PRODUCTION OF EXOPOLYSACCHARIDES FROM *Humphreya coffeata* UNDER DIFFERENT CULTURE MEDIUM AND CONDITIONS IN SHAKE FLASKS

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Introduction. Macrofungi have been consumed for their nutritional value and comprise a vast and a large untapped source of powerful new pharmaceutical products [1]. Polyporaceae families have compounds with antibacterial, antifungal, phytotoxic, nematocidal, cytostatic, antiviral and other pharmacological activities [2]. Exopolysacharides (EPS) are one of the more interesting metabolites from mushrooms [1]. It has been reported that *H. coffeata* produces metabolites in the bulk supernatant with genotoxic, cytotoxic and antioxidant activities [3]. This study aims to obtain and characterize the EPS of *H. coffeata* and to determine their cytotoxic activity against tumorogenic cells (K562) in submerged culture by using two flaks geometries and two culture mediums.

Methods. *Humpreya coffeata* was collected from Tierra Alta (Colombia) and subcultured in Universidad EAFIT-Colombia [3]. *H. coffeata* is inoculated from slants into a 250 mL baffled shake flask with 50 mL of medium and cultured in darkness at 150 rpm, 30°C, for 4 days. All experiments were performed at least in duplicate in 250 mL conventional shake flasks (**CF**) and baffled shake flasks (**BF**) containing 50 mL of culture media (with 2 mL of seed culture), at 150 rpm, 30°C for 20 days in darkness. Two culture medium with different carbon sources were evaluated glucose (**FZM**, glucose 35 g/L, pH 5.5), and lactose (**MPO**, lactose 50 g/L, pH 4.5). Biomass and EPS were quantified by gravimetric methods, carbon source consumption by colorimeter assays (DNS), and average molecular weight by gel permeation chromatography (GPC) [4].

Results. There are no significant differences in specific growth rate and by culture medium (CF 0.77 day^{-1}, BF 0.71 day^{-1}). With respect to the two culture medium, a higher concentration of biomass is observed using MPO (CF 11.40 and BF 11.50 g/L) and a higher concentration of EPS (BF 3.40 and CF 4.50 g/L). When using FZM concentrations of biomass (CF 8.02 and BF 8.20 g/L) and EPS (CF 2.0 and BF 2.20 g/L) were lower (Fig. 1). However, using FZM where less concentration of EPS were obtained, a higher molecular weight were determined by GPC (FZM: CF 22,059 Da, BF 19,315 Da. MPO: PCF 12,077 Da, BF 12,293 Da). Preliminary results presents that higher molecular weight EPS exhibits cytotoxic activity against tumorigenic cells.

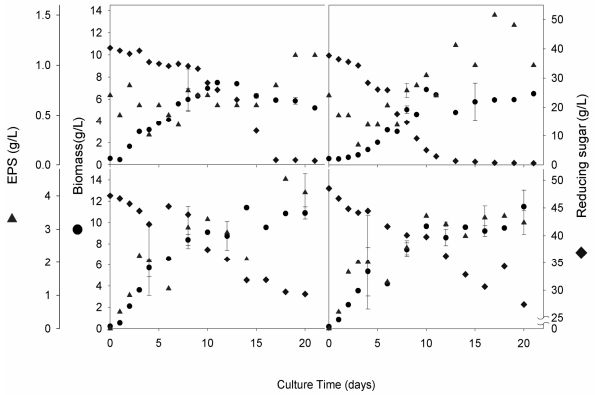

Fig. 1 Biomass growth, EPS production and reducing sugar concentration of *H. coffeata* cultured in CF (right) and BF (left), using FZM medium (up) and MPO (down).

Conclusions. A higher production of EPS was found in *H. coffeata* cultures using MPO medium but high molecular weight of EPS was obtained using FZM medium. Cytotoxicity assays are now in course to test the effectiveness of the EPS obtained in tumor cells.

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References. **1**. Fazenda, ML, Serviour R, McNeil B, Harvey LM (2008) *Adv Appl Microbiol* 63:33-104. **2.** Wasser SP (2002) *Appl Microbiol Biotechnol* 60:258-274 **3.** Porras-Arboleda S, Valdez-Cruz NA, Rojano B, Aguilar C, Rocha-Zavaleta L, Trujillo-Roldan MA (2009). *Int J Med Mush* 11(4):335-350 **4.** Trujillo-Roldán MA, Moreno S, Espín G, Galindo E (2004) *Appl Microbiol Biotechnol* 63(6):742-7