Improving feruloyl esterases production by nutrients modification using *Aspergillus niger* and *A. versicolor* cultured in solid state fermentations

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Introduction. Feruloyl esterases (FAEs, E.C. 3.1.1.73) are enzymes cleaving ester links between polysaccharides and monomeric or dimeric ferulic acids. Some fungi produce FAE, mainly: *A. awamori*, *A. niger* and *A. oryzae* (1). Recently, FAEs have received considerable interest due to their biotechnological applications such as: food, drugs, pulp and paper, and bioethanol (2).The aim of the present work was to improve the FAE production of *A. niger* and *A. versicolor* by modification of the media composition, using solid state fermentations (SSF).

Methods. Fungal strains: *A. niger* (PCS-6) and *A. versicolor* (MIIIgc-2b) were acquired from CIATEJ (Guadalajara, Jal.) microbial collection. Culture media compositions were (g/L): (**a**) Glucose, 7.5; starch, 2.0; urea, 6.0; K_2HPO_4, 7.5; $MgSO_4$, 1.5. (**b**) Glucose, 7.5; starch, 2.0; $NaNO_3$, 4.5; K_2HPO_4, 0.72; $MgSO_4$, 0.37. pH were adjusted at 6.5. Also, the effects of different carbohydrates (Fig. 1) and starch concentrations (2, 4, 8, 16 g/L), on the FAEs production were studied.
Media for SSF were prepared in conical flasks (250 ml) by mixing 0.5 g of polyurethane, 1.5 g of maize bran and 6 ml of culture medium. Moisture was adjusted to 75 %. SSF was performed by inoculating $3x10^7$ spores/g of Dry Matter and incubating at 30 °C. Each experimental unit (flask) was carried out in duplicate and sampled daily for further assays. Feruloyl esterase was assayed by quadruplet according to Ramírez et al. (3).

Results.
Two culture media were compared to produce FAEs using both fungal strains. For *A. versicolor*, medium (b) was found to be the best, while for *A. niger*, both media were equally convenient. Remarkably, FAE production for *A. versicolor* was six times increased using medium (b). This result suggested that *A. versicolor* prefers nitrate as nitrogen source; while *A. niger* does not have preference between nitrate or urea. Different carbon sources (starch, glucose, xylose and xylan) were also tested to improve feruloyl esterase production. Among the tested carbohydrates, starch and a mixture of starch and glucose, were the best options for *A. niger* (Fig. 1), while for *A. versicolor*, similar results on enzyme production were obtained for any carbon source. Finally, the effect of different starch concentrations, on the FAE production, was studied. Results showed that no significant differences were observed for the FAE activities at any tested starch concentration for both fungal strains, suggesting a non repressive effect by starch on the FEA synthesis on solid cultures.

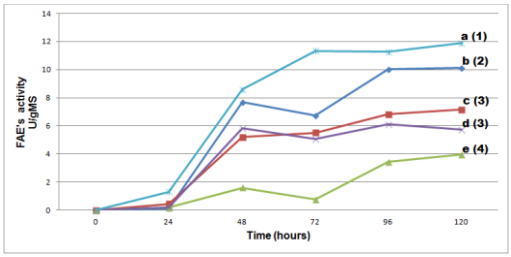

Fig.1 Effect of different carbohydrates (in g/L) on the *A. niger* feruloyl esterase production. a) Glucose 7.5 + starch 2, b) Starch 9.5, c) Glucose 9.5, d) Xylan 9.5, e) Xylose 9.5. Letters followed by different numbers are significantly differed P<0.05.

Conclusions. Nitrate was found to be the best nitrogen source for feruloyl esterase production by *A. versicolor*, increasing six times its production. Starch was found to be the best carbon source for FAE production by both fungi. High starch concentrations in the media did not affected feruloyl esterase production on solid cultures.

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