THE ISOLATION AND MOLECULAR CHARACTERISATION OF DIFFERENT *Pseudomonas* spp. FROM WASTE ENVIRONMENTS IN SRI LANKA

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Phenol demonstrates widespread occurrence in the environment and hence microorganisms have evolved with the capacity to utilise phenol as a carbon source. Such biodegrading microorganisms can be used as bioremediation agents to treat phenol contaminants in wastewater. The focus of this study was to isolate *Pseudomonas* species as this genus is the most utilised and popular bacterial agent that is used in the bioremediation of phenol. Bacteria were isolated from wastewater collected from petroleum-contaminated environments in the Kurunegala, Kandy, Colombo and Gampaha districts in Sri Lanka. Bacterial isolation and culturing were done in Mineral Salt Media, supplemented with 200 mg/L phenol as the sole carbon source. Bacterial identification was done using 16 S rRNA gene analysis up to the species level. The Phenol degradation efficiency of the identified *Pseudomonas* spp. was measured using 4 - aminoantipyrine spectrophotometric assay. The identified bacterial isolates were screened for the presence of catabolic gene, *LmPH* that codes the large subunit of phenol hydroxylase enzyme responsible for the initial ring activation of phenol by using a primer set designed based on the gene sequence of *P. putida*. The species *P. aeruginosa* (MH031762), *P. monteilii* (MH636875.1) *and Pseudomonas* sp. (MH027519) were isolated in this study. The phenol degradation assay showed that *P. aeruginosa* degrades 1800 mg/L phenol within 120 h, *P. monteilii* degrades 1700 mg/L phenol completely within 144 h and *Pseudomonas* sp. showed complete degradation of 1700 mg/L phenol in 144 h. Furthermore, the gDNA amplification of *P. aeruginosa*, *P. monteilii* resulted in expected amplicons of 684 bp for *LmPH* gene specific primers in PCR, confirming the presence of the *LmPH* gene. Nucleotide sequences of amplicons showed ≥ 99% homology to *LmPH* gene in the BLAST analysis. Laboratory assay on phenol degradation, followed by the characterising catabolic gene of phenol, confirmed the potential of the phenol degradation of isolated *Pseudomonas* spp.

Keywords: Phenol, *Pseudomonas* spp., waste environments

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