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IG: cultivating the fungi in pure cultures, their identification, writing the manuscript;
MT: collecting the stones, identification of lichen species, measuring the stone water-holding capacity, writing the manuscript.

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SHORT COMMUNICATION

Basaltic stones with epilithic lichens as a novel substrate for an osmotolerant fungus, *Aspergillus glaucus*

Isabella Grishkan*, Marina Temina

Institute of Evolution, University of Haifa, 199 Aba Khoushy Ave, Mount Carmel, Haifa 3498838, Israel

* Corresponding author. Email: grishkan@research.haifa.ac.il

Abstract

Aspergillus glaucus is a fungus able to tolerate low water activity of the environment. Its dense growth and sporulation were found on basaltic stones with epilithic lichens after 14 years of storage at a temperature of 4–7°C and relative humidity of 14–18%. Dust and soil particles deposited on the lichen thalli and dissolved in the water condensed on the stones during the storage period, apparently served as a nutrient source for the fungus. Probably, strongly xeric water regime on basaltic stones suitable for *A. glaucus* did not allow mesophilic fungi to develop and prevented the xerotolerant fungus from competition with other microfungi for nutrient sources. It is also possible that specific cellular mechanism associated with the production of chaotropic compounds (such as glycerol) supported germination and development of *A. glaucus* at low temperatures, which were considered non-optimal for the fungus.

Keywords

basaltic stones; epilithic lichens; low temperatures; osmotolerant fungus; sporulation

Introduction

Aspergillus glaucus (formerly *Eurotium herbariorum*) belongs to the group of osmo-, halo-, and xerotolerant fungi, which are known by their ability to tolerate low water activity and poor growth in high water activities (e.g., [1]). *Aspergillus glaucus* is a cosmopolitan fungus, mostly distributed in tropical and subtropical regions [2]. It was found in a great variety of substrates: soil of different types, sand dunes, salt marshes, outdoor and indoor air, pulp and paper, leather and cotton fabrics, etc. (see [2] and references therein). *Aspergillus glaucus* is a member of the native fungal communities in hypersaline water of salterns [3] and deep-sea sediments [4]. In Israel, *A. glaucus* was found in the Dead Sea water [5] and its coastal sand [6]. The fungus is known as one of the most important agents causing food spoilage when food products are stored at moisture contents with a range of 14–23% (e.g., [1]). It may produce biologically active secondary metabolites [7,8], including antimicrobial compounds [9]. The fungus can cause some infectious diseases in immunocompromised patients [10] and can also be dangerous even for immunocompetent persons [11]. Therefore, *A. glaucus* is a biologically and ecologically important fungus and any additional information about its habitats and peculiarities can be interesting and essential.

Our present study revealed a novel habitat for *A. glaucus* – basaltic stones with epilithic lichens stored in the cold room of the Institute of Evolution, University of Haifa (Israel), at a temperature of 4–7°C and a relative humidity of 14–18%. The aim of the study was to determine and to describe growth of the fungus on the stones covered by lichens. We hypothesized that the two types of stones stored in the cold room – basaltic

and chalk, could be overgrown by different fungal species due to different water-holding capacity of these stones and that a specific surface water regime of the basaltic stones could be appropriate for the development of a xerotolerant fungus.

Material and methods

Fifty basalt and fifty chalk stones completely covered by lichens were collected in Dalton, Upper Galilee, Israel, in January 2002. The stones were brought to the laboratory and dried at room temperature (23°C) for 2 weeks. After identification of lichen species, the stones were packed in paper bags, put in plastic boxes, and placed into the cold room for storage. In 2016, the stones were checked under the binocular magnification of ×45, and the growth of fungi was observed over lichen's thalli. For proper identification of the fungi, they were transferred into Petri dishes with malt extract agar (MEA) and MEA containing 30% of the Dead Sea water. In order to test the relationship between surface water regime of the stones and fungal growth, water-retaining capacity of the basalts and chalks was measured according to Aho and Weaver [12] and Kidron and Temina [13].

Results and discussion

After 14 years of storage, the dense colonies of *A. glaucus* were found on the lichen's thalli on all 50 basaltic stones (with *Aspicilia contorta* subsp. *hoffmanniana*, *Immersaria athroocarpa*, *Lecanora muralis*, *L. rupicola*, and *Xanthoria calcicola* as the most abundant species). The fungal colonies consisted mainly of the well-developed conidial sporulation (Fig. 1a) producing long chains of spinulose conidia (Fig. 1b). The sexual state of the fungus was also found (Fig. 1c), but cleistothecial fruit bodies were immature and did not contain any asci and ascospores. On MEA with 30% of the Dead Sea water, the fungus developed well-grown colonies (Fig. 2a) producing both asexual (Fig. 2b) and sexual (Fig. 2c) sporulation. The morphological structure and size of both kinds of sporulation as well as colony appearance fully corresponded to the description of *A. glaucus* (e.g., [14]). Notably, this fungus was completely absent on lichens covered the chalk stones (with *Aspicilia calcarea*, *Caloplaca aurantia*, *C. citrina*, *C. oasis*, and *Lecanora albescens* as the most abundant species). These stones were overgrown by a thin network of another fungal species – *Penicillium aurantiogriseum*, a typical soil fungus with worldwide distribution, which was frequently and abundantly isolated from various types of Israeli soils.

To the best of our knowledge, such specific substrate as basaltic stones covered by epilithic lichens and stored under continuous cold conditions is firstly reported as a habitat for the osmo- and xerotolerant species from the genus *Aspergillus*. Apparently, dust and soil particles deposited on the lichen thalli and dissolved in the water, which was condensed on the stones during the storage period, served as a nutrient source for *A. glaucus*. The reasons why different types of stones, basalts and chalks, stored at the same conditions during the same period of time were overgrown by fungal species with different life-history strategies [15], stress-selected (*A. glaucus*) and ruderal-selected (*P. aurantiogriseum*), are not fully understood. Such a phenomenon could be probably initiated by the different water-holding capacity of basalt and chalk. Our measurements of water-holding capacity showed that the basaltic stones absorbed 1.2-fold greater amounts of water, which evaporated 1.5-fold faster as compared to the chalk stones. Similar results were obtained when the water-holding capacity of basaltic and limestone substrates was compared [16]; the results were explained by less porous nature of basalt than that of limestone. It is likely, therefore, that a more xeric water regime on basaltic stones suitable for *A. glaucus* does not allow mesophilic fungi, such as penicillia, to develop and prevents the xerotolerant fungus from competing with other microfungi for nutrient sources.

The optimal temperatures for the formation of conidia and cleistothecia of *A. glaucus* were found at a range of 25–30°C [17]. In the experiment on the effect of water

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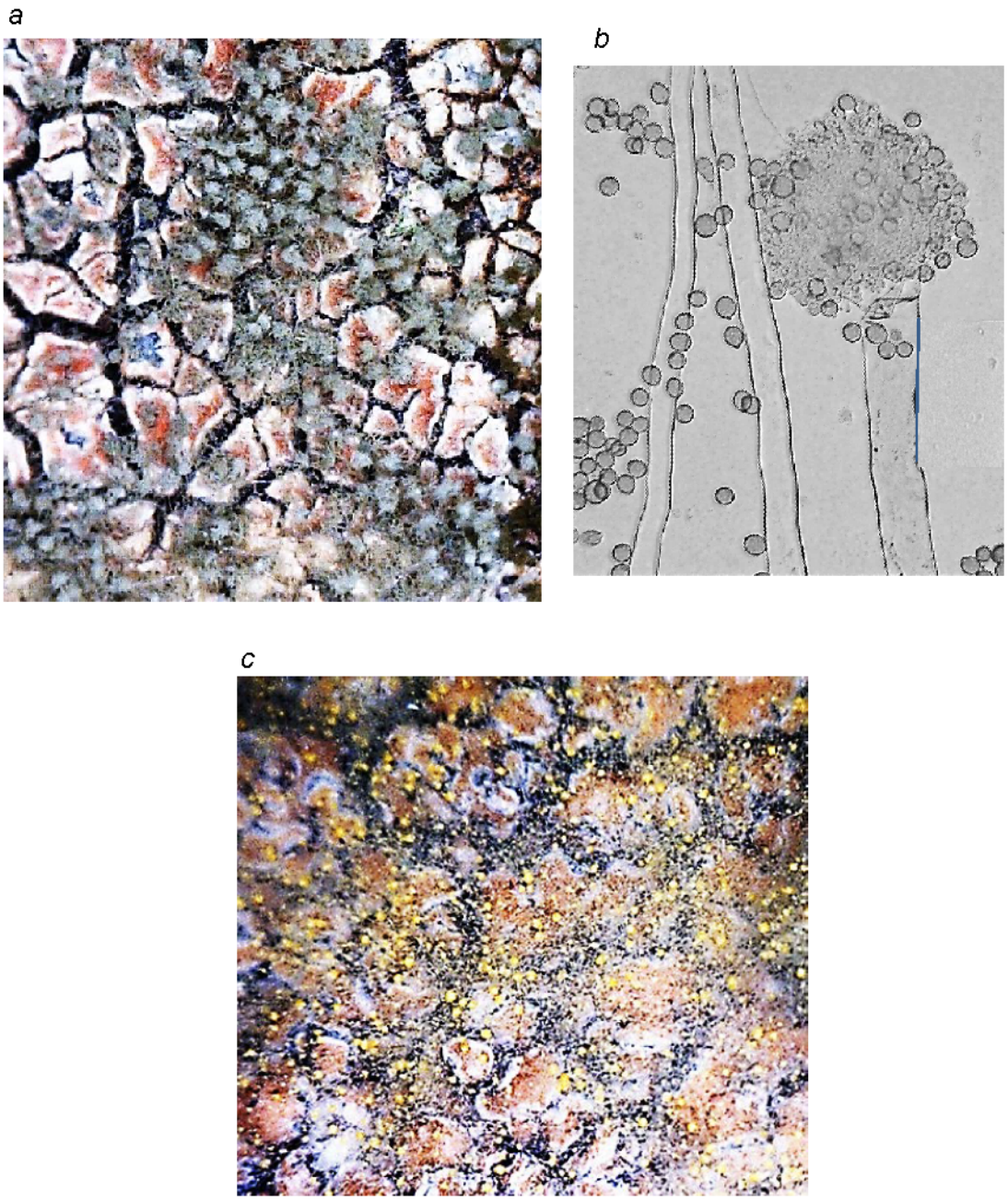

Fig. 1 Growth of *Aspergillus glaucus* on basaltic stone covered by epilithic lichens. **a** Conidial sporulation with long chains of conidia. **b** Conidial head and conidia. **c** Immature cleistothecia.

activity and temperature on germination and growth of *A. glaucus*, no growth of fungal strains occurred at 5°C [18], and only a few isolates of the fungus were able to grow at 10–15°C with less than 0.5 mm per day. By contrast, on the stones with lichen thalli, fully developed conidial sporulation and immature ascomata of the fungus were formed at a temperature of 4–7°C. Recent findings have shown that at low temperatures (+5°C and +1.7°C), growth rates of the *A. glaucus* strains were optimal on media containing fructose, which is known as a chaotropic compound (as well as glycerol, a compatible solute which is accumulated by osmophilic fungi in order to balance the internal and external osmotic pressure) [19]. The authors associated this phenomenon with the ability of chaotropic substances to enhance cellular activity at suboptimal growth temperatures and in that way extend the temperature borders for fungal survival and growth. In our case, it is possible, therefore, that the above cellular mechanism supported germination and development of *A. glaucus* at a temperature range which was considered non-optimal for the fungus. Thus, *Aspergillus glaucus*, which can survive

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at high osmotic pressure, increased concentration of salts, and xeric conditions, is able also to tolerate another kind of stress – continuous low temperatures. Moreover, at these temperatures, the fungus can successfully colonize a specific substrate – basaltic stones covered by different species of epilithic lichens.

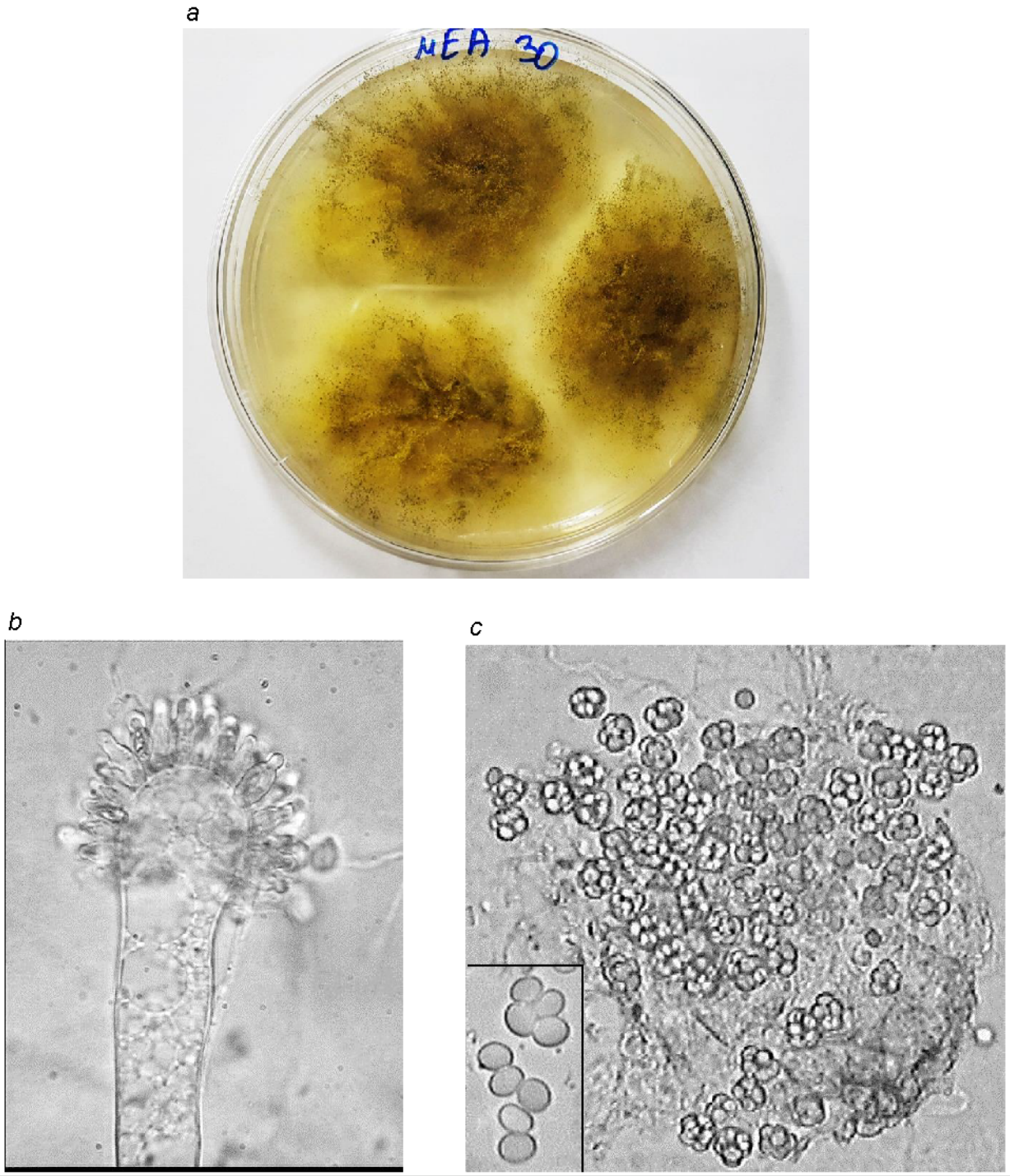

Fig. 2 Growth of *Aspergillus glaucus* on malt extract agar with 30% of the Dead Sea water. **a** Colony appearance. **b** Uniseriate conidial head. **c** Cleistothecium with asci and ascospores.

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