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Alteration of protein patterns in black rock inhabiting fungi as a response to different temperatures

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ABSTRACT

Rock inhabiting fungi are among the most stress tolerant organisms on Earth. They are able to cope with different stressors determined by the typical conditions of bare rocks in hot and cold extreme environments. In this study first results of a system biological approach based on two-dimensional protein profiles are presented. Protein patterns of extremotolerant black fungi – *Coniosporium perforans*, *Exophiala jeanselmei* – and of the extremophilic fungus – *Friedmanniomyces endolithicus* – were compared with the cosmopolitan and mesophilic hyphomycete *Penicillium chrysogenum* in order to follow and determine changes in the expression pattern under different temperatures. The 2D protein gels indicated a temperature dependent qualitative change in all the tested strains. Whereas the reference strain *P. chrysogenum* expressed the highest number of proteins at 40 °C, thus exhibiting real signs of temperature induced reaction, black fungi, when exposed to temperatures far above their growth optimum, decreased the number of proteins indicating a down-regulation of their metabolism. Temperature of 1 °C led to an increased number of proteins in all of the analysed strains, with the exception of *P. chrysogenum*. These first results on temperature dependent reactions in rock inhabiting black fungi indicate a rather different strategy to cope with non-optimal temperature than in the mesophilic hyphomycete *P. chrysogenum*.

Introduction

'Exposure of cells to suboptimal growth conditions or to any environment that reduces cell viability or fitness can be considered stresses' (de Nadal *et al.* 2011). Stress has been classified as either biotic or abiotic, these including thermal (hot or cold) and non-thermal stress, such as acid, water, or pressure (Mafart *et al.* 2001). Both the physiological state and the natural environment in which an organism has been evolutionarily selected, influence its adaptive responses and rapid adaptations are crucial to maximizing cell survival (de Nadal *et al.* 2011). Eukaryotic cells have evolved sophisticated cellular mechanisms in response to the stresses that regulate several aspects of cell physiology as e.g. gene expression,

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metabolism, cell cycle progression, cytoskeletal organization, protein expression and homeostasis, and modification of enzymatic activity. These stress tolerance responses can generate both immediate and long-term adaptations, which are especially crucial for the survival of organisms in environments with extreme physicochemical parameters. Within eukaryotes, a specialized group of fungi – the black yeasts and microcolonial fungi (MCF) – have been identified as conquerors of an extremely stressful habitat: the bare rock in hot and cold environments (Staley *et al.* 1982; de Hoog & Grube 2008; Sterflinger *et al.* 2012).

Due to their stress tolerance, MCF and black yeasts have a wide distribution that includes some of the most extreme environments of the Earth as well as extraterrestrial conditions (Onofri *et al.* 2012). Originally black fungi – also named dematiaceous fungi – were described as inhabitants of living and dead plant material. However, in the last 30 y they have been isolated from hypersaline waters (Gunde-Cimerman *et al.* 2000), acidic environments (Baker *et al.* 2004), radioactive areas (Dadachova *et al.* 2007), as human pathogens or opportunists (Matos *et al.* 2002) and as a dominant part of the epi- and endolithic microbial communities (Friedmann 1982; Sterflinger 2000; Burford *et al.* 2003; Ruibal *et al.* 2005; Sert *et al.* 2007; Selbmann *et al.* 2008). Together with cyanobacteria and lichens, they contribute to the global biogeochemical cycling by active weathering of natural rocks and stone monuments (Sterflinger & Krumbein 1997).

These habitats share some important characteristics: osmotic stress, UV and oxidative stress and rapid variation of temperature, water supply, and nutrient availability (Sterflinger *et al.* 1999; Vember & Zhdanova 2001; Sterflinger 2005). To withstand these changes, organisms living in such environments need either permanently existing or exceptionally fast adaptive cellular or metabolic responses. Although MCF and black yeasts are a diverse taxonomic group having polyphyletic origins within the Ascomycota, they have similar morphological and physiological characters. These similarities were interpreted as a 'principle of uniformity' by Urzí *et al.* (2000) being an obligate basis to tolerance of physical and chemical stress on rock and plant surfaces. Slow growth rates, an optimal surface/volume ratio of the cauliflower-like colonies, thick and strongly melanised cell walls, exopolysaccharides production, the high intracellular content of trehalose, and polyoles as well as lack of sexual reproductive structures, are considered as adaptations to the extreme environments (Sterflinger 1998; Selbmann *et al.* 2005; Onofri *et al.* 2007; Gostinčar *et al.* 2010).

Temperature is undoubtedly one of the major factors affecting the growth and survival of any microorganism (Deegenaars & Watson 1998): for this reason it is of great interest to investigate how MCF and black yeasts withstand temperatures that are significantly out of their growth range. Unlike in other Ascomycetes as *Neurospora crassa*, *Candida albicans*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Kraus & Heitman 2003; Bahn *et al.* 2007; Alonso-Monge *et al.* 2009), the stress-response mechanisms of MCF have not yet been investigated, either on the genomic or on the proteomic level. A very recent investigation has revealed the complexity of protein composition in cosmopolitan fungi as *Penicillium chrysogenum* and *Aspergillus* sp. (Jami *et al.* 2010a; Rizwan *et al.* 2010). Further studies that analyse the ecological differences and analogies among these fungi in a systematic approach and on the molecular level are missing. The production of molecular chaperons (MC), so called 'heat shock proteins' (HSPs), small HSPs, and also 'cold shock proteins' (CSPs) belong to the most important stress reactions of cells in general (Becker & Craig 1994; Albanese *et al.* 2006; Nakamoto & Vígh 2007; Nevarez *et al.* 2008) and are known to represent the main effect to temperature stress in mesophilic fungi such as *P. chrysogenum* (Raggam *et al.* 2011). Thus, protein expression profiling was chosen as the first tool to shed light on the biological response of MCF and black fungi towards suboptimal temperatures.

The main goal of the present paper was to reveal if black fungi and mesophilic hyphomycetes present a similar reaction to temperature stress, as reflected by the protein patterns. Three strains of black rock inhabiting fungi were chosen for this study: *Exophiala jeanselmei* MA 2853, *Coniosporium perforans* MA 1299 and *Friedmanniomyces endolithicus* CCFEE 5208. Fungi were grown at different temperatures and the protein profiles were analysed in comparison with each other and with *Penicillium chrysogenum* (strain MA 3995), as reference strain.

Materials and methods

Fungal strains

Three strains of black fungi, clustering within two different orders of Dothideomyceta (Chaetothyriales and Capnodiales), were used in the present study (Fig 1). The isolates were selected according to their bio-ecological characteristics. They all colonize rock epi- or endolithically but they have a diverse geographical distribution:

(1) *Exophiala jeanselmei* (MA 2853) is a mesophilic black yeast detected as a frequent colonizer of rock in moderate climates (Warscheid & Braams 2000; Sterflinger & Prillinger 2001). It has a close phylogenetic and physiological relation to human opportunists and pathogens (de Hoog 1993) which makes this strain a highly interesting model to study the evolution of virulence (Gostinčar *et al.* 2011).
(2) *Coniosporium perforans* (MA 1299) is a widely distributed microcolonial rock inhabitant fungus in both moderate and Mediterranean climates (Sterflinger *et al.* 1997; M. Owczarek, in preparation). Although it can be considered as mesophilic with respect to its growth optimum, this strain has a remarkable high temperature and desiccation tolerance (Sterflinger 1998).
(3) *Friedmanniomyces endolithicus* (CCFEE 5208) is a psychrophilic fungus with an outstanding and unique ecology and phylogeny. The considerable sequence deviation from known taxa, reflected by the phylogenetically isolated position, suggest *F. endolithicus* as an endemic species for the Antarctic (Selbmann *et al.* 2005), where it occurs cryptendolithically in rocks, having a strong degree of extremotolerant specialisation (Onofri *et al.* 1999).

The mesophilic hyphomycete *Penicillium chrysogenum* (MA 3995) was chosen as a reference strain since it is very well