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Characterization and biological prospecting of
phyllosphere microorganisms capable in
aromatic hydrocarbon degradation

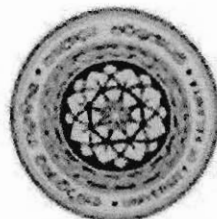


Submitted by

L.J. S Undugoda

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Abstract

Thirty-eight phyllosphere fungal strains and twenty-four bacterial strains were isolated from the leaves of four ornamental plants (*Ixora chinensis*, *Ervatamia dervaticata*, *Hibiscus rosa-sinensis* and *Amaranthus cruentus*) collected from five polluted sites, Colombo Fort, Maradana, Orugodawattha, Panchikawattha and Sapugaskanda. Plate assay, colorimetric and HPLC analysis results revealed that only nineteen of these fungal strains and eight bacterial strains had the AH degradation ability. Then the best AH (Naphthalene, phenanthrene, xylene and toluene) degrading phyllosphere fungi were identified upto species levels (*Penicillium oxalicum*, *Aspergillus aculeatus*, *Aspergillus oryzae* and *Colletotrichum siamense*) using molecular techniques followed by PCR amplification, amplicons sequencing and BLASTN search. The best AH degrading bacteria were *Alcaligenes faecalis* and *Alcaligenes* sp.DN25. The bacterial strains belonging to genera, *Bacillus*, *Serratia*, *Klebsiella* and *Pseudomonas* showed low degradation rate of phenanthrene, naphthalene, toluene and xylene degradation ability.

Four AH degrading bacterial strains, *Alcaligenes faecalis*, *Alcaligenes* sp.DN25, *Bacillus cereus* and *Bacillus methylotrophicus* harboured the plasmids. Transformation and plasmid curing experiments revealed AH degradation ability of *Alcaligenes faecalis* and *Alcaligenes* sp.DN25 was a plasmid encoded character. The catabolic plasmids of *Alcaligenes faecalis*, *Alcaligenes* sp.DN25, *Bacillus cereus* and *Bacillus methylotrophicus* harboured *nahR*, *xylQ* and *phnG* genes, but did not have *nahU*, *xylM* and *phnAc* genes. Nucleotide sequence alignment and RFLP patterns of *xylQ* gene revealed the presence of two allele types of *xylQ* gene. Nucleotide sequence alignment and dendogram of *nahR* gene revealed the presence of three allele types of *nahR* gene. These genes can be used to create modified organisms which can remediate AH pollutants in the ambient air.

Keywords: Phyllosphere fungi, Plasmid, *nahR* gene, Ornamental plants, Aromatic hydrocarbon.