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Isolation and Identification of Heavy Metal Tolerant Soil Fungi from the Ussangoda Serpentine

Kumari G.K.A., Saputhanthri P.S.*

Department of Plant Sciences, Faculty of Science, University of Colombo, Colombo 03
**pradee@pts.cmb.ac.lk*

Abstract

'Serpentine' soils derived from certain ultramafic rocks occur throughout the globe. These soils are high in Fe and Mg concentrations; consist heavy metals such as Ni, Cr, Cd, and Co; are nutrient poor and ionically imbalanced for plant growth. Serpentine life forms typically contain specialised physiological mechanisms that allow tolerate the adverse chemical conditions imposed by the substrate. Heavy metal hyper-accumulating and/or tolerating plants and micro-organisms from serpentines represent a rare and valuable biological resource. These unique habitats and their exceptional species must be characterised and conserved. This study aimed to isolate and identify heavy metal tolerating soil microorganisms from the Ussangoda serpentine site (Hambantota District). Soil samples from ten locations within the serpentine area (to represent various aspects of the terrain such as flat plain, shrubby patches in the middle, and periphery) were collected and analysed separately. Low soil organic matter (1.02% to 1.71%) and moisture (highest being 1.21%) contents were observed with soil pH ranging between 5.47-8.46 (majority of the samples being acidic). Metal analysis of soil Cr, Fe and Cu indicated 0.1-0.4 ppm, 10.4-19.0 ppm and 5 ppm-11 ppm, respectively. Serpentine soil microorganisms were isolated using standard methods. Twenty fungal isolates and twelve bacterial isolates were obtained by the preliminary study. To investigate their tolerance of heavy metals, all twenty fungal isolates were grown in PDA media containing different concentrations of selected metal solutions ($Ni(NO_3)_2$ and $CuSO_4(H_2O)_5$). Nine out of twenty fungal isolates survived up to 800 µg/mL $CuSO_4(H_2O)_5$ and four isolates tolerated up to 2,000 µg/mL $Ni(NO_3)_2$. Based on the highest tolerance shown to the tested metals, two fungal isolates were selected and identified using molecular techniques. Genomic DNA extracts of two fungal isolates were sequenced using Internal Transcribed Spacer (ITS) region reverse and forward primers. From the sequences, the two isolates were identified as *Aspergillus terreus* and *Gongronella butleri*. *A. terreus* has many previous records on heavy metal tolerance and being used in cleaning up industrial effluents contaminated with certain metals. *G. butleri* is known to produce the amino-polysaccharide chitosan which is a metal chelator and it may be one reason for the observed heavy metal tolerance capacity of the fungus. This study has provided evidence-based information which may be useful in developing green technologies to remediate soils contaminated with heavy metals. The results also reiterate the importance of conserving serpentine habitats that harbor unique species and communities of organisms.

Keywords: Serpentine, Ussangoda, Heavy-metal tolerant fungi