## D-26 Isolation of plasmid DNA from a locally isolated heavy metal ion resistant strain of *Bacillus cereus*

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Micro-organisms are increasingly being used in bioremediation of waste water contaminated by heavy metal ions, and in the commercial extraction of metal ions from solution. Resistance to heavy metal ions may be due to the presence of a plasmid, or a genetic modification linked to the sex factor, or chromosome.

The objective of this investigation was to analyse 3 species of metal tolerant *Bacillus* strain isolated from Sri Lanka, *B. cereus, B. megaterium,* and *B. pumilus,* for the presence of plasmids. DNA was extracted by alkaline lysis method from a mini-preparation and subjected to agarose gel electrophoresis. The *Bacillus* strain *B. cereus* revealed 2 plasmid (one parallel to, and the other greater than the 23 kb marker). Since the amount of DNA obtained was not adequate to perform restriction enzyme digestions, a large scale preparation of plasmid DNA was made and purified on a CsCl/ethidium bromide gradient.

*Hind III* and *EcoRI* digests of purified DNA, when subjected to agarose gel electrophoresis, revealed a discrete banding pattern, confirming the existence of plasmid DNA. However, since plasmid DNA was found to be also contaminated with genomic DNA, a Southern blotting was performed on the same gel. Hybridization with electro-eluted purified plasmid DNA band (23 kb) oligolabelled by $^{32}P$ revealed a clear banding pattern with 3 bands for *EcoRI* and 4 bands for *Hind III* digested samples. Analysis of the banding pattern is consistent with the presence of a plasmid of approximate size 18 kb.

This is the first report of an isolation of a plasmid from a metal-tolerant *Bacillus* species from Sri Lanka.