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Characterisation of bioactive protein-bound polysaccharides from *Amanita ponderosa* cultures

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Different bioactive compounds of edible mushrooms are responsible for their antioxidant, antitumor, antimicrobial, immunomodulatory, antiatherogenic and hypoglycemic reported properties [1, 2]. These properties are mostly due to the ability to synthesize different polysaccharides, namely protein-polysaccharide (PPS) complexes. The antioxidant capacity of these compounds present great interest in preventing innumerous diseases, including cancer, cardiovascular, auto-immunes diseases and accelerated aging. *Amanita ponderosa* are wild edible mushroom (Fig 1a_1, a_2, b), growing in some Mediterranean microclimates, namely in Alentejo region (Southern Portugal) and Andalusia (Southern Spain) [3], and establishes a mycorrhizal symbiosis with holm oaks and cork trees like *Quercus ilex* and *Q. suber*. There are few studies with respect to this species, however in this work was possible to obtain *A. ponderosa* pure cultures (Fig 1c, d_1, d_2) from strains collected in different areas of Alentejo.
This study focused on the characterisation of the PPS complexes produced in liquid cultures of *A. ponderosa*. Batch cultures (Fig 1e_1, e_2) were performed during 15 days, and polysaccharides concentrations were determined by the phenol–sulphuric method. A combined FTIR-ATR (Fourier-transform infrared using the attenuated total reflection) and Raman spectroscopy was used for the screening of bioactive PPS compounds present in the culture extracts. After identification of these bioactive compounds, PPS extracts were fractionated by size exclusion chromatography (SEC) using Sephacryl S-300 as stationary phase. Then, the chromatographic fractions and extracts were analysed by SEC, using an HPLC system coupled to UV (280 nm) and RI detectors in order to determine polysaccharide average molecular weights (Mw). The toxicity of the dried mushrooms, cultures and PPS extracts was assessed using *Artemia salina* test kit (Artoxkit MTM, Microbiotest). Acute toxicity was evaluated *in vivo* using *Swiss* mice. Samples of dried fruiting bodies and lyophilized mycelia were orally administered, by means a gastric probe, at a concentration of 5000 mg kg^{-1} and LD_{50} was evaluated.
The aim of this study was the purification and characterisation of PPS complexes produced by *A. ponderosa* cultures using a new microanalytical approach to monitoring the production, *in situ*. Microanalysis using FTIR-ATR (Fig. 2) and Raman of PPS samples showed spectra compatible with identification of this type of compounds in culture extracts [4]. IR band between 3000–3500 cm^{-1} arise from OH stretching vibrations of hydroxyls and water, moderate IR bands between 1600–1400 cm^{-1} arise from stretching vibrations of carboxylate. Strong IR/Raman bands in the region of 900–1200 cm^{-1} are characteristic for carbohydrates and can be attributed to coupled CO and CC stretching and COH bending vibrations in polysaccharides. PPS separated by size exclusion chromatography (SEC) showed seven mainly complexes with molecular weights

ranging between 1.5–20 kDa. Dried fruiting bodies, lyophilized mycelia and PPS extracts did not present toxicity against *A. salina*. Mycelia of cultures and fruiting bodies also did not show toxicological effects in *Swiss* mice with LD_{50}>5000 mg Kg^{-1}.
PPS compounds have displayed important antioxidant properties and further studies are in progress in order to investigate their antitumural activities. The application of microanalytical approach to monitoring the production of PPS compounds can be successful applied in biotechnological processes.

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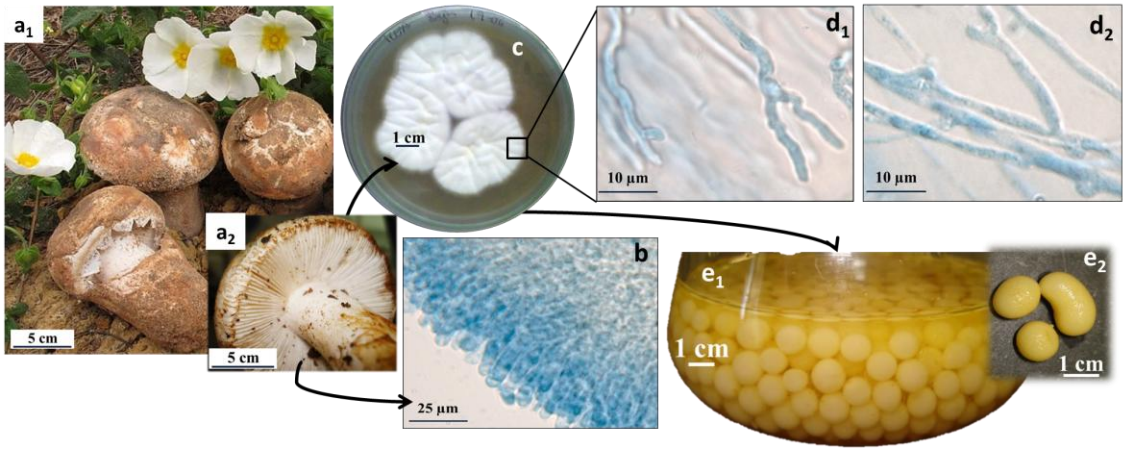

Figure 1: a_1 and a_2) Macroscopic features of wild edible mushrooms *A. ponderosa*, b) micromorphological features of *A. ponderosa* hymenium with basidia, c) the mycelium of *A. ponderosa* pure cultures in solid medium, d_1 and d_2) micromorphological features of mycelium with observation of septate hyphae forming a "fork-like" characteristic structures in the end regions and clamp connections, e_1 and e_2) liquid cultures of *A. ponderosa* mycelium.

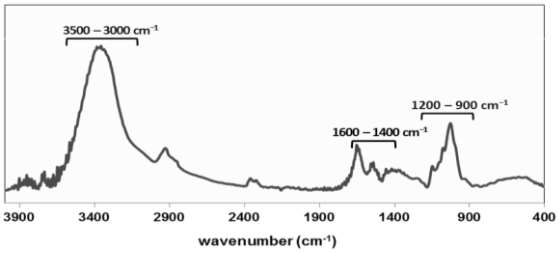

Figure 2: FITR-ATR spectrum of protein-polysaccharide complexes obtained from cultures.

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