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Salinity induced effects on the growth rates and mycelia composition of basidiomycete and zygomycete fungi☆

C. Venâncio [a,*], R. Pereira [b], A.C. Freitas [c], T.A.P. Rocha-Santos [d], J.P. da Costa [d], A.C. Duarte [d], I. Lopes [a]

[a] Department of Biology & CESAM, Campus de Santiago, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Department of Biology, Faculty of Sciences of the University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[c] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina, Rua Arquiteto Lobão Vital, Apartado 2511, 45202-401 Porto, Portugal
[d] Department of Chemistry & CESAM, Campus de Santiago, University of Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

Soil salinization, as the combination of primary and secondary events, can adversely affect organisms inhabiting this compartment. In the present study, the effects of increased salinity were assessed in four species of terrestrial fungi: *Lentinus sajor caju*, *Phanerochaete chrysosporium*, *Rhizopus oryzae* and *Trametes versicolor*. The mycelial growth and biochemical composition of the four fungi were determined under three exposure scenarios: 1) exposure to serial dilutions of natural seawater (SW), 2) exposure to serial concentrations of NaCl (potential surrogate of SW); and 3) exposure to serial concentrations of NaCl after a period of pre-exposure to low levels of NaCl. The toxicity of NaCl was slightly higher than that of SW, for all fungi species: the conductivities causing 50% of growth inhibition (EC_{50}) were within 14.9 and 22.0 $mScm^{-1}$ for NaCl and within 20.2 and 34.1 $mScm^{-1}$ for SW. *Phanerochaete chrysosporium* showed to be the less sensitive species, both for NaCl and SW. Exposure to NaCl caused changes in the biochemical composition of fungi, mainly increasing the production of polysaccharides. When fungi were exposed to SW this pattern of biochemical response was not observed. Fungi pre-exposed to low levels of salinity presented higher EC_{50} than fungi non-pre-exposed, though 95% confidence limits overlapped, with the exception of *P. chrysosporium*. Pre-exposure to low levels of NaCl also induced changes in the biochemical composition of the mycelia of *L. sajor caju* and *R. oryzae*, relatively to the respective control. These results suggest that some terrestrial fungi may acquire an increased tolerance to NaCl after being pre-exposed to low levels of this salt, thus, suggesting their capacity to persist in environments that will undergo salinization. Furthermore, NaCl could be used as a protective surrogate of SW to derive safe salinity levels for soils, since it induced toxicity similar or higher than that of SW.

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1. Introduction

Salinization of soils may occur as a natural process due to the accumulation of salts resulting from the degradation of the soil parent rock. However, considering the most recent reports on climate change, increasing sea level, and severe and frequent weather events can also act as main drivers for salt intrusion in coastal regions (IPCC, 2013). Furthermore, these same reports point to a global increase in mean temperatures and scarce rainfall events. All these factors will eventually promote salinization of coastal soils, which may be exacerbated by other factors, such as uncontrolled water consumption by society in general and inappropriate irrigation practices (European Soil Portal, 2012). The excessive amount of salts in the soils may change water-holding capacity and promote ions imbalance, thus impacting life cycle traits of the groups of organisms that inhabit it (e.g. Pereira et al., 2015). Within this framework of soil salinization, it is pertinent to understand its effects on fungi, since these organisms play a pivotal role in the functioning of soil communities. Namely they contribute to the availability of water and nutrients to plants and other edaphic organisms (Genre and Bonfante, 2012), to soil's aggregation (Ritz and

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* Corresponding author. Centro de Estudos do Ambiente e do Mar, Departamento de Biologia da Universidade de Aveiro, Campus de Santiago, P-3810-193 Aveiro, Portugal.
E-mail address: a32884@ua.pt (C. Venâncio).

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Young, 2004; Genre and Bonfante, 2012) due to a complex net of hyphae that are able to spread and cover a large soil area, to carbon and nutrient recycling (Dighton, 2007; Baldrian and Valášková, 2008) or even contribute as a food source (e.g. rhizosphere bacteria) (Ballhausen and de Boer, 2016). Furthermore, their almost unique capacity to degrade lignin (only shared with bacteria) makes them relevant players in the degradation of plant material (Schwarze et al., 2013). Following this context, an increasing attention has been given to this group of organisms within the research field of ecological risk assessment studies. Within the context of salinization scenarios, some works have already reported the effects of increased salinity in soil fungi. Castillo and Demoulin (1997) observed that the growth of *Micropory xanthopus* was inhibited at salinities between 35 and 40 gL^{-1} NaCl (corresponding to 69.3 and 79.2 $mScm^{-1}$) and that the growth of *Schizophyllum commune* never decreased below 50% even when it was exposed to salinities of 35 (salinity of natural seawater). Aksu and Balibek (2010) reported that the growth rates of *Rhizopus arrhizus* only declined by 20–25%, even after being exposed to 50 gL^{-1} NaCl (conductivity of 98.9 $mScm^{-1}$). These published results suggest a high tolerance of terrestrial fungi to salinity. Such high tolerance may be related with their capacity to produce many extracellular compounds that helps to control the excessive uptake of chemical compounds present in the surrounding environment, either by immobilizing them externally or by metabolizing them (Fink-Boots et al., 1999; Nesci et al., 2004). For instance, Fink-Boots et al. (1999) observed an increased production of extracellular enzymes and consequent degradation of metabolites (phenol compounds and superoxide anion radicals) in basidiomycete species exposed to temperature stress. Furthermore, in four food spoilage fungi strains (*Aspergillus* section *Flavi*) exposed to NaCl (osmotic potential of −3.0, −7.0, −10.0 MPa), the total content of polyols (mainly glycerol) and sugars (mainly glucose) increased with increasing salt concentration (Nesci et al., 2004). These fungi stress-induced responses have been associated with its high tolerance to salinity.

Though some knowledge already exists on the sensitivity and mechanisms of response of fungi to salt stress, little is known regarding the effects that prolonged exposure to low salinity levels may exert on fungi. One of the few published works focusing on long-term effects of salinity in fungi was done by Langenfeld-Heyser et al. (2007). These authors monitored the growth and stress responses of *Paxillus involutus* when exposed to NaCl. They found that a liquid culture of this basidiomycete was able to growth at NaCl concentrations as high as 100 mM (approximately 11.56 $mScm^{-1}$). Furthermore, although the growth of *P. involutus* started to be affected at 200 mM NaCl (23.13 $mScm^{-1}$), it could tolerate up to 500 mM NaCl (57.84 $mScm^{-1}$) after being exposed for three weeks to NaCl.

Regarding salinity tolerance observed in wood decay fungi, information available is scarce if not inexistent. Accordingly, the present work aimed to assess the effects of increased salinity on the mycelial growth and biochemical composition of four species of terrestrial fungi (*Phanerochaete chrysosporium*, *Trametes versicolor*, *Rhizopus oryzae* and *Lentinus sajor caju*). For this, two specific objectives were delineated: (i) to assess if NaCl could be used as a surrogate of natural seawater for risk assessment purposes and (ii) to evaluate the effects of prolonged exposure to low salinity levels on the growth and mycelial composition of fungi.

2. Materials and methods

2.1. Test solutions

Increasing levels of salinity were set up by using a gradient of sodium chloride (NaCl) and of natural seawater (SW) conductivities. Conductivity values were employed as a measure of salinity. The salt NaCl was supplied by Merck (St Louis, MO, USA) and the SW was collected at a site located in the East coastal region of the North Atlantic Ocean (40°38′33″N, 8°44′55″W: Aveiro, Portugal), monitored under the European Union regulation (Directive, 2006/7/CE) for its water quality. For each NaCl concentration, culture medium powder [3.9% Potato Dextrose Agar (PDA), Merck, Darmstadt, Germany] and the previously calculated quantities of NaCl (to reach the desired final conductivity) were weighed separately and added to the adequate amount of ultrapure water (1 L). Before being used for ecotoxicological assays, SW was filtered through cellulose nitrate membranes with a pore size of 0.20 μm (ALBET-Hannemuehle S.L., Barcelona, Spain) to remove associated natural biota. Each SW dilution (in which the culture medium powder was also directly dissolved) was prepared by adding ultrapure water to seawater. All concentrations/dilutions were sterilized prior to its use in an autoclave during 20 min, at 121 °C and 1 Bar (Uniclave 88, AJC).

2.2. Test species

Four terrestrial fungi species, including three basidiomycetes [*Phanerochaete chrysosporium* (Burdsall 38388), *Lentinus sajor caju* (Fries) Fries and *Tramete versicolor* (Pilát 38412)] and a zygomycete (*Rhizopus oryzae,* Went & Prins. Geerlings, 31002) were used in this study. *Phanerochaete chrysosporium*, *T. versicolor* and *R. oryzae* were obtained from the BCCMTM/MUCL Culture Collection (Belgium) whereas *L. sajor caju* was obtained from UNESP (São Paulo State University, Brazil). Individual cultures of each species were preserved in the laboratory, in PDA, in the dark, at 4 °C. Prior to the beginning of the tests, new individual cultures were obtained. Each fungi species was cultured in PDA at 28 °C in the dark for 3–5 days depending of the species.

2.3. Growth inhibition assays

Growth inhibition assays were carried out by exposing each of the four fungi species to gradients of salinity constituted by seven to eight conductivities of NaCl and SW, in 90 mm Ø Petri dishes. Detailed descriptions of the conductivities ranges ($mScm^{-1}$) tested for each species and time (days) of exposure to NaCl and SW are shown in Table 1. Negative controls (only PDA medium) were also performed for each assay.

For each fungal species, the assay was started by placing, at each replicate, a single circular 7 mm Ø agar disk collected from the edge of an active growing culture. Four replicates were performed per conductivity and control. Time of exposure was dependent on the growth rate of fungi, thus, an assay ended when the control Petri dishes were totally covered by the respective mycelium. According to this, exposure occurred during three (for *P. chrysosporium*) or eight (*L. sajor caju*, *T. versicolor* and *R. oryzae*) days, at 28 °C and in the dark (Table 1; NPE). The average diameter of the disks was measured at the beginning and at the end of the assays, by measuring the minimum, maximum and diagonal diameters, as highlighted in Fig. 1. Daily growth rate (*DGR*; $mmday^{-1}$), for each species was determined by using Equation (1):

$$DGR_{ab} = \frac{D_b - D_a}{t_b - t_a}, \ \ mmday^{-1} \tag{1}$$

where D_b is the average of the three measured diameters at the end of the assay, D_a is the diameter of the disk at the beginning of the assay (7 mm) and t_b-t_a is the exposure time interval (in days).

At the end of the assays, the mycelium of each fungus was collected, stored and frozen in sterile tubes at −80 °C until further analysis. Afterwards, these samples were lyophilized (at −85 °C,

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