PE 13 - Detection of Exo-Chitinase Activity from Submerged Culture of *Lecanicillium fungicola*

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Lecanicillium fungicola has been studied as responsible of dry bubble disease of *Agaricus bisporus*, mainly due to the production of extracellular cell wall degrading enzymes, including chitinases, glucanases and proteases (CWDE). The pH has been reported as a very important signal for expression of these CWDE. The CWDE obtained from submerged fermentation (SmF) of *L. fungicola* have been applied for production of oligomers from chitins with several degree of acetylation, however, reports of chitinases purification and their properties are scarce and they are needed for specific applications. The aim of this paper was to produce chitinases from *L. fungicola* and detection of its chitinolytic enzymes.

L. fungicola was cultivated in SmF using Czapeck medium supplemented with chitin, the pH during fermentation was set at initial value of 5, and increased one unit until pH 8 in 144 hours. The change of pH during SmF produced firstly proteases followed by endochitinases and N-acetylhexosaminidases. The enzymatic extract was obtained from the culture broth after centrifugation, ultrafiltration and freeze dried (2). The specific activities for N-acetylhexosaminidase, endochitinase and protease of the enzymatic extract were determined as 0.33, 9.72 and 1079.6 U/mg protein, respectively.

After electrophoretical separation using a 12% (w/v) SDS-PAGE using Coomassie blue for staining, showed that the crude enzymatic extract contain at least eight protein bands assigned to molecular weights (MW) of 63, 55, 48, 40, 36, 30, 28 and 24 kDa.. In-gel chitinase assays were used with chitin of 0.64 degree of acetylation as substrate and revealed with Calcofluor white M2R. The electrophoretical separation displayed four protein bands with chitinolytic activity and MW of 63, 55, 40 and 24 kDa, the latter two bands detected from the enzymatic extract of *L. fungicola* did not coincide with previous reports, for instance, chitobiase and chitosanase of *Verticillium alboatrum* were reported with MW of 64 kDa and 58 kDa and enzymes of *V. lecanii* presented up to 45 kDa. In gel chitinase assay of isoelectrical focusing (5% acrylamide gel and ampholytes pH 3-10) it was detected the presence of a chitinase with an isoelectrical point of pH 4. More studies about specificity of these enzymes with substrates with several degrees of acetylation, as well as further characterization such as type of chitinases (endo or exo), optimal pH and temperature of activities are under way.