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Extracellular lipase enzymes from zygomycetes fungi: production, isolation and examination of biotechnologically relevant properties

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Lipase enzymes (E.C. 3.1.1.3) hydrolyze the triacylglycerols, which are the major constituents of fats and oils, to produce free fatty acids, glycerol and partial acylglycerols. Moreover, many lipases can catalyze the synthesis and translocation of ester linkages resulting biotechnologically important ester compounds. Recently, there is a growing interest for microbial lipases due to their low production costs and wide range of industrial applicability. Accordingly, identification and biochemical characterization of novel microbial lipases have special importance for industrial process development purposes. Zygomycetes are good producers of lipase enzymes; however, only a few enzymes have been isolated and characterized from this fungal group to date. Our knowledge regarding to their synthetic activity in organic media is also limited.

In our studies, 204 zygomycetes fungi were tested on culturing media contained tributyrin for preliminary detection of their extracellular lipase activities. Many *Rhizomucor, Rhizopus, Mucor, Umbelopsis* and *Mortierella* strains showed high enzyme activity and selected for further submerged and solid-state fermentation assays. In those studies, effect of different inductor oils on the enzyme activity is also tested. Enzyme yield of some isolates was outstanding when wheat bran was used as supplement in both submerged and solid state fermentations. Addition of mineral salt solution and olive oil to the solid fermentation medium resulted in at least 1.5-fold increment in the enzyme activities. Lipase production was also tested using oat bran, pressed hempen-, line-, poppy-, pumpkin seed as substrate with high lipid-content. The pumpkin-, and poppy seed residues proved to be promising substrates for lipase induction.

Transesterification assays were performed in non-aqueous conditions using lyophilized crude lipases of selected 11 strains. Enzymes from *Rhizomucor miehei, Rhizopus oryzae, Rhizopus stolonifer, Mucor corticolus* and *Mortierella echinosphaera* exhibited the highest transesterification activity in *n*-heptane using *p*-nitrophenyl palmitate as fatty acid donor and ethanol as acceptor. To characterize the reaction, effect of incubation time and temperature, various reaction media and acceptor alcohol on synthetic activity were also tested. Results showed that prolonged incubation time and high temperature (40 and 50 °C) generally enhanced the product yield. Considering the results of fermentation and transesterification tests, purification and biochemical characterization of *R. miehei*, *Rh. oryzae*, *M. corticolus* and *Mo. echinosphaera* lipases were carried out. SDS-polyacrylamide gel electrophoresis indicated a molecular mass of about 52, 56, 20 and 30 kDa for the purified enzymes, respectively. Biochemical characterization assays including temperature and pH tolerance studies, substrate specificity determination, and examination of the effect of some ions, alcohols and organic solvents on the activity were also performed. Purification of lipase produced by *Rh. stolonifer* is in progress, and synthetic esters formed by transesterification and esterification reactions are also being researched using gas chromatography technique.

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Molecular characterization of ROP GTPase activated kinases in *Arabidopsis*

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The Rho-type GTPases have central roles in cellular processes associated with cytoskeletal dynamics (e.g. cell movement, cell division, cell shape, and cell polarity). These proteins operate as molecular switches: they activate signal transduction pathways when they are in GTP-bound conformation, but their signalling activity cease when they are GDP-bound. If the Rho GTPase is in the GTP-bound form, it can further activate a diverse set of downstream signalling effector proteins.

Plants has a specific group of Rho-type GTPases, the „Rho of plants" (ROP) family. Our knowledge about the signalling pathways associated with ROPs is yet incomplete. ROPs differ from other Rho-type GTPases in the regions which are responsible for effector binding, suggesting that ROP GTPases have specific effectors. Indeed, plants lack the Rho GTPase-activated PAK kinases, which are very important mediators of Rho GTPase signalling in yeast as well as in animals. Therefore our question was: are there any ROP GTPase-activated kinases, which may have PAK-like functions in plants? Due to a yeast two-hybrid screening approach two ROP-interacting kinases could be identified. These kinases interacted with the GTP- but not with the GDP-bound ROP form what is typical for ROP effectors. Furthermore, the in vitro activity of these kinases was dependent on the presence of GTP-bound ROP. These ROP-activated kinases belong to the subfamily VI of receptor-like cytoplasmic kinases (RLCKs) of *Arabidopsis*. They have a receptor kinase-like catalytic domain, but they don't have extracellular or transmembrane regions and that's why they can found in the cytoplasm. Based on their primary structure, the 14 *Arabidopsis* RLCK VI kinases can be classified into two groups (A and B). Only the members of group A have ROP GTPase-binding ability. Based on in silico comparison, several positions were identified where the amino acids are characteristically different in the sequences