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Summary

Over the last decade, the consumption of heavy metals in the industrial activities, instance electroplating plants, mining, nuclear and electronics industries, has increased dramatically and poses a major environmental and human health hazard. This threat of heavy metal pollution has led to an increased interest in developing systems that can remove or neutralize its toxic effects in soil, sediments and wastewater.

Microbial population in metal polluted environments contain micro-organisms which have been adapted to toxic concentrations of heavy metals and become metal resistant. The use of heavy metal resistant bacteria for bioremediation of polluted water has attracted growing attention because of several problems associated with pollutant removal using conventional methods.

The present study was conducted to identify and characterize the bacterial isolates with superior ability to accumulate heavy metal ions. Identification of some heavy metals tolerant–genes in such bacteria species was also considered.

The present investigation comprises two studies:

- Microbiological & Ecological studies.
- Molecular study.

So gram-positive and gram-negative water bacteria isolated from industrial wastewater that exhibited resistance to range of metal ions including copper, cadmium, zinc, and cobalt. Different techniques have been used for genes detection and characterization. PCR and DNA sequencing were used to analyses the genetic systems involved in metal resistance in these bacteria.

To accomplish this goal the following steps were performed:

- The wastewater samples were collected from industrial wastewater ponds found in Sadat City, Egypt.
- Different species of bacteria were isolated from wastewater.
- Some chemical properties of wastewater samples were determined.
- Some of heavy metals contents in wastewater were evaluated.
- Bacterial identification using bacteriological and biochemical methods were performed.
- The minimum inhibitory concentrations (MICs) for each bacterial isolates were determined.

- The minimum inhibitory concentrations (MICs) for each isolates grow in mixture of metals were determined

- The metals biosorption performance was evaluated.

- *Pco*R, *Czc*D resistance genes were detected using PCR to amplify DNA fragment of 630 bp, 400 bp in *Cu*, *Czc* genes respectively.

- *Pco*R, *Czc*D amplification products were cloned and transformed into *E.coli* host cells.

- The DNA sequences for pACu1, pACzc1 clones were conducted.

- The DNA sequence was subjected to computer analysis to identify the cloned genes.

- Computer analysis revealed that the amplified DNA fragment is similar to *Cu*, *Czc* genes isolated from other strains.

From the previous steps the author could conclude that:

- Five bacterial isolates resistant to Cu, Cd, Co and Zn have been isolated from industrial wastewater ponds at Sadat city, Egypt.

- MICs determination indicated that the bacteria used in the present investigation have developed resistance to several of the metal ions tested..

- Some of the isolates showed high resistance to certain ions such as the gram positive isolates *B. cereus* (A1) was more resistant to cadmium, cobalt and zinc.

- The gram negative *E.aerogenes* (A3) showed the highest level of resistance to copper.

- A partial length of *Cu* and *Czc* resistant genes have been detected and amplified from two isolates *Bacillus cereus* and *Enterobacter aerogenes*.

- Sequencing of *pACu1*, and *pACzc1* clones were performed

- The sequence of these clones were compared to those in public database.

- The alignment indicated that there is 98 % similarity ratio between *pACu1* obtained from *E.aerogenes* clone and *Cu* gene of *E.coli* plasmid pRJ1004 DNA.

- Also there is 99 % similarity ratio between *Bacillus cereus* of p*ACzc*1 clone and *Czc* gene of *Ralstonia* sp.