Molecular characterization of bacteria involved in bioremediation of heavy metals and elucidation of possible bioremediation mechanisms

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ABSTRACT

Health effects associated with potentially toxic elements highlight the risk related to toxicants like Cd, Ni, Cr, Zn, Pb, Hg, Cu and As. Traditional methods of metal removal have several drawbacks and therefore there is a need to find a sustainable and eco-friendly solution. Microbes provide an alternative cost effective, eco-friendly approach for metal remediation.

An effluent from a textile dyeing factory was screened for bacterial strains capable of metal bioremediation. Twenty four bacterial strains were isolated and identified using 16S rRNA analysis. They belonged to 5 genera including *Bacillus* (09), *Staphylococcus* (10), *Paracoccus* (3), *Aeromonas* (1) and *Fictibacillus* (1). Metal resistance capacity, bacterial growth in metal-containing media and the metal removal ability of the bacterial strains were assayed. Assays were initially carried out for Cd, Cu and Pb and then for Cr, Zn and Fe.

To elucidate molecular mechanisms of metal tolerance, isolated strains were screened for any possible metal resistant determinants using PCR. Several genes and gene clusters (operons) were identified and cloned. Bacterial strains that harboured metal resistant determinants showed significant metal resistance and metal removal ability in spiked cultures as well as in an industrial effluent. A *cadD* recombinant bacterial strain developed in this study was also found to have enhanced metal removal ability, confirming involvement of CadD in metal detoxification.

All bacterial strains isolated showed high metal resistance capacity for Cu, Cd, and Pb. The highest Minimum Inhibitory Concentration (MIC) was shown for Pb (≥1000 mg/L) by several strains. Most strains isolated grew significantly ($p<0.0001$) in metal containing media and were able to remove >90% of Pb (10 strains), Cu (10 strains) and Cd (5 strains) from metal spiked media compared to the control ($p>0.05$) within 7 days.

A set of metal resistance genes (*copA, cadA, copZ, cadC, cadD*, and *MT*-like) were identified using PCR and confirmed by sequence analysis. The functions of the genes involved in metal homeostasis was bioinformatically determined by identifying conserved regions/ functionally active sites/motifs present. Several complete genes and operons from strains TWSL_4 (*B. megaterium*), TWSL_6 (*S. warnei*), TWSL_17 (*S. epidermidis*,) and TWSL_22 (*S. epidermidis*) involved in metal remediation were isolated and cloned in the vector, pGEM®-T and three genes in the expression vector, pET-21a(+).

Bacterial strains (TWSL_4, 5. 6, 9, and 17), and the *cadD* recombinant clone pD7d16`/18` when assayed using an industrial effluent sample had an elevated metal removal capacity compared to the control effluent (abiotic control). IPTG induced pD7d16`/18` clone showed higher bioremoval ability compared to the un-induced control, confirming the involvement of CadD in metal homeostasis. Formation of biofilms by the bacterial strains (isolated and recombinant) were observed and scanning electron microscope (SEM) and energy-dispersive X-ray (SEM-EDX) analysis revealed the immobilization of Fe. Molecular Dynamics simulation studies revealed the presence of three regions (Cys6-Cys10, Asp100-His102 and Lys113-Asp116) in CadC as good binding sites for Cd^{2+} and Zn^{2+}.

The study clearly demonstrated the multi-metal removal capacity and metal bioremediation ability of the bacterial strains. These have the potential to be developed into an efficient metal removal system. Furthermore, operons and genes identified in the study, could be used for the construction of a biosorbent or biosensor to monitor the presence of Zn, Cu, Cd and Pb in effluents or in the environment.