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Cellular responses of microcolonial rock fungi to long-term desiccation and subsequent rehydration

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Abstract: Melanised rock-inhabiting fungi are astonishingly resistant to environmental stresses. Also known as micro-colonial fungi (MCF), they are ubiquitous and even colonise bare rocks in deserts. To survive in nutrient poor and extremely stressful conditions, MCF have reduced morphogenetic complexity to a minimum, and rely on a broad spectrum of stress protection mechanisms. Although visual signs of carotenoid presence are masked by heavily melanised black cell-walls, we were able to isolate and characterise a variety of carotenoids (ß-carotene, ζ-carotene, phytoene, torularhodin and torulene) in the rock-inhabiting, relatively fast-growing strain A95. The desiccation/rehydration stress response was used to measure the ability of A95 to adapt to slow or fast changes in external conditions. Revival of MCF after prolonged desiccation and rehydration was documented by biochemical (analyses of lipids and protective pigments), cultivation, and microscopic methods. Survival of MCF is enhanced when desiccation is rapid and mycostasis is instant rather than following prolonged periods of low metabolic activity.

Key words: Anhydrobiosis, carotenoids, lipids, mycostasis, protective pigments, stress responses.

INTRODUCTION

Micro-colonial fungi (MCF) are the only inhabitants of varnished rock surfaces in arid regions (Staley *et al*. 1982) as well as ubiquitous settlers on sub-aerial rock surfaces in other climatic zones (Gorbushina *et al*. 1993, Urzí *et al*. 1995, Wollenzien *et al.* 1995, Sterflinger & Prillinger 2001, Ruibal 2004, Selbmann *et al*. 2005, Gorbushina 2007). Rock-inhabiting ascomycetes form a peculiar ecological group with simple morphology that exhibits a remarkable tolerance to stress (Palmer *et al.* 1987, Sterflinger & Krumbein 1995). Often stress resistance in micro-organisms is strongly correlated with an easy to simulate desiccation challenge, and here we chose desiccation / rehydration stress to investigate the capability of rock inhabiting MCF to adapt to slow or fast changes in external conditions.

Different pro- and eukaryotic organisms are able to withstand almost complete desiccation (Billi & Potts 2002). To test whether MCF are capable of surviving the removal of all but 0.1 g water / g dry weight (a condition that occurs during matric stress as well as through travel in simulated space), we took a representative strain of rock-inhabiting fungi (*Sarcinomyces petricola* strain A95) and measured its ability to revive. A matric stress (physical removal of water by desiccation in air) characteristic of the natural habitat of these fungi was applied for eight wks followed by sudden rehydration. Biochemical and ultra-structural changes in strain A95 were followed by analysing lipid- and pigment-composition as well as by microscopy.

MATERIAL AND METHODS

Strain

The black microcolonial fungus *S. petricola* strain A95 (= CBS 123872) was isolated from a marble rock surface near the Philopappos monument on Musaios Hill, Athens (Greece). This relatively fast growing strain belongs to the *Chaetothyriales* (Gueidan *et al*., 2008) and is maintained in the Geomicrobiology culture collection at the University of Oldenburg (ICBM, Oldenburg University, Germany).

Media, growth and desiccation conditions

Inocula were taken from two-wk-old pre-cultures grown on 2 % malt−extract agar (MEA) and suspended in physiological saline using a homogeniser (Ultra-Turrax T25, IKA Labortechnik, Staufen, Germany). Sterile nitrocellulose filters (Sartorius 0.22 µm, 25 mm diam) laid on MEA were inoculated with 50 µL of this suspension and fungal colonies allowed to develop in the sub-aerial environment (Fig. 1A). After eight wks of growth, the supporting filters were transferred to a desiccator. Two types of desiccation were employed: (i) fast removal of free water (to imitate environmental conditions on rock surfaces), and; (ii) slow removal of water. Phosphorus pentoxide was used as the desiccant in both cases, but for fast desiccation the nitrocellulose filters were placed in dry Petri dishes while for slow desiccation the filters were placed in Petri dishes containing a layer of an agar medium. After one wk all colonies (both desiccation types) had dried down to a constant weight (water content less then 0.1 g water / dry weight). Eight wks later, the colonies were sub-sampled for lipid and pigment analysis, as well as for ultra-structural studies by light- and transmission-

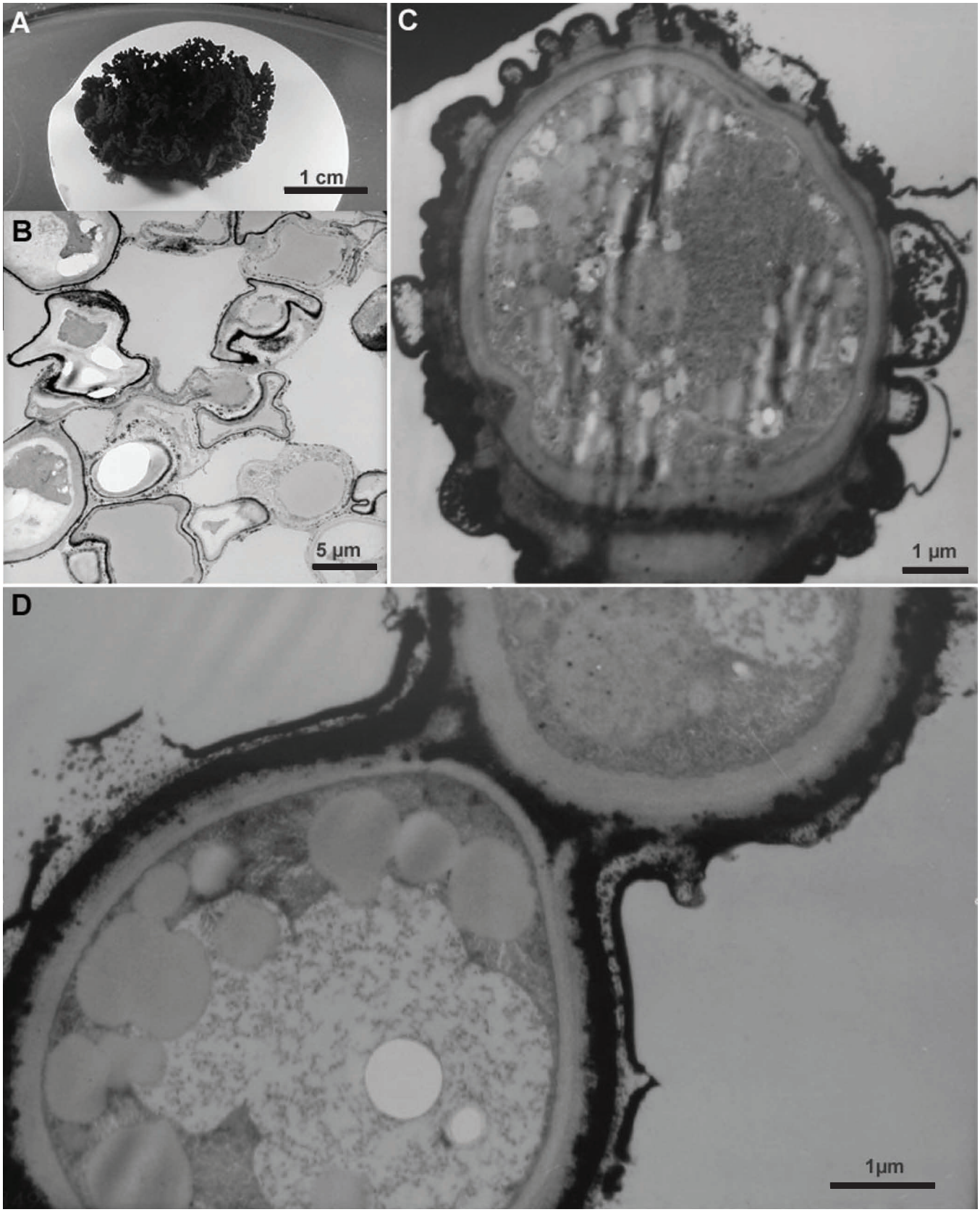

Fig. 1. Colonies of rock-inhabiting strain A95 were grown on nitrocellulose filters (A), and subjected to fast and slow desiccation (8 wk in a desiccator containing P_2O_5 with and without underlying agar) and analysed microscopically. A. experimental setup, well-developed single colony just before treatment; B. TEM micrograph of a colony sectioned after 8 wk of desiccation (overview showing collapsed dehydrated cells filled with coalescent lipid inclusions). Differences were not observed between fast and slow desiccated colonies; C, D. TEM micrographs of restored cells just 24 h after rehydration.

electron microscopy (TEM).

Determination of dry weight and rate of water loss

A separate set of colonies was used to determine the rate of water loss. After transfer to a desiccator, colonies (fast- and slow-desiccated) were removed on consequent days and weighed until constant weight was achieved. These experiments were carried out in triplicate.