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MOLD ON THE ROCKS: A LITHOBIONTIC FUNGUS FROM THE SEDIMENTS OF RADIOACTIVE THERMAL WATERS IN THE GASTEIN VALLEY, AUSTRIA

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The waters from the radioactive thermal springs at the lower western slope of mount Graukogel arrive at the Austrian spa Bad Hofgastein via an eternite pipeline and are then distributed to various localities by means of (smaller) iron tubes. The main duct from the springs to the spa, as well as the local distribution system, were both found to contain sediments. They are formed by the rock debris originating from the microbial decay constantly occurring at the colonized rocks of the springs. This process results in the leaching of silica, uranium, zinc and other metals and minerals and the formation of miniscule rock particles, resulting from the weathering of the stone (HEINEN & LAUWERS in press, LAUWERS & HEINEN 1985). The sediment from the main pipeline was found to contain considerable amounts of uranium (unpubl.), derived from living and dead bacteria, settled within the sediment, which accumulate this metal. They are also responsible for the relatively high percentages of organic matter in the samples from sediments and springs (HEINEN & LAUWERS in press).

In samples from sediments and the rock substratum of the thermal springs, fungi had been observed frequently with light- and electron microscopy (HEINEN & LAUWERS 1985), but so far we failed to isolate them from the complex rockcolonizing microflora, because they were always overgrown by fast-growing bacteria. This could finally be overcome by applying the (slightly modified) method of WEBLEY et al. (1960): About 15 ml of sterilized agar solution (1%) were poured over 0.6 ± 0.1 g of sediment in petridishes just before hardening and the plates were then incubated at 50 °C. After two days, hyphae with and without sporangia began to develop on, around and from inside the rock particles from the main pipeline sediments (*fig. 1*). Fungal growth on the sediment from an iron tube was less and considerably slower. Because the medium contained no organic substrates, the by definition heterotrophic fungi must obtain their carbon and energy supply from low-molecular organic compounds present in the sediments. Soils in general and these kinds of sediments in particular, always contain extracellular enzymes that catalyze the degradation of organic macromolecules

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Table 1. Enzymic equipment of the fungus isolated from the sediments of the main pipeline. The mold was harvested from cherry-agar after 24 h at 50 °C, ground in a Potter tube and used for api-test, api-zym and conventional assays at 50 °C.

substrate/enzyme		substrate/enzyme	
sacch., ara, man	+	leucine arylamidase	20
glu, mal, fru	+	valine arylamidase	5
rha, gal, mne	–	cystine arylamidase	<5
sor, gly	–	trypsin	0
amylose	++	chymotrypsin	<5
H_2S	(+)	phosphoamidase	>40
urease	+	α-galactosidase	20
nitrate reductase	–	β-galactosidase	0
acid phosphatase	>40[1]	β-glucuronidase	0
alkaline phosphatase	>40	α-glucosidase	5
esterase C-4	20	β-glucosidase	30
esterase C-8	5	α-mannosidase	10
esterase C-14	0	α-fucosidase	0
protease[2]	0	N-acetyl-β-glucosamidinase	>40

[1] This and all following enzymes: nanomoles substrate hydrolyzed (api-zym test).
[2] Tested on caseine-containing cherry agar.

from dead cells and other sources. These enzymes are secreted by the microbes present in the soils and sediments and are also released by dead or dying cells together with a variety of intracellular degrading enzymes (SKUJINS 1967). This way compounds are provided which can serve as substrates for the molds. The fungus itself is also well-equipped to convert complex substrates with especially high activities of phosphatases, esterases, α-galactosidases, β-glucosidases and other enzymes suitable to utilize bacterial remnants (*table 1*).

The molds growing from both sediments developed aerial hyphae, often branched and topped by sporangia, with mineral particles clustered around them (*fig. 2*). That these crystals indeed adhere to the surface of the hyphae could be concluded from stereomicroscopic observation and plates prepared for scanning electron microscopical examination (*fig. 3* and *4*). The mass of minerals attached to the surface varied considerably: some hyphae were almost entirely covered either by amorphous layers or crystalline minerals, others showed no contamination at all, or only an irregular succession of free and covered areas. This means, that the mold produces a sticky outer layer, which most probably attacks (and partly dissolves) the rock material. The intricate interaction between mold and rock observed in several samples (*fig. 5* and *6*) even suggests, that this fungus belongs to the endolithic type which can penetrate the material by boring tunnels into the rock (GOLUBIĆ et al. 1975, GOLUBIĆ 1979).

Taxonomically the fungus was determined as belonging to the genus *Aspergillus*, assuming a blue-green appearance after sporulation.

Fungi are common members of rock-degrading microbial communities (WEBLEY et al. 1963, MÜLLER & FÖRSTER 1963) and often participate in the leaching and/or accumulation of heavy metals like iron, titanium, uranium and others.

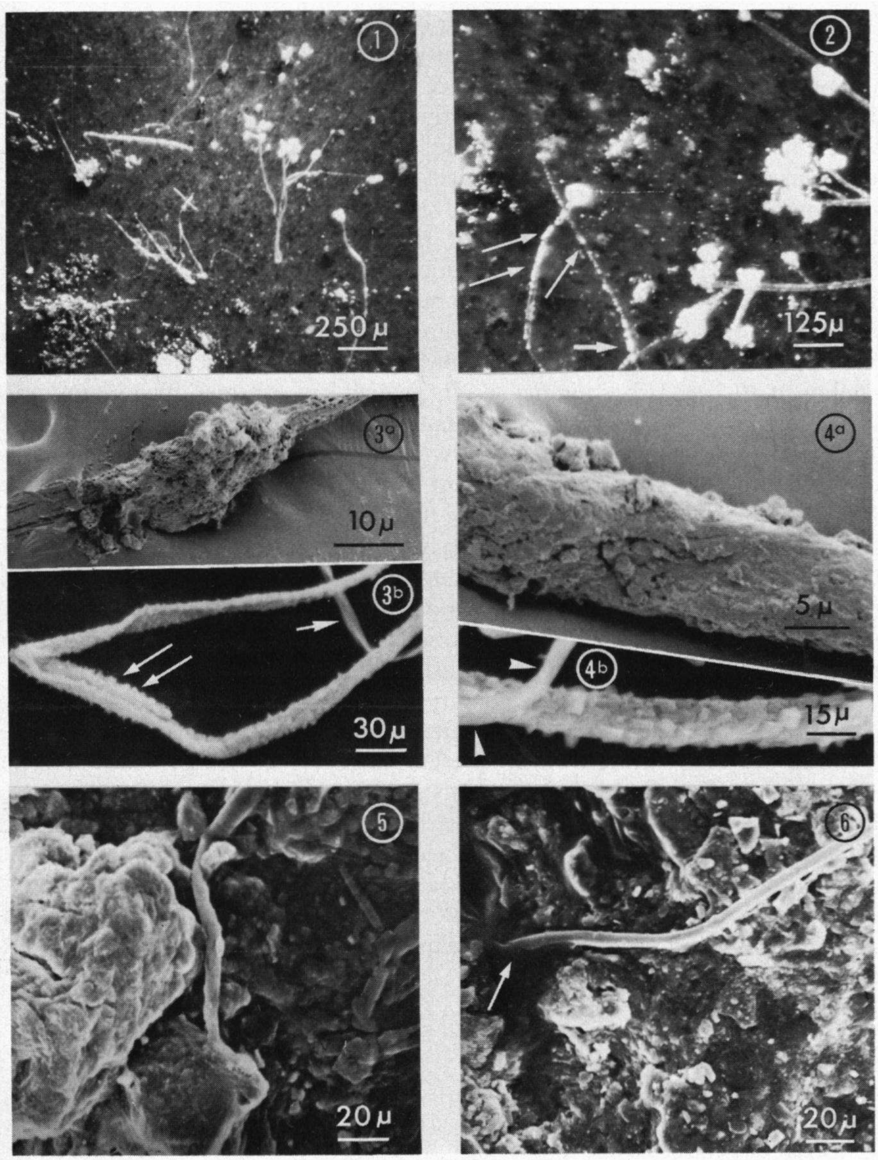

Fig. 1: Hyphae growing out of the sediment-agar medium at 50 °C; many are branched and have formed sporangia at the top.

Fig. 2: To a varying extent the hyphae are covered with mineral particles (arrows).

Fig. 3: At certain areas of the hyphae the attached material was found to extend to big chunks (a), other parts are continuously covered (b); at one part the hypha is visible within the coverage (double arrow), but there are also regions free of mineral particles (arrow).

Fig. 4: The hyphae can be covered by amorphous layers (a) as well as crystalline material (b), but free areas are also always present (arrows).

Fig. 5: A hypha from within the sediment-agar originating from (or growing into) a rock particle (arrow).

Fig. 6: Another hypha disappearing into the rock material is embedded in a smooth (organic?) mass at the location of penetration (arrow).

This process is enhanced when pyrite or other sulfides are available for the chemoautotrophic bacteria of the community, as in the thermal springs under study (HENDERSON & DUFF 1963, SILVERMAN & MUNOZ 1971, SCHEMINSKY et al. 1972, RALPH, 1979). In that case, the bacteria can oxidize the pyrite (in the ore or water) to ferric sulfate and sulfuric acid. At the resulting (locally) high acidity uranium, for instance, can be solubilized as uranyl sulfate (TAYLOR 1979).

In order to examine a possible involvement of the fungus in such processes, hyphae from the plates were collected, rinsed in distilled water to remove the bulk of the adhering mineral particles and dried at 115 °C. The uranium content was determined by liquid scintillation counting. Cut-out sections from the sediment-agar, on which the fungus had grown, were treated similarly to obtain the background uranium concentration. The results showed that the hyphae contained 10.2 μg U per mg dry weight, which is a considerable amount and in the same order of magnitude as found in the microbial mats of the thermal springs at Badgastein (HEINEN & LAUWERS in press). However, if this value is correlated to the 24,2 μg U/mg present in the sediment-agar from which the fungus developed, one has to conclude that the fungus contains only 42,2% of the uranium available. In contrast to the bacteria, which (under slightly modified conditions) were found to accumulate uranium while growing on the sediment (unpubl.), the mold probably does not partake in the acquisition of the metal. The radioactivity measured in these samples could just as well originate from the mineral particles clustering to the surface of the hyphae.

The data obtained on the elementary composition of the mold hyphae by X-ray micro-analysis confirmed this interpretation (*table 2*): the bioelements P, S and K (and also Ca) were always present, but all other elements showed wide variations. In different hyphae, but also at different locations of single hyphae, the presence of Si, Fe, Al (but also S) varied from trace amounts to abundance, while Mg, Mn, Cr, Zn and Cu were often clearly detectable but definitely absent at other locations. This again seems to be due to the varying amounts of metal-containing sediment particles that stick to the surface of the mold (*fig. 3* and *4*). Clad in its mineral armor, this fungus appears to grow not only on, but almost in the rocks.

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Table 2. Elements determined in hyphae collected from sediment-agar medium, according to X-ray micro-analysis.

	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Cu	Zn
hypha 1, area a	–	?	++	+++	+	+	(+)	+	++	(+)	–	?	+	+	(+)
hypha 1, area b	(+)	(+)	+	++	+	++	+	+	(+)	–	–	–	(+)	–	(+)
hypha 1, area c	–	+	(+)	+	+	+++	+	+	(+)	(+)	–	+	+	–	?
hypha 2, area a	+	(+)	++	++	+	+	(+)	++	++	+	+	(+)	+	+	+
hypha 2, area b	(+)	(+)	+	++	++	++	+	+	(+)	–	–	–	(+)	–	–
hypha 3, center	–	+	(+)	(+)	+	++	(+)	++	+	–	–	–	(+)	–	–
hypha 4, area a	(+)	?	+	+	++	+	(+)	+	+	–	–	–	+	–	–
hypha 4, area b	+	(+)	++	+++	+	+	+	+	++	+	+	(+)	+	+	+

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