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Characterization of two heavy metal removal Bacillus strains from Sri Lanka into species level

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Bacteria are organisms that have the capability to adapt to changing environments. Though the development of antibiotic resistance of bacteria has become a threat to animals including human beings, acquired heavy metal resistance and tolerance capability of bacteria is of immense benefit as they can be used in bioremediation of man polluted natural environmental bodies such as water and soil. In this study we have characterized a few bacterial strains which were isolated from industrial waste water with different heavy metal concentrations with the aim of using them in industrial waste water treatment for heavy metals. Heavy metal resistance and bio-removal capacity was determined prior to species identification. Bacterial strains were grown in Cu^{2+}, Cd^{2+} and Pb^{2+} metal ion (1 mg/ L) containing LB media and metal ion removal by the bacteria per day was determined along with the growth rate. Two bacterial strains having similar Cu^{2+}, Cd^{2+} and Pb^{2+} resistance were characterized morphologically, biochemically and molecularly and identified to the species level by 16S ribosomal DNA sequence analysis. The phylogenetic tree was constructed after multiple sequence alignment using Clustal W tool with the aid of MEGA7.0.14 software. Sequence comparisons were made with sequences available in NCBI database using BLAST tool after analyzing the sequences using BioEdit software v7.1.11. Two bacterial isolates TWSL-10 and TWSL-7 showed 83.685 ± 1.152% and 92.091 ± 0.361% Cu^{2+} ion removal capacity while Pb^{2+} removal capacity were 93.101 ±0.803% and 97.257 ± 0.627 % after the 7th day of inoculation. The Cd^{2+} ion removal of the strain TWSL-7 was 83.859 ± 0.906 % while it was 63.101±0.416% for the strain TWSL-10. Strain TWSL-7 showed 99% homology to the 16S rDNA sequences of both *Bacillus marisflavi* while TWSL-10 showed 99% homology to *Bacillus aquamaris* sequences available at NCBI. Strains TWSL-7 and TWSL-10 were confirmed as *Bacillus marisflavi* strain TWSL-7 (Accession number: gb|KR027925.1) and *Bacillus aquamaris* strain TWSL-10 (Accession number: gb|KT184891.1), respectively, based on molecular and morphological characteristics and their sequences were deposited in the NCBI database.

Keywords: 16S rDNA, *Bacillus*, bioremediation, industrial waste water, metal resistance

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