INTERFACIAL ACTIVITY OF FUNGAL HYDROPHOBIN PLYHD OF *Paecilomyces lilacinus* AND STABILIZATION OF OIL IN WATER (O/W) EMULSION

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Introduction. Filamentous fungi produce amphipatic proteins called hydrophobins, which are characterized by a low molecular weight (~10 kDa) and by surface activity. Hydrophobins have a distribution of eight residues of cysteine and can be adsorbed on hydrophobic surfaces and onto both hydrophobic (air, oil and waxes) or hydrophilic interfaces (water and the cell wall). These proteins form a protective hydrophobic coating on the surface of hyphae and spores allowing the adhesion of these structures onto the surface of the host cuticle. Furthermore, the interfacial activity of these proteins is of great interest for biotechnological applications such as the immobilization of biosensors in solid surfaces and as surfactant in liquid systems with two phases [1].
This study was focused to isolate the hydrophobin (PLHYD) from *Paecilomyces lilacinus* and evaluate its surface activity on solid-air and liquid-air interfaces.

Methods. *P. lilacinus* was cultivated on nitrocellulose membranes with defined medium and sucrose at 30℃.
The hydrophobicity of the mycelium was measured with sterile water droplets. Surface hydrophobicity on the mycelium was determined by measuring the contact angle of a sterile water drop (1µL) on a fungal mat. PLHYD was purified according to method reported by Vigueras *et al.* [2] with some modifications. Protein profiles were analyzed by SDS-PAGE. The total protein content was determined using a NanoDrop ND-1000.
A hydrophobin-coated surface was prepared by drop-casting onto a Teflon membrane and surface hydrophobicity was determined by measuring the contact angle of a water drop with a goniometer. Micrographs were obtained by scanning electron microscopy (SEM). The critical micelle concentration (CMC) was estimated by optical tensiometry, using the pendant drop method. An oil-in-water emulsion was prepared with oleic acid, water and 0.45mg/mL of hydrophobin as stabilizing agent. The emulsion was sonicated during 20 minutes. The particle size and stability of the emulsion were analyzed in a Zetasizer Nano ZSP.

Results.
The hydrophobin was extracted after day 7, when the maximum concentration was reached as verified with the increase in the contact angle (θ) of the mycelium as follows: 4th day θ=85°, 5th day θ=102°, 6th day θ=109.8° and 7th day θ=116.6° Other authors reported similar values in the seventh day (112.6°) with *Penicillium expansum* [3]. SDS-PAGE analysis showed one band of ~7 kDa. The hydrophobin yield was 6 mg PLHYD/g protein. The PLYHD was able to modify the surface hydrophobicity of Teflon, decreasing the contact angle from 130.1(±2)° to 47 (±2)°. The SEM micrographs (f ig. 1a, b) show a hydrophobin deposit onto Teflon which presented a mosaic-like pattern and zones with microfibrils <0.5µm. The PLHYD lowered the surface tension of water from 72 to 34.8 mN m^{-1}, with a CMC of 0.45 mg mL^{-1}. The PLHYD was able to stabilize an oil-in-water emulsion during 48 h (fig. 2a, c). The average particle size of the emulsion was ~2 µm of diameter (fig. 2b, c). The emulsion shows one predominant particle size distribution with changes over time.

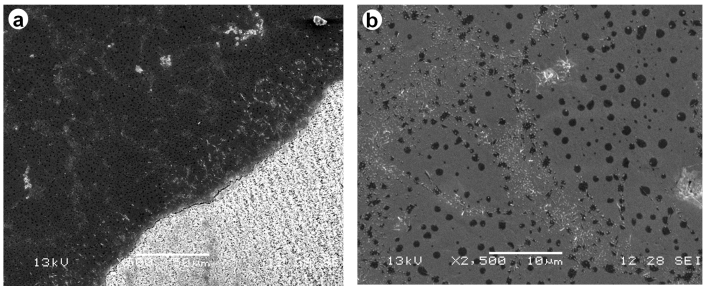

Fig. 1. Scanning electron micrographs of layer of PLHYD deposited onto Teflon a) x 500; b) x 2500

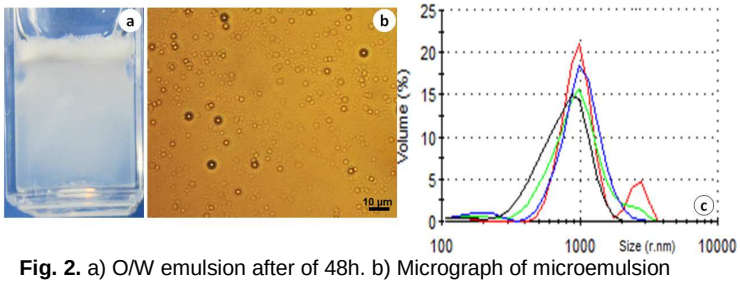

Fig. 2. a) O/W emulsion after of 48h. b) Micrograph of microemulsion with a 40x objective. c) Particle size at 0h (—), 1h (—), 2h (—), 3h (—).

Conclusions.
The PLHYD showed interfacial activity on Teflon-air, water-air and water-oil interfaces. These interfacial studies are the basis for the use of this protein to stabilize other hydrophobic compounds in emulsions or to modify polymeric surfaces.

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