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Short communication

Guttation droplets of the edible mushroom *Suillus bovinus* as a new source of natural antioxidants

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ABSTRACT

Although guttation is a well-known feature of several filamentous fungi, their potential as natural antioxidant sources is unknown. This study investigates the antioxidant capacity of the exudate produced by the wild edible mushroom *Suillus bovinus* and the effect of the temperature and of light/darkness exposure on antioxidants synthesis. The fungus was grown on solid media at 18 °C or 25 °C, under light/darkness conditions. The antioxidant activity of the exudate was evaluated by using 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) and reducing power assays. The fungal exudate proved to have antioxidant activity, showing values of reducing power ranging from 0.450 to 0.528, and of scavenging effects on DPPH radicals from 75.68 to 82.38%. Colonies grown under continuous light at 18 °C had the lowest growth rate and exudate volume, but these environmental conditions proved to be the most appropriate to increase antioxidant activity of the droplets. These results indicated that the droplets of *S. bovinus* are a promising source of natural antioxidants and provide useful information on how to manipulate the production of such compounds.

1. Introduction

All around the world, both wild and cultivated mushrooms are becoming increasingly important in the human diet, especially due to their nutritional and medicinal benefits (Cheung, 2010). These benefits can be partly explained by their richness on antioxidants, which have the capacity to scavenge free radicals and reactive oxygen and nitrogen species (Cheung, 2010). Although the antioxidant potential of mushrooms has been recognized (Cheung and Cheung, 2005; Barros et al., 2007), no data is available about antioxidant properties of guttation droplets produced by some mushrooms species. The production of guttation droplets is a common feature of several species of Basidiomycota (Sun et al., 1999; Rangel-Castro et al., 2002) and Ascomycota (Gareis and Gareis, 2007; Hutwimmer et al., 2010). Despite the widespread occurrence of this phenomenon, very few works have been devoted to guttation formation and to the chemical characterization of these droplets. Available works refer that their composition is influenced by environmental factors (*e.g.* temperature, pH), being frequently rich on primary and secondary metabolites, inorganic substances, and proteins/enzymes (Sun et al., 1999; Rangel-Castro et al., 2002; Gareis and Gareis, 2007). As far as we know, antioxidant proprieties of fungal guttation droplets or the influence of environmental conditions in such proprieties are still unknown. Therefore, the aim of this work was to evaluate the antioxidant capacity of the exudates produced by the edible ectomycorrhizal mushroom *S. bovinus* (Pers.) Roussel, and the effect of temperature and light/darkness on such proprieties as well as on the guttation formation. It is expected from this study to assess, for the first time, the potentially of fungal guttation to be an additional source of natural antioxidant for medicinal, pharmaceutical and food application.

2. Materials and methods

2.1. Fungal strain

The mycelium of *S. bovinus* was isolated from sporocarps collected under *Pinus pinaster* stands in Bragança (Northeast Portugal) on Melin-Norkrans (MMN) agar medium at pH 6.6. The identification of fungal isolate was confirmed by sequencing of the internal transcribed spacer region, using the primers *ITS1* and *ITS4* (White et al., 1990). The strain was maintained in MMN agar medium at 25 ± 1 °C, in the dark, being regularly sub-cultured.

2.2. Effect of environmental factors on mycelia growth and production of exudate

Petri dishes (9 cm diameter) containing 10 ml of MMN medium (pH 6.6) were inoculated with two agar plugs of 5-mm diameter, each one cut from the actively growing margin of *S. bovinus*

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Table 1
F values and probability levels of the effect of light/darkness exposure and temperature on *Suillus bovinus* growth, production of exudate and antioxidant activity of the exudate (total phenols, reducing power and DPPH).

	Light/darkness	Temperature	Light/darkness × temperature
Growth rate	n.s.	$F_{3,55}$ 18.268***	$F_{3,55}$ 5.112*
Exudate	$F_{3,51}$ 49.547***	n.s.	$F_{3,51}$ 24.763***
Total phenols	$F_{3,11}$ 1,564.913***	$F_{3,11}$ 27,001.701***	$F_{3,11}$ 11,297.055***
Reducing power	$F_{3,11}$ 17.814**	$F_{3,11}$ 197.938***	$F_{3,11}$ 178.639***
DPPH	$F_{3,11}$ 962.730***	n.s.	n.s.

Probability levels: ns – no significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

subcultures. The Petri dishes were then incubated in four different conditions: in continuous light intensity of 100 mE m^{-2} s^{-1}, at 25 °C (i) and 18 °C (ii), and in continuous darkness, at 25 °C (iii) and 18 °C (iv). Fifteen replicates of each treatment were performed. Eighteen days after inoculation, the colony diameter and the quantity of exudate produced by the fungus in each treatment were evaluated. The volume of exudate (in μl) was determined by pipetting the guttation droplets from the fungal colonies. The droplets from five plates of each treatment were combined, diluted 1:3 (v/v) in ultrapure water and analyzed for total phenols, scavenging effect on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, and reducing power assay (see below).

2.3. Antioxidant activity

All chemicals used in the antioxidant activity were purchased from Sigma Chemical Co. (St. Louis, USA). Water was treated in a Mili-Q water purification system (TGI Pure Water System, USA). Total phenols quantification was achieved according to Singleton and Rossi (1965), with minor modifications, described by Oliveira et al. (2011). The ability to scavenge DPPH free radical was monitored according to Oyaizu (1986). The reducing power was determined following the procedure described by Berker et al. (2007). Ascorbic acid, a common preservative of natural origin used in the food industry, was used as reference compound.

2.4. Statistical analyses

Data from radial fungus growth (mm/day), fungus exudate production (μl) and antioxidant activity of exudate are presented as the mean of 3 or 15 independent experiments displaying the respective standard deviation (SD) values. Differences among means were determined by analysis of variance (ANOVA), using SPSS v.18 software and averages were compared using Tukey test ($p < 0.05$).

3. Results and discussion

3.1. Effect of temperature and light/darkness exposure on the mycelial growth and exudate production

The radial mycelial growth rates of *S. bovinus* were significantly affected by environmental conditions, in special by the temperature (Table 1). Light/darkness exposure only had a significant effect on the fungus growth when interacted with temperature. This study corroborates other reports (Vargas-Isla and Ishikawa, 2008) in which the light/darkness exposure has been showed to have relatively little effect on fungus growth. The temperature of 25 °C was found to be the most favorable to the growth of *S. bovinus* (Fig. 1). Similar result was previously reported for other macrofungal species (Mswaka and Magan, 1999; Daza et al., 2006). The amount of exudate produced by *S. bovinus* was also showed to be significantly influenced by the light regime, whereas the temperature seemed not to have had any effect (Table 1). Volume of exudates was higher when *S. bovinus* was grown under continuous darkness (Fig. 1). Earlier studies focused mainly on the nutritional medium conditions causing fungal guttation (Hutwimmer et al., 2010), or on environmental factors affecting their chemical composition (Rangel-Castro et al., 2002; Gareis and Gareis, 2007; Hutwimmer et al., 2010). The current study reports, for the first time, the effect of light regime and temperature on the production of exudate.

3.2. Antioxidants proprieties of the exudate produced by S. bovinus

The exudate produced by *S. bovinus* grown under the different environmental conditions proved to have antioxidant activity (Table 2). The reducing potential of the fungus exudate was very similar between treatments, showing an average value of 0.489 and 0.477 under continuous darkness and light conditions, respectively. However, under continuous darkness, temperature seemed to have some influence on the reducing potential of the exudates, since significantly higher values were obtained at 25 °C than at 18 °C. The analysis of variance corroborates this result (Table 1), by showing that temperature had a higher effect than light/darkness exposure. Comparing values of reducing power to the ones obtained with the reference compound, a similar activity was observed between exudates and ascorbic acid at a concentration of 0.075 mg/ml. In the DPPH assay, regardless of the temperature value, the scavenging effect was observed to be significantly higher under continuous

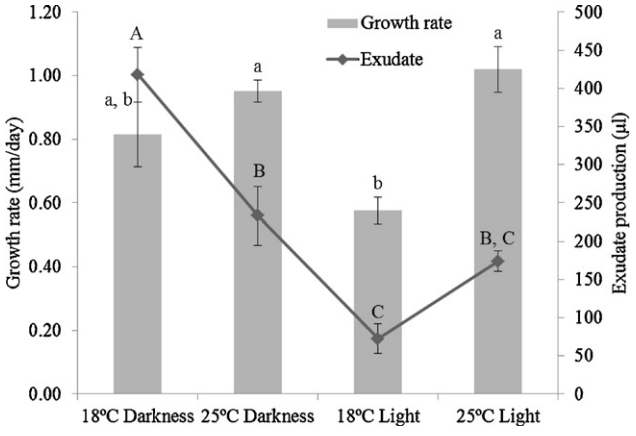

Fig. 1. Growth rate of *Suillus bovinus* and exudate production per colony, after 18 days of cultivation under continuous light, at 18 °C or 25 °C, or under continuous darkness, at 18 °C or 25 °C. Each value is expressed as mean ± SD ($n = 15$). Bars with different lowercase and uppercase letters indicate means values with significant differences, respectively on fungus growth rate and fungus exudate production ($p < 0.05$).