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Biodeterioration of low density polyethylene by *Montagnula scabiosae*

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Endolichenic Fungi (ELF) are a group of fungi adapted to live within lichen thalli. Present study investigates the ability of *Montagnula scabiosae*, an ELF, for biodeterioration of low density polyethylene (LDPE) by determining the activity of three extracellular fungal enzymes, reported to have potential roles in depolymerizing polyethylene, using enzymatic assays. Liquid medium screening assays, using four potato dextrose agar (PDA) plugs (0.5 cm diameter) of ELF cultures, inoculated into Erlenmeyer flasks containing sterile mineral salt medium broth (MSMB). Eight sterile LDPE strips (10 cm x 3 cm) were added into each flask and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 21 days. MSMB flasks with plain PDA plugs and sterile LDPE strips were used as the controls. Solid medium biodeterioration studies also were conducted using three PDA plugs of the ELF cultures inoculated into mineral salt medium agar (MSMA) plates. Eight sterile LDPE strips were aseptically placed on the surface of each MSMA plate and incubated at room temperature for 45 days. MSMA plates with plain PDA plugs and sterile LDPE strips were used as the controls. Both liquid and solid media assays were carried out in four replicates. LDPE biodeterioration was analyzed based on reductions in weights and tensile properties, changes in peaks of Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectra, changes in carbonyl index (CI) and Scanning Electron Microscopic (SEM) images. A distinguishable LDPE deterioration ability was shown by *Montagnula scabiosae*, isolated from the host lichen *Pyrenocarp* sp. Results showed that isolate reduced weights of strips by 13.22% in liquid medium and by 12.06% in solid medium. Tensile strength of liquid medium treated strips, reduced by 6.64% and solid medium treated strips, by 3.00%. The control LDPE strips on ATR-FTIR analysis showed four prominent peaks at 2916 cm^{-1} , 2848 cm^{-1} , 1463 cm^{-1} and 720 cm^{-1} and a few minor peaks. Changes visible in the ATR-FTIR spectra of treated strips, manifested new peaks, fluctuations in percentage transmittance and deletions of existing peaks. CI value of the LDPE strips in liquid medium were found to be increased by 2.70% and in solid medium increment was 2.31%. SEM images of treated strips showed erosion zones on LDPE matrix. Qualitative enzymatic assays using Agar plate-based screening methods, indicated the presence of laccase and peroxidases, in this ELF isolate. In the quantitative enzymatic assays, *M. scabiosae* showed laccase, lignin peroxidase and manganese peroxidase activities as $1.07 \times 10^{-7}\text{ kat L}^{-1}$, $1.22 \times 10^{-8}\text{ kat L}^{-1}$ and $2.10 \times 10^{-8}\text{ kat L}^{-1}$ respectively. Remarkable LDPE deterioration ability shown by this ELF isolate indicates that it has a high potential in degrading LDPE waste.

Keywords: Biodeterioration, Laccase, LDPE, *Montagnula scabiosae*, Peroxidases

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