal landfill sites [17], a rare pathogenic dermatophyte, *Microsporum racemosum* Borelli, was isolated from the ash heap ground. In this article, we present its macro- and microscopic characteristics as well as discuss possible ecological factors affecting the incidence of the fungus in the ground.

MATERIAL AND METHODS

The ash heap investigated is situated in Sosnowiec at a distance of ca. 100 m from the municipal landfill site. On this heap, coal combustion ash from the coal mine „Kazimierz-Juliusz" was dumping for a period of ca. 100 years. The dumping was ceased in 1990. Up-till-now, the ash heap surface (ca. 1.5 ha) has not been treated in any way. Actually, it is covered with the black „coal" ground (loose sand in the ground classification by the Polish Standard BN-78/9180–11), on which only few plants (lichens, mosses, herbs, grasses and shrubs) are possible to be found. Ash heap's material is also used as inert material for municipal waste disposal.

Ash heap ground was sampled 4 times (April, October 1993; May, September 1994). Altogether, 7 locations on ash heap's surface were investigated. The way of sampling is described by *Ulfig* [17]. The samples were examined for selected physico-chemical parameters (Tab. 1). Full physico-chemical characteristics of the ash heap material were presented by *Terakowski* [15]. The human hair baiting technique [19] was used for KF recognition in the samples. Isolated fungi were identified according to selected keys and monographs [3, 8, 13].

RESULTS

Out of 90 *Petri* dishes set up, 63 (70%) were positive for KF. Altogether, 158 fungal appearances belonging to 18 species were observed. L index (number of appearances divided by the number of *Petri* dishes set up) was 1,76. *Aphanoascus durus* with its anamorph *Chrysosporium* sp. and *Ch. europae* were the prevailing species in the ash

Table I. Physico–chemical characteristics of the ash heap ground

Parameter	Unit	Min	Max	Mean	St. Error	St. Deviation
Humidity	%	2,3	15,3	6,9	4,1	7,2
pH in H_2O	–	6,5	7,6	7,1	0,3	0,5
Conductivity	µS/cm	48	339	202	84	146
Ignition losses in 600°C	%dw	19,2	30,2	25	3,2	5,5
Organic carbon	as above	1,4	17,8	10,1	4,7	8,2
Total nitrogen	as above	0,24	0,39	0,29	0,04	0,08
C:N	–	5,7	46,3	32,3	13,3	23
Total sulphur	% dw	0,12	0,25	0,17	0,04	0,07
S–So_4	mg/100 g	3,4	7,7	4,9	1,3	2,3
P_2O_5 total	% dw	0,009	0,076	0,038	0,019	0,034
P_2O_5 available	mg/100 g	0,1	0,4	0,2	0,1	0,17
K_2O available	as above	3,7	6,7	5	0,88	1,53
Iron	as above	2,1	2,7	2,5	0,2	0,3
Zink	mg/kg (ppm)	285	646	475	104	181
Lead	as above	10,2	68,5	36,1	17,1	29,6
Cadmium	as above	0,8	1,5	1,1	0,2	0,35
Copper	as above	7	53,6	28,1	13,6	23,6
Chromium	as above	3,7	19,8	14,1	5,2	9,07

dw – dry weight of ground

heap ground (Fig. 1). *Microsporum racemosum* were found to grow on 4 *Petri* dishes. The species appeared in April 1993. Two strains were isolated and introduced to the IEIA (Institute for Ecology of Industrial Areas) collection.

Microsporum racemosum Borelli

On *Sabouraud* glucose agar (SGA), colony growing moderately rapidly, with a mean daily growth rate of 4 mm in the dark at 28°C, flat, with a folded and pinkish umbo, downy through floccose to granulose or powdery, initially colourless, later becoming dark cream to pink; margin defined, irregular, fimbriate; reverse initially yellowish but slowly becoming brownish, brown to pink-violet; pigment not diffusing into the agar. The pigment also produced on potato dextrose agar (PDA) and, though only brown, on phyton yeast extract agar (PYE). No pigment observed on malt extract agar (MEA) and *Sabouraud* 1:10 + salts (*Takashio* agar).

Hyphae hyaline, septate, branched, thin- and smooth-walled, 1.8–4.5 µ wide. No racquet and spiral hyphae observed. Microconidia extremely numerous, disposed in clusters, usually arises from the conidiogenous hyphae at right angles, hyaline, thin- and smooth-walled, stick- and cartridge-shaped, with truncate basal ends, 1-, rarely

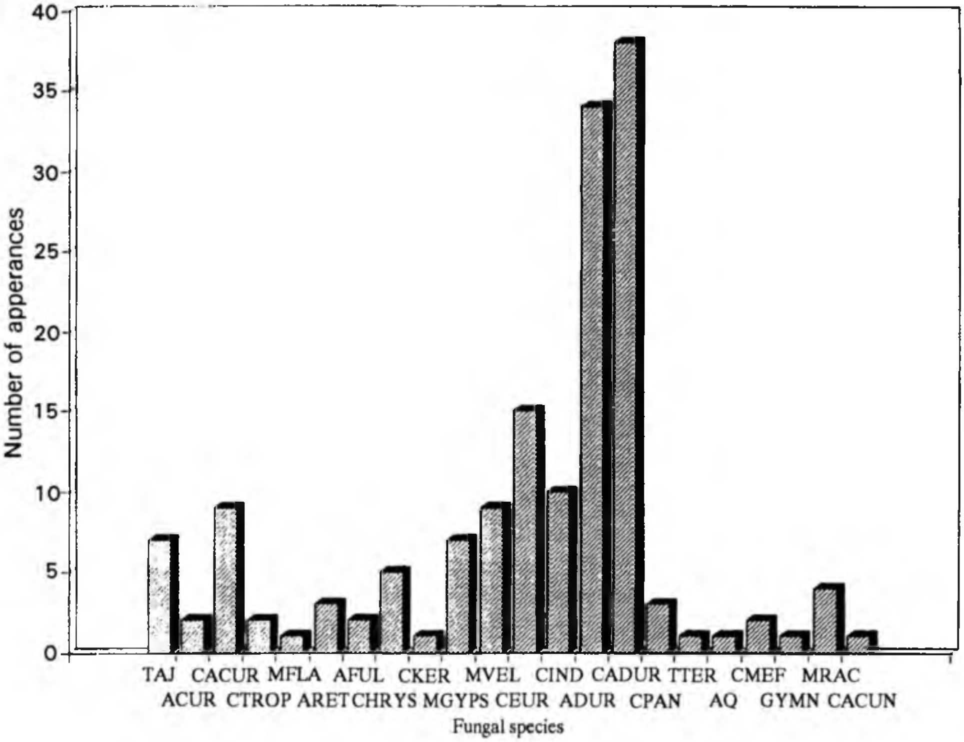

Fig. 1. Occurence of keratinolytic fungi in the ash heap ground in Sosnowiec
Abbreviations: TAJ – *Trichophyton ajelloi* ACUR – *Arthroderma curreyi* CACUR – *Chryso-sporium* an. *Arthroderma curreyi* CTROP – *Chrysosporium tropicum* MFLA – *Malbranchea flava* ARET – *Aphanoascus reticulisporus* AFUL – *Aphanoascus fulvescens* CHRYS – *Chrysosporium* an. *Aphanoascus reticulisporus/fulvescens* CKER – *Chrysosporium keratinophilum* MGYPS – *Microsporum gypseum* complex MVEL – *Myceliophthora vellerea* CEUR – *Chrysosporium europae* CIND – *Chrysosporium indicum* ADUR – *Aphanoascus durus* CADUR – *Chrysosporium* an. *Aphanoascus durus* CPAN – *Chrysosporium pannicola* TTER – *Trichophyton terrestre* complex AQ – *Arthroderma quadrifidum* CMEF – *Chrysosporium mephiticum* GYMN – *Gymnoascus sp.* MRAC – *Microsporum racemosum* CACUN – *Chrysosporium* an. *Arthroderma cuniculi*

2-celled, 2.7–9.7 × 1.3–3.3 μm. Macroconidia less numerous, growing on simple, unbranched conidiophores, hyaline, multiseptate, fusiform, elliptical and cigar-shaped, relatively thin-walled (0.8–1.6 μm), generally smooth, some of them verruculose or verrucose, on SGA regularly shaped and (1–6) – celled, on Sabouraud 1:10 + salts (1–12, usually 7–9) – celled, often curved or even branched, 24.4–71.0 × 8.0–12.6 μm (Fig. 2, 3). No chlamydospores observed. No ascamata observed on the hair bait and when crossed the strains isolated. One of the strains (IEIA 672A) was sent to the Microfungus Collection and Herbarium, University of Alberta, Edmonton, Canada (UAMH).

The fungus does not grow at 37°C but displays slow growth at 7°C, with a mean daily growth rate of 0.37 mm. Strongly keratinolytic, producing penetrating organs in the hair.

Material examined.

Living single-spore strains (obtained by the dilution method): IEIA 673A and IEIA 674B, ash heap ground, Sosnowiec, Poland.

DISCUSSION

M. racemosum was first described in 1965 in Venezuela where was isolated from the healthy hair of a forest rat [2]. Subsequently, the species was recovered in Amazonias soil, and also from forest soil and animal hair (squirrel, badger) in Romania [1, 12]. In 1982, soil isolates of *M. racemosum* were also reported in Czechoslovakia [2]. Pathogenic properties of the fungus were demonstrated both experimentally and naturally [1, 2, 4, 5, 11, 12]. Its perfect state, *Nannizzia racemosa* Rush-Munro, Smith *et* Borelli, was described by Rush-Munro *et al.* [12]. In 1986 [21], a new combination, *Arthroderma racemosum*, was proposed.

M. racemosum is very close to the members of the *M. gypseum-fulvum* group. The first is characterized by the abundant stick- and cartridge-shaped microconidia disposed in clusters whereas in the *M. gypseum-fulvum* group the microconidia are usually clavate and never the prevailing micromorphological elements [14]. Because of the abundance of microconidia, the micromorphology of *M. racemosum*, at first glance, is more characteristic for *Trichophyton* than *Microsporum* species. Subsequently, examination of macroconidia reveals that in *M. gypseum-fulvum* group they are typically verruculose to verrucose while in *M. racemosum* a considerable part of them is smooth that also resembles *Trichophyton*. Additionally, the number of septa, generally uniform in *M. gypseum* (up to 6), is usually higher in *M. racemosum* (up to 12). Finally, the production of the characteristic pink-violet pigment by *M. racemosum* is of particular taxonomic significance. The pigment appears after 3 weeks of incubation but only on SGA and PDA.

Although KF in soils and other habitats in Poland have been a subject of extensive investigation for more than thirty years. there is no reference to *M. racemosum* isolates

Fig. 2. Microconidia of *M. racemosum* (× 400; phase contrast)

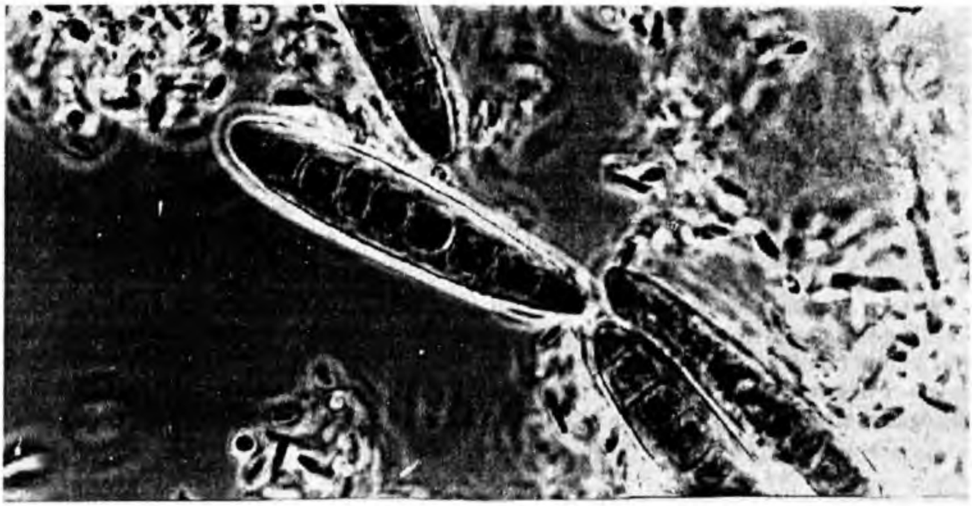

Fig. 3. Macroconidia of *M. racemosum* (× 1000; phase contrast)

in available literature [6, 7, 9, 10, 16]. This is, therefore, the first report on its isolation in our country. It is also to note that a similar fungus was previously isolated from sewage sludge but misidentified as a member of the *M. gypseum-fulvum* group [18]. This data indicates that *M. racemosum* is a rare but widely distributed pathogenic fungus.

The habitat of the ash heap ground is of particular ecological interest. This ground is heterogenous as regards physico-chemical features. In general, it is characterized by the high ignition losses and organic carbon (Tjurin), derived from the partly combusted coal, low salt concentration, and pH close to neutral. Apart from the Zn content, the concentration of the other heavy metals were low. The untoxic character of the ground was showed by the watercress sprout test [15]. Under these rather favourable conditions, lower and higher plants as well as micro-organisms were poorly represented [17]. This was probably caused by the low nutrient concentration and the deficiency of available carbon sources in the surface layer of the ground. In addition, the ground tended to get dried rapidly during the intensive insolation and dry whether.

In contrast, Kf were found to occur relatively aboundantly in the ash heap ground. *M. racemosum* was strictly associated with this habitat. This finding is rather opposed to previous observations indicating the association of *M. racemosum* chiefly with forest soils and animal hair. The presence and good growth of the species in the ash heap ground can be explained in the following way: The fresh coal combustion ash a material devoid of living organisms and keratin remains needed for the growth of KF. The only important source of keratin and KF in the surroundings of the ash heap is the municipal landfill site. This object considerably influenced, *via* the air, the ash ground mykoflora. Some KF, including *M. racemosum*, found favourable conditions to survive in this habitat. However, this dermatophyte did not occur in municipal landfill site's ground. This can be explained by the unfavourable ecological conditions of the ground and the possible restricted abilities of the fungus to compete with other KF on hair bait.

Municipal solid wastes and the habitats influenced by municipal landfill sites can be real sources of pathogenic fungi [17]. Isolation of pathogenic dermatophytes from this environment cannot be ignored from an epidemiological point of view.

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PIERWSZA IZOLACJA *MICROSPORUM RACEMOSUM* BORELLI

Streszczenie

W toku badań grzybów keratynolitycznych w glebach zanieczyszczonych przez wysypiska odpadów komunalnych z hałdy popiołów kopalnianych wyizolowano rzadki gatunek chorobotwórczego dermatofita – *Microsporum racemosum* Borelli. W niniejszym artykule przedstawiono makro- i mikroskopowe właściwości tego gatunku, jak również przedyskutowano potencjalne ekologiczne czynniki wpływające na występowanie grzyba w gruncie badanej hałdy.

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