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# Has the prehistoric ice-man contributed to the preservation of living fungal spores?

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**Abstract:** There are arguments against the conclusion drawn by Haselwandter and Ebner that fungal spores have survived for some 5300 years on hay padding in the leather boots of a frozen body discovered in the Austrian Alps. According to cryobiological experience, long-term survival of fungal spores is very unlikely at temperatures fluctuating between zero and  $-40^{\circ}\text{C}$ . It is quite possible that living spores of these common species have recently reached this substratum.

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**Key words:** Ice-man; *Absidia*; *Chaetomium*; Fungal spore; Longevity; Cryobiology

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Haselwandter and Ebner [1] succeeded in isolating *Absidia corymbifera* and *Chaetomium globosum* from hay used as padding in leather shoes of a prehistoric man whose frozen body was found in the Austrian Alps. They concluded that the fungal spores giving rise to these cultures had survived for some 5300 years on this substratum. However, doubts about their conclusion have appeared in the popular press [2]. The purpose of this article is to articulate the doubts in more detail. From cryobiological experience [3] we know that temperatures between zero and  $-40^{\circ}\text{C}$  are not suited for long-term preservation of fungi. In a moist environment the surrounding water will freeze between 0 and  $-10^{\circ}\text{C}$ . When a thin

layer of water with a high osmotic value remains present, the spore will dehydrate. In this condition it can survive for several years, but at temperatures above  $-40^{\circ}\text{C}$  there is still some metabolic activity and damage will occur to proteins and membranes. When spores freeze in dry conditions and no dehydration occurs, ice crystals will form inside the spores at temperatures between  $-20$  and  $-40^{\circ}\text{C}$ . These crystals will increase in size and the resulting damage is usually lethal. Under both conditions fungal spores will not survive for 5000 years at temperatures above  $-40^{\circ}\text{C}$ . It is only at temperatures of liquid nitrogen or at least at  $-130^{\circ}\text{C}$  that a permanent preservation of living fungal material is now possible, and only after a carefully determined freezing protocol [4]. While under dry conditions spores of *Chaetomium* and some other ascomycetes, and also conidia of genera like *Aspergillus* and *Penicillium* are known to survive for some decades, it

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is unlikely that this would be the case under periglacial conditions. All kinds of fungal spores are much more sensitive to temperature damage than bacterial endospores.

Is there any other way for the two observed fungal species to have reached the hay in the ice-man's shoes? The fungi isolated by Haselwandter and Ebner, *Absidia corymbifera* and *Chaetomium globosum*, are common species representing two different ecological groups.

*A. corymbifera* has been grouped as psychrotolerant thermophilic [5]. It is the commonest agent of mucormycosis of lungs, nasal sinuses, the cornea and other organs in both man and warm-blooded animals, and also of mycotic abortion in cattle; but it is also commonly isolated from soil, compost, dung, hay and similar substrata [5]. The fact that sheep are herded every year in the area where the ice-man was found might be a sufficient explanation for the isolation of this fungus from spores translocated by melting waters.

*Chaetomium globosum* is a very common, ubiquitous cellulolytic fungus. It is most often found on all kinds of plant substrata and dung [5]. Its temperature minimum for growth is around 4°C [6]. There is at least one report of its isolation from soil of a snow bed in 2630 m altitude in the Southern Alps [7]. Temperatures suitable for its sporulation would even be reached at the locality where the ice-man was found during a few days a year. Though its spores are not normally airborne, they are easily spread by all kinds of

animal and they do not depend on an ice-man carrying spores from the valley into the mountains.

It is known that the ice-man's body was initially handled with few precautions to avoid contamination; it also is inevitable that all body parts got into contact with melting water in the superficial ice layers of recent years before the body was discovered. Therefore a microbial contamination of the hay sample becomes very likely, which can account for the presence of viable fungal spores throughout this material. We are therefore very reluctant to ascribe a very high age to such fungi.

## References

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