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Enzymatic approach to green air: Depolymerization of polycyclic aromatic hydrocarbons (PAHS) by *Aspergillus* sp. isolated from phyllosphere of urban areas

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Polycyclic aromatic hydrocarbons (PAHs) are hazardous air pollutants that are toxic to many life forms. Biodegradation is an eco-friendly efficient option found to remediate these toxic PAHs. Air pollutants from many sources get settled over the phyllosphere through atmospheric deposition. Phyllosphere is a large niche for many fungal species and some of them metabolize many PAHs to nontoxic concentrations. The present study was to determine the PAHs (phenanthrene, anthracene, naphthalene and pyrene) degradation capability of phyllosphere inhabited Aspergillus species. Fungal isolations were made from leaf samples (Amaranthus cruentus, Hibiscus rosa-sinensis, Ervatamia divaricate, Plumeria sp., and Ixora chinensis) grown in Panchikawatta, Orugodawatta, Pettah, Maradana, Colombo Fort and Sapugaskanda oil refinery sites in Sri Lanka. Out of morphologically different thirty-five fungal isolations, *Aspergillus* spp. were identified to the genus level using identification keys and pre-existing identified reference cultures. PAHs degradation ability of isolated Aspergillus spp. was screened using a plate assay and confirmed by High Performance Liquid Chromatography (HPLC). Further, phyto-toxicity assays were performed using Vigna radiata seeds to test environmental toxicity and toxicity to the degrading fungal cells in the medium from the produced metabolites. Furthermore, manganese-dependent peroxidases (MnPs), lignin peroxidases (LiPs), and laccases enzyme activities of them during the PAHs depolymerization were analysed parallel to the PAHs degradation percentages. According to HPLC analysis, Aspergillus sp. $P_{21}B - 77$ showed the most efficient degradation of anthracene (80%), Aspergillus sp. $P_{11}B - 34$ was the most efficient degrader for naphthalene (82%) and Aspergillus sp. $P_{22}T - 82$ was the most efficient degrader for pyrene (84%) and phenanthrene (86%). MnP enzyme activity dominated the highest anthracene depolymerization ability of Aspergillus $P_{21}B - 77$ However; LiPs activity dominated the highest phenanthrene and pyrene depolymerization in Aspergillus sp. $P_{22}T$ – 82. Moreover, Aspergillus sp. $P_{11}B - 34$ showed the best naphthalene degradation, and laccases enzyme activity dominated the degradation. The toxicity assay revealed that the generated metabolites were not toxic to the growth of Aspergillus spp. and, also verified that those by-products were not destructive compounds to the phyllosphere. Aspergillus spp. could be useful as a potential biological agent for an effective bioremediation process in polluted environments contaminated with phenanthrene, anthracene, naphthalene and pyrene like polycyclic aromatic hydrocarbons.

Keywords: Aspergillus spp., Bioremediation, HPLC, Phyllosphere, Phytotoxicity

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