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SCREENING OF FUNGAL SPECIES ISOLATED FROM DIFFERENT SOIL SAMPLES FOR EXTRACELLULAR LIPASE PRODUCTION

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Lipases are remarkable biocatalysts that hydrolyze triacylglycerols to glycerol and fatty acids. Along with other reactions like acidolysis, alcoholysis, and aminolysis, these ubiquitous enzymes also catalyze reversible reactions, including esterification, transesterification, and interesterification. Filamentous fungi are excellent extracellular lipase producers, and fungal lipases can function at extreme temperatures and pH, are chemo-selective, stable in organic solvents, and are abundant. Extraction of fungal lipases is relatively easy and cost-effective. Although many studies have been conducted in this area, many more lipolytic fungal species still need to be discovered and studied. The objective of the current work was to isolate and characterize novel lipolytic fungi from oil-contaminated soil. The collected soil samples from four different areas (Normal soil sample from mini Sinharaja area, Faculty of Science, University of Peradeniya, Compost sample from Gampaha area, Oil-contaminated soil sample from coconut oil mill in Gampaha, Oil-contaminated soil sample from coconut oil mill in Kandy) were cultured on PDA medium, and fungal species were isolated. Para-nitrophenyl palmitate assay was used to examine each species' individual lipolytic activity in the fungal strains that tested positive in the phenol red and Tween 20 tests. Following the morphological identification based on colony morphology and microscopic view, molecular identification (sequencing followed by BLAST search and phylogenetic analysis) was done to determine the most efficient fungal species. Out of the 12 isolated species, 10 species gave positive results for lipolytic activity, and based on the results of the para-nitrophenyl palmitate assay, DNA from three species with the highest lipolytic activity were extracted using the phenol-chloroform method and heating method. According to the sequences of ITS regions, the most efficient lipolytic fungal species were identified as *Rhizopus arrahizus* (0.5363) followed by *Aspergillus niger* (0.1980), and *Aspergillus nomius* (0.1963). Enzyme characterization and media optimization are necessary to use these fungal species as potential sources of lipase for industrial use.

Keywords: Fungal lipase, Industrial application of lipase, ITS gene, Phenol red test and Tween 20 test, pNPP assay