Assessing the Role of Microorganisms Present in the Gut of the Earthworm *Pontoscolex corethrurus* in Metal Uptake from Soil

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Earthworms have been involved in waste and environment management for many years. Heavy metals like copper, cadmium, zinc and lead are known to be major contaminants of soil. Endogeic earthworms are often exposed to various kinds of stress, which in turn could have a profound effect on the microflora of their gut. The present study aims at determining the role of the gut microflora of the endogeic earthworm *Pontoscolex corethrurus* in detoxifying heavy metals present in the soil. The earthworms were exposed to different concentrations of Cd, Cu and Zn under semi-natural conditions. Microorganisms from the gut of earthworms grown under control conditions and those exposed to heavy metals were isolated and characterized for various parameters like multiple metal tolerance, presence of plasmids, resistance to common herbicides, presence of metal resistance genes, and changes in protein expression.

The bacteria specifically harboured in the gut of earthworms subjected to metal stress (cadmium, copper and zinc) were identified by comparing the microbes isolated from the gut of earthworms subjected to metal treatment with the gut microflora of control earthworms. *Staphylococcus* sp. *and Delftia* sp. were found to be selectively accumulated in the gut of earthworms exposed to Cd stress*.* The microorganisms which were selectively harboured in the gut of earthworms subjected to Cu treatment were *Bacillus* sp. and *Staphylococcus* sp*. Aeromonas* sp. *Bacillus* sp*.* were found to be selectively accumulated in the gut of earthworms exposed to Zn stress. The microorganisms were accurately identified using the 16S rDNA sequencing technique.

The earthworms' ability to take up heavy metals from the soil was analyzed by carrying out atomic absorption spectroscopy studies on soil samples. The results indicated a drastic decrease in all the three heavy metals (Cd, Cu and Zn) in the soil after the growth of *Pontoscolex corethrurus*. The isolated microorganisms *Staphylococcus aureus, Delftia* sp., *Bacillus cereus* and *Aeromonas* sp. were assessed based on their ability to tolerate different concentrations of heavy metals ($CdCl_2$, $CuCl_2$ and $ZnCl_2$). It was observed that all the four bacterial strains were able to tolerate Cu and Zn, but were sensitive to Cd. All the microorganisms tested (*Staphylococcus sp., Delftia sp., Bacillus cereus and Aeromonas hydrophila*) were found to be resistant to the herbicides Atrazine and 2,4-D, but were sensitive to Glyphosate (concentration 26.7 g/L).

In the presence of Cd, all the four bacterial strains (*Staphylococcus* sp., *Delftia* sp., *Bacillus cereus and Aeromonas hydrophila*) showed the over-expression of certain proteins compared with the control. In *Bacillus cereus,* there was over-expression of a protein corresponding to a molecular weight of 97 kDa. *Delftia* sp. showed the over-expression of three different proteins with molecular weights of approximately 43 kDa, 66 kDa and >200 kDa. In *Staphylococci*, protein bands corresponding to molecular weights of approximately 80 kDa, >97 kDa and >200 kDa were over-expressed in the presence of Cd. No visible protein bands were seen in the presence of Cu and Zn. Plasmids were not present in any of the four bacterial strains. The bacterial strains were analyzed for the presence of the metal resistance genes Znt R, AHA_2962, Czc A and Czc C/D. The *Aeromonas* strain exhibited the presence of the Znt R gene as confirmed by amplification and sequencing.