Sugarcane endophytic fungi as a source of new strains producing lipase

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Background

Lipases are enzymes widely distributed in nature, which catalyze the hydrolysis of fats and oils into free fatty acids, acting on lipid substrates emulsified (DHEEMAN et al., 2011). The industrial demand for new lipase sources with different enzymatic characteristics promote the isolation and detection of new strains with lipase activity (COLEN et al., 2006). These enzymes are considered alternative catalysts for biodiesel synthesis. However, its high cost and low efficiency, hamper its implementation on industrial scale. Endophytic microorganisms inhabit, for at least one period of their life cycle, the interior of the host plant without inducing disease symptoms or producing external structures (AZEVEDO; ARAÚJO, 2007). Our group has previously shown the capacity of some endophytic fungi to secrete hydrolytic enzymes such as lipase, specifically fungal strains isolated from *Saccharum officinarum* leaves (FÁVARO et al., 2011). In this context, this study aimed to characterize a collection of endophytic fungi from sugarcane, to identify novel lipase-producing strains.

Methods

A collection of 205 endophytic fungi isolated from sugarcane leaves was evaluated. Lipase/esterase activity was detected using culture media containing the substrates olive oil, triolein, tributyrin, Tween 20, and Tween 80. Plates were incubated at 25°C for 4 days. The enzymatic index (EI = diameter of halo/diameter of colony) was calculated in duplicate. Besides the detection of lipase/esterase activity, fungal strains were also analyzed at conditions of low water activity (aw), in order to selecting/select strains more adapted to bioprocess conditions. The procedure was to cultivate fungal strains the culture media MY50G (with 50% glucose) and DG18 (with 18% glycerol), indicated to grown xerophilic fungi. For that evaluation, the tests (triplicate) were performed by measure the colony diameter after incubation at 25°C for 10 days. Selected strains were identified by sequencing and phylogenetic analysis of ITS1-5.8S-ITS2 region of ribosomal DNA.

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Results and Conclusions

Olive oil is suitable for the detection of lipase activity, whereas the other substrates may be degraded by both lipases and by esterases. The simultaneous use of different substrates allowed to trace the phenotypic profile of each strain. This approach helps reducing the number of strains to be further evaluated by quantitative assay. In this regard, it was prioritized the selection of lipase producing fungi over esterase, for their subsequent application in biodiesel synthesis. The results showed that fungal strains were categorized in different phenotypic classes according to the substrate degradation profile. The exclusive degradation of only one of the five substrates was observed to 33% of the strains. From a total of 205 strains, 5.0% degraded olive, 11.0% Tween 20, 2.0% Tween 80, 7.0% tributyrin, 8.0% triolein, and 51.0% didn't show any degradation. As for simultaneous degradation, in other words, degradation of more than one substrate: 8 strains showed enzymatic activity in olive and triolein; 2 strains in olive and tributyrin; 1 strain in olive and Tween 20; 3 strains in olive, triolein and Tween 20; 1 strain in olive, triolein, Tween 20 and Tween 80; 1 strain in triolein and Tween 80; 2 strains in olive and tributyrin; 7 strains in Tween 20 and Tween 80; 2 strains in tributyrin and Tween 20 and 1 strain in all of the substrates. The tolerance to conditions of low water activity was investigated. 57% of the strains were capable of growing on culture medium MY50G (aw = 0,91) and 59% at culture medium DG18 (aw = 0,94). Some strains that presented high enzymatic index were also capable of growing on low water activity, indicating that they could be candidates for producing lipases at solid state fermentation. Analysis of the sequence of the ITS1-5.8S-ITS2 region of the ribosomal DNA showed that selected strains belong to the class Ascomycota, specifically to the genera *Ampelomyces, Bipolaris, Gibberella, Bionectria, Colletotrichum, Fusarium, Trichoderma, Penicillium, Myrmecridium, Talaromyces*, and *Gaeumannomyces.* The lipase production of selected isolates will be further evaluated using quantitative tests before their application in the synthesis of biodiesel from palm oil.

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