Microscopic fungi in the Zbrašov aragonite caves
Mikromycety Zbrašovských jeskyní

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The aragonite coating in some parts of the caves in Zbrašov (northern Moravia, ČSFR) is strewn with fragments of textile fibres, originating probably from the clothes of cave visitors. These filaments as well as the aragonite in their close vicinity are colonised by microscopic fungi. About fifty taxa have been identified. Spores of fungi in the aeroplankton in the caves mostly belong to other species than do occur on the aragonite. The species composition of the fungal community on aragonite is probably influenced by the temperature inside the caves.

Aragonitová výzdoba ve Zbrašovských jeskyních je znatelně znečištěna úlomky textilních vláken pocházejících s největší pravděpodobností z oděvů návštěvníků. Tato vlákna i aragonit v jejich bezprostřední blízkosti jsou osídleny mikroskopickými houbami. Celkem bylo identifikováno asi 50 taxonů mikromycetů. Spory hub ve vzdušném prostoru jeskyní patří velkou většinou k jiným druhům než jsou ty, které se vyskytují na aragonitu. Na formování společenstva mikromycetů na aragonitu má pravděpodobně vliv také teplota v jeskyních.

Introduction

The aragonite caves of Zbrašov were discovered in 1912 and made open to the public in 1926. The number of visitig people is relatively high – about 50.000 in a year – and has a rising trend. Aragonite occurs there in the form of druse-shaped aggregates of thin needles up to 8 cm long and 0.2 to 3 mm thick. Needles form bushy formations sometimes classified as aragonite anthodites. These aragonite druses are more or less grown through and about by wart-like aggregates of calcite and amorphous aragonite. Mixtures of magnesite and huntite are further minerals forming part of aragonite aggregates. A continuous aragonite coating is known practicaly only in the so called Jurik's dome, where it covers part of the roof and walls of the formation called ''Curtain'' (''Opona'').

In the last 15 years the cave operators have observed progressive greying of the aragonite druses and in places also their red and brown pigmentation. Later, falling away of small needles of aragonite crystals and globules of magnesite with huntite, several mm in diam., has been seen. The overall appearance of the ''Curtain'' in Jurik's dome evoked an impression as if covered with dust (Morávek 1992).

In a preliminary analysis of samples of aragonite from Zbrašov in 1990 in the Czechoslovak Collection of Microorganisms, fragments of textile fibres were found on the surface of aragonite as well as on amorphous minerals. Several species of microscopic fungi and bacteria were isolated from these substrates. Linking up with these preliminary results, in our present study we turned our attention above all to the identification of textile fibres and to the isolation and determination of micromycetes.

Material and Methods

Sampling was performed on May 28th 1991 in the Zbrašov caves in Jurik's dome, from the "Curtain". Samples (pieces of crystalline and amorphous minerals and textile fibres) were collected with forceps into sterile bottles. Inside the caves, there is a constant temperature of 15°C. Crystal fragments, pieces of amorphous aragonite and other minerals and minute tufts of textile fibres were put onto two sets of petri dishes with three kinds of nutrient media: malt extract agar, soil agar and water agar (Gams et al. 1987). One set was incubated for one week at 25°C, the other for three weeks at 15°C. During the incubation the dishes were currently inspected, the fungi isolated into pure culture and identified.

Beside this, four petri dishes 9 cm diam. (two with soil agar, two with malt extract agar in a 0.5 cm thick layer, were exposed in the Jurik's dome under the "Curtain" for 4 hours. During the exposition there was normal operation (at that time an expedition of school children passed round). After transfer to the laboratory, two of the dishes (one of each medium) were incubated for one week at 25°C and two at 15°C for three weeks. The obtained colonies were treated as explained above.

Samples for evaluation by electron microscope consisted of textile fibres and pieces of crystalline and amorphous minerals. They were divided into smaller portions which were metal plated by pulverizing Au cathode in argon atmosphere. Micrographs were taken with a scanning electron microscope JSM-35 of the Co. JEDL.

Results and Discussion

A. Documentation by means of the scanning electron misroscope.

The "dust" on the mineral surface consists of tufts of intertwined textile fibres. Cotton as well as wool and hairs of Angora rabbits were demonstrated. Beside natural, also synthetic fibres were identified, with greatest probability of polyester or polypropylene, some of them with typical fibrillation at the end due to mechanical damage when using the clothing - wearing it. Viscose fibres with a hint of certain degree of degradation were also present. The fibre fragments originate most probably from clothing of cave visitors: during the movement of persons they fall away freely and by streaming air are carried onto the surface of aragonite on the roof of the "Curtain", thus forming the above "dusty" coat. The continuing process of crystallization causes that these fibres become covered with mineral particles and actually incorporated into the cave decoration. From Pl. I Fig. 1 one can judge that textile fibres under the conditions in the cave represent a suitable substrate for the growth of microscopic fungi which in turn can also pass onto the surface of aragonite (Pl. I Fig. 2).

B. Microscopic fungi in the air of the Zbrašov caves and on the aragonite decoration.

The list of micromycetes identified is given in Tab. 1. According to the source of isolates, the micromycetes found in the cave fall into two groups: a) taxa whose spores are a part of the cave aeroplankton (A25 + A15); b) taxa occurring in close vicinity of aragonite, either directly on the minerals or on textile fibres (M25 + M15). These two groups have only 4 taxa in common (c. 8 %). This suggests that at present the microflora on aragonite is so far not dependent on the presumed continuing delivery of spores from

outside in connection with the operation regime of the caves; it probably forms a stable community which is renewed from its own sources.

Inside of these two groups a further differentiation may be stated according to the incubation temperature preference. In the aeroplankton, the number of psychro- and mesophylic species (in the sense of Ainsworth 1943, i.e. with the growth optimum below 20°C or above it, respectively) is roughly balanced, whereas the number of species from the mineral and from textile fibres obtained after incubation at 15°C (the obvious temperature in the Zbrašov caves) is 1.5 times as high as that obtained after incubation at 25°C. This also points to a more or less specialized group of microfungi occurring in this habitat.

A conspicuous fact in comparing the species from the aeroplankton with those from the aragonite is the relative frequent occurrence of *Verticillium sp., Aspergillus versicolor, Acremonium cf. atrogriseum, A. cf. verruculosum,* less *A. murorum* also directly on fragments of aragonite and the other minerals. Of particular interest are *A. cf. verruculosum* and *A. cf. atrogriseum*, the former appearing in abundance only among the isolates from 15°C, the latter from 25°C. According to available literature it was possible to determine them only approximately and the fact that they might be new species cannot be excluded. In cultures of *Verticillium sp.* an ascomycete closely resembling the insufficiently known *Torrubiella minutissima* Lagarde (1917) appeared repeatedly. It was described as a mycoparasite on *Cordyceps* from French caves. White fusiform ascomata grew in our cultures particularly at 15°C under slow drying. Single perithecia without stroma, long cylindrical asci with thick truncate apex and needle-shaped ascospores are in accordance with the concept of *Torrubiella* Boudier accepted by Samson et al. (1989). However, Müller and von Arx (1973) stress the separation of ascospores into part-spores inside the ascus as generic feature. Two species of this genus are reported in connection with *Verticillium* spp. sect. Prostrata (Gams 1971), but our fungus does not match the description of any.

The aeroplanktic spores belong partly to typical air-borne taxa like *Cladosporium* Link, *Aspergillus* Micheli and *Penicillium* Link, partly to representatives of fungi on plant debris, in soil, or to entomopathogenous species. Remarkable is the occurence of *Aspergillus speluneus* which was described from caves in the U.S.A. (Raper et Fennell 1965).

When comparing the ecological characteristics known from the literature (Domsch et Gams 1972, Domsch et al. 1980) for species isolated by us from aragonite, we find that most of them belong to xerotolerant ones, occurring in physically (sand dunes, desert soils) or physiologically (salty soils) dry habitats. Other species are known from calcareous soils (*Botrytis cinerea, Penicillium aurantiogriseum, P. canescens*) and a number was isolated

from caves or uranium mines (*Alternaria alternata, Aspergillus versicolor, A. ustus, Botrytis cinerea, Cladosporium spp., Paecilomyces marquandii, Penicillium aurantiogriseum, P. brevicompactum, P. chrysogenum, P. implicatum, P. viridicatum*).

Of interest is also a relatively frequent occurrence of basidiomycete anamorphs: all of them are of the arthroconidial type. Basidiomycetes are known as having complex enzyme equipment for decomposing hardly degradable substrates such as cellulose, lignin etc. In the Zbrašov caves they probably colonize textile fibres.

A special position in the cave microflora is that of entomopathogenic fungi, which probably enter the caves with their hosts. Papers on this theme were published from French and Algerian caves (Lagarde 1913, 1917, 1922). Some of his findings have recently been confirmed. Rombach et Samson (1983) and Samson et al. (1984) point out the connection of the so called troglophilic insects with some genera like *Beauveria* Vuill., *Hirsutella* Pat., *Paecilomyces* Bain., *Stilbella* Lindau and *Tritirachium* Limber. From our isolates *Engyodontium* (syn. *Beauveria*) *album*, and *Paecilomyces farinosus* belong here.

A negative effect of micromycetes on mineral substrates was reported from Spain (De la Torre et al. 1990). Scanning electron microscope studies showed coating and corrosion of mineral particles of a historical building wal material by hyphae of *Penicillium glabrum* (as *P. frequentans*) and also aggregation of particles around the mycelium. The fungus produced organic acids in vitro. Also fungi isolated from sandstone monuments (Kuroczkin et al. 1990) produced organic acids, caused dilution of calcium carbonate and formation of calcium oxalate. Grote et al. (1990) reported oxidation of divalent manganese to its insoluble tetravalent salts with the aid of micromycetes isolated from rocks and stone buildings. The ability of producing manganese salts and accumulating manganese in hyphae was also demonstrated.

In the Zbrašov caves no traces of ferric chloride have been found (Morávek 1992), and thus the origin of red and brown pigmentation of aragonite remains so far unexplained. Due to a relatively large amount of micromycetes isolated from its surface, the discolouration might rather be ascribed to their activity. Some of the species found are known as producers of exogenous pink or red pigment: *Emericellopsis terricola, Aspergillus versicolor*, and some species of *Penicillium*. During decomposition of organic substances such as wool, cotton or insect bodies, brown humic compounds may be formed.

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Tab. 1 Genera and species of fungi isolated from air (A25, A15) and from aragonite aggregates as well as from textile fibres (M25, M15), at incubation temperature 25 and 15°C.

	A25	A15	M25	M15
Acremonium murorum (Corda) W. Gams			+	+
Aspergillus versicolor (Vuill.) Tiraboschi			+	+
Basidiomycetes sp. div. (anam.)			+	+
Paecilomyces farinosus (Holm:Fr.) A.H. Brown et G. Smith			+	+
Penicillium janczewskii Zaleski			+	+
Penicillium viridicatum Westling			+	+
Acremonium cf. *atrogriseum* (Panasenko) W. Gams			+	
Acremonium cf. *verruculosum* W. Gams et Veenbaas-Rijks				+
Acremonium sp.			+	
Alternaria alternata (Fr.:Fr.) Keissler				+
Doratomyces nanus (Ehrenb.) Morton et G. Smith			+	
Emericellopsis terricola van Beyma			+	
Gliocladium catenulatum Gilman et Abbott			+	
Monocillium granulatum (Fuck.) W. Gams				+
Mortierella alpina Peyronel				+
Mortierella hyalina (Harz) W. Gams				+
Mucor hiemalis Wehmer				+
Paecilomyces marquandii (Massee) Hughes				+
Penicillium aurantiogriseum Dierckx				+
Penicillium brevicompactum Dierckx			+	
Penicillium canescens Sopp				+
Penicillium chrysogenum Thom				+
Penicillium citreonigrum Dierckx			+	
Penicillium expansum Link				+
Phoma chrysanthemicola Hollos				+
Sporothrix sp.				+
Verticillium psalliotae Treshow			+	
Verticillium sp.				+
Botrytis cinerea Pers.: Fr.	+	+		+
Cladosporium cladosporioides (Fres.) de Vries	+	+		+
Cladosporium herbarum (Pers.: Fr.) Link	+		+	+
Cladosporium macrocarpum Preuss	+			+
Penicillium crustosum Thom	+		+	
Basidiomycetes sp. div. (anam.)	+	+		
Engyodontium album (Limber) de Hoog	+	+		
Phoma eupyrena Sacc.	+	+		
Acremonium kiliense Grütz		+		
Aspergillus niger van Tieghem		+		
Aspergillus speluneus Raper et Fennell		+		
Aspergillus ustus (Bain.) Thom et Church	+			

Tab. 1. continued

	A25	A15	M25	M15
Aspergillus sp. (A. nidulans group)		+		
Aureobasidium pullulans (de Bary) Arnaud		+		
Chrysosporium merdarium (Link) Carmichael	+			
Cladosporium sphaerospermum Penzig	+			
Engyodontium rectidentatum (Matsushima) W. Gams et al.	+			
Epicoccum nigrum Link	+			
Oidiodendron truncatum Barron		+		
Paecilomyces lilacinus (Thom) Samson	+			
Penicillium griseoroseum Dierckx	+			
Penicillium implicatum Biourge		+		
Zakatoshia sp.	+			

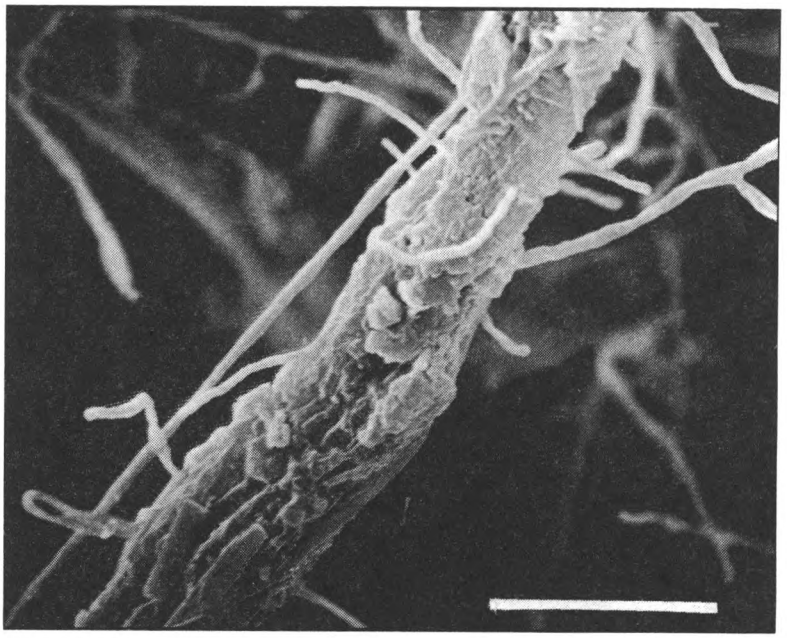

Plate I, Fig. 1. Textile fibre with microorganisms on the surface. Bar = 10 μm. Photo V. Kalousková.

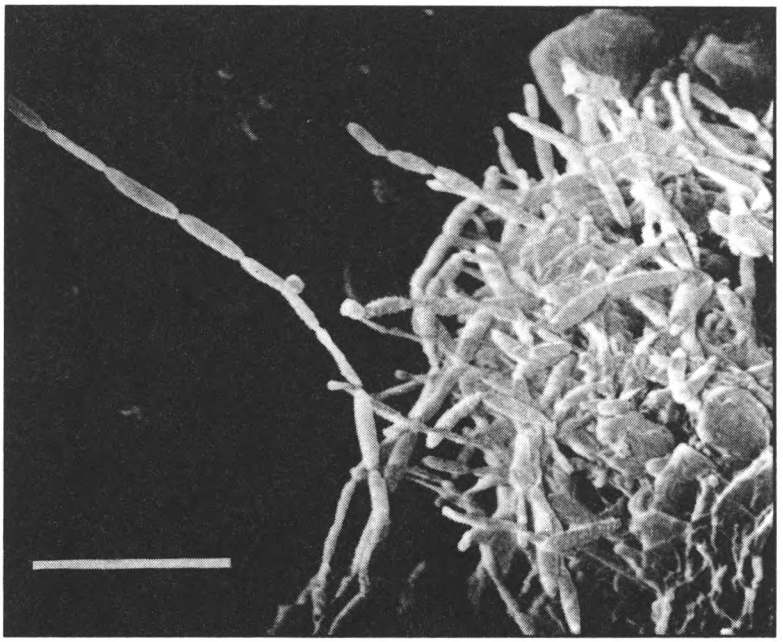

Plate I, Fig. 2. Microorganisms on the aragonite surface. Bar = 10 μm. Photo V. Kalousková.