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Growth and oxidative enzymatic activity of in-vitro cultures of *Ciliochorella buxifolia*

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ABSTRACT

To get a better insight into the physiological capabilities of *Ciliochorella buxifolia*, the most frequent fungus occurring on *Scutia buxifolia* leaf-litter in a native forest from Argentina, its in-vitro ability to use 10 carbon sources and to produce extracellular enzymes, including its response to tannic acid and to the addition of a water-soluble fraction of *Scutia buxifolia* leaf-litter, was analyzed. Growth, colony morphology and extracellular enzyme activity as well as differentiation of pycnidia were a function of the C substrate. The fungus responded to the presence of tannic acid in a range between 0.001 and 0.1% (w/v), by increasing growth, but higher phenol concentrations like 0.5% were inhibitory. The activity of extracellular oxidative enzymes increased with the concentration of tannic acid. Furthermore, the fungus showed extracellular laccase and peroxidase activity, being the former increased by water-soluble fraction in association to pycnidia development. Based on these results, *Ciliochorella buxifolia* is a fungus growing on *Scutia buxifolia* leaf-litter that is able to metabolize soluble phenolic compounds, which triggers the synthesis of extracellular oxidative enzymes possibly involved in sporulation and detoxification reactions.

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[1] Post-mortem.

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1. Introduction

One of major pools of organic matter in forest soils is leaf-litter, which is recycled by microorganisms and becomes an enormous source of nutrients, regulating, in this way, forest productivity (Osono 2007; Valásková et al. 2007; Wurzburger and Hendrick 2007). Although the soil microflora, as a whole, is involved in organic matter transformation, fungi play a major role in mineralization, mainly during the early stages of decay (Dighton 2003; Lensing and Wise 2007; Paul 2007; Valásková et al. 2007; Osono et al. 2008). However, richness, abundance and activity of litter-degrading fungi are a function of substrate quality, as well as of their competitive and reproductive ability, which are additionally modified by abiotic and biotic interactions (Cooke and Rayner 1984; Coleman and Whitman 2005; Hättenschwiler et al. 2005).

Allegrucci et al. (2005, 2007) and Cabello and Arambarri (2002) as well as Elíades et al. (2010, 2011) found a complex assemblage of fungi associated with leaf-litter of *Scutia buxifolia* Reiss (Rhamnaceae, Rhamnales). This perennial tree grows in a native temperate forest of Argentina that is characterized by alkaline calcareous (Rendolls) soils (Arturi et al. 1996). The debris from this tree, which is of a dark-brown color, is recalcitrant to degradation due to its content in polyphenols such as tannins (1.1%) and lignin (41.7%; Saparrat et al. 2008, 2010). This conditions the colonization and abundance of fungi (Saparrat et al. 2007b, 2008, 2010; Allegrucci et al. 2011). Among them, *Ciliochorella buxifolia* is the only fungus that grows exclusively in *S. buxifolia* leaf-litter and because of this, is the more frequent one (Allegrucci et al. 2005, 2007; Elíades et al. 2010). While analyzing the role of *C. buxifolia* LPSC 847 on *S. buxifolia* leaf-litter at the early stages of degradation we found that the fungus had a low saprotrophic ability on cell wall polymers, low levels of β-1,4 endoglucanase activity and lack of oxidative enzymes related to lignin degradation (Saparrat et al. 2010), unless the fungus was cultured in liquid medium (Troncozo et al. 2008).

These findings raised some questions about the eco-physiological characteristics of *C. buxifolia*, which might be related to degradation of *S. buxifolia* leaf-litter. The working hypotheses were: 1. *Ciliochorella buxifolia* has higher saprotrophic ability on soluble phenolic compounds than on other C sources, which leads to the specific colonization and high frequency on *S. buxifolia* leaf-litter. 2. Growth of this fungus is modulated by tannin. 3. Soluble phenolic compounds like those released by *S. buxifolia* leaf-litter in water induce in *C. buxifolia* the synthesis of laccases and peroxidases. Therefore, the aim of this work was to know further about the physiological capabilities that allow this fungus to use C sources that might be or are released by leaf-litter and to produce extracellular degrading enzymes. Furthermore, the response of the fungus to tannic acid, a model phenolic compound, and to the addition of a water-soluble fraction (WSF) of *S. buxifolia* leaf-litter, on its growth and the synthesis of extracellular oxidative enzymes, also was analyzed.

2. Materials and methods

2.1. Fungal isolate and inoculum source

Ciliochorella buxifolia LPSC (Culture collection of the La Plata Spegazzini Institute) 847 strain is a type specimen. It was isolated from leaf-litter of *S. buxifolia* of a natural dry forest at the Biosphere Reserve "Parque Costero del Sur" (MABUNESCO), located in eastern Buenos Aires province, Argentina (35°11′S, 57°17′W; Allegrucci et al. 2011). Stock cultures were maintained as slants on malt extract agar (MEA) medium at 4 °C.

2.2. Carbon substrates as sources for fungal growth

The utilization of organic substrates as the sole C source in association to the enzyme's activity involved in their degradation was evaluated by cultivating the fungus on a basal agar (2%, w/v) mineral salts medium (5 g $NH_4H_2PO_4$, 2.5 g K_2HPO_4,

Table 1 – Organic compounds used in this study, their concentration and fungal enzymes involved in their degradation.

C substrate type[a]	Organic compound	Concentration (%, w/v)	Major enzymes involved in degradation
Readily available C	Glucose	1	Hexokinase (EC 2.7.1.1)
	Sucrose	1	Invertase (EC 3.2.1.26)
Moderately recalcitrant ('slow') C	Apple-pectin	0.1	Pectin lyase (EC 4.2.2.10) and polygalacturonase (EC 3.2.1.15)
	Birch-wood xylan	0.2	1,4-β-Xylan xylanohydrolase (EC 3.2.1.8)
	Soluble starch	1	α-Amylase (EC 3.2.1.1)
Highly recalcitrant C	Gallic acid	0.05	Catechol oxidase (EC 1.10.3.1), laccase (EC 1.10.3.2) and monophenol monooxygenase (EC 1.14.18.1)
	Humic acid	0.05	Manganese peroxidase (EC 1.11.1.13)
	Indulin-AT (Kraft lignin)	1	Laccase (EC 1.10.3.2), lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13) and versatile peroxidase (EC 1.11.1.16)
	Sodium-carboxy-methylcellulose (CMC)	0.5	Endo-(1,4)-β-d-glucanase (EC 3.2.1.4)
	Tannic acid	0.05	Catechol oxidase (EC 1.10.3.1), monophenol monooxygenase (EC 1.14.18.1) and tannin acyl hydrolase (EC 3.1.1.20)

[a] Based on its degree of degradability due to their molecular weight, structure, and the need for enzymatic digestion prior to assimilation (Cooke and Whipps 1993; Mondini et al. 2006; Goldfarb et al. 2011; Hoeksema and Classen 2012).