TITLE: A *Enterobacter clocae* grows at high cadmium concentrations and protect *Salvinia auriculata*

AUTHORS: Mota, G.P.; Freitas, M.C.; Souza, G.R.; Rocha, L.O; Olivares, F.L.; Berbert-Molina, M.A.; Intorne, A.C.

INSTITUTION: Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ (Avenida Alberto Lamego, 2000, CBB/LFBM, CEP 28013-602, Brasil)

ABSTRACT:

Cadmium (Cd) contamination is a global problem and a growing threat due to its high toxicity**.** Bioremediation mediated by Cd-resistant bacteria in association with aquatic macrophytes is an alternative for recovering contaminated areas. *E. cloacae* UENF P7 was isolated from an aquatic plant and was shown to remove Cd. In this work, it was verified the cultivation of this strain in high concentrations of Cd and its ability to protect the aquatic plant *S. auriculata* exposed to this metal. For this, the bacteria was cultivated in LB medium with 55 and 110 mg L^{-1} Cd, and without addition (control), in Erlenmeyer flasks, at 30 ºC and 140 min^{-1}, evaluating growth (OD=600nm, CFU .mL-1 and dry weight), pH, live/dead assay and morphology by optical and electron microscopy (transmission and scanning). The test with the plant, submitted or not to Cd stress, was carried out in a greenhouse with pots containing 500 mL of Hoagland solution ¼, 1 g of plant, bacterial inoculum (10^8 cells mL^{-1}) and Cd (0,5 mg L^{-1}). At the end of 8 days, fresh weight (FW) was evaluated. In medium with 55 mg L^{-1} Cd there seemed to be an increase in cell production (OD_{600nm}=15), being 3.5 times greater than the control after 10 h of culture. This was confirmed by cell plate count, cell dry weight and microscopy. With 110 mg L^{-1} Cd, there was an extensive lag phase and the final cell production also seemed superior to the control, although slightly lower than with 55 mg L^{-1} Cd. Under all conditions, cells were viable at the end of culture and there was a decrease in the pH of the medium (pH ≈ 3.8) during culture. However, in media with Cd, the pH rose again when the culture reached the stationary phase. In all treatments, the cells were viable at the end of the culture and there was a decrease in the pH of the medium (pH ≈ 3.8) during growth. However, in media with Cd, the pH increased when the culture reached the stationary phase. In electron microscopy images with Cd, the cells had eléctron-dense granules and biofilm formation that were absent in control. Plants treated with the bacteria obtained FW 8.3% higher than the control plants. The addition of Cd significantly affected the plant, with 44.7% reduction in FW. However, when inoculated with the bacteria, the damage caused by Cd was lower (FW=35.1%). To the best of our knowledge, this work is the first to suggest a greater production of cells in an organism at these Cd concentrations. Thus, the association of *S. auriculata* and *E. cloacae* UENF P7 shows potential for bioremediation of this metal.

Keywords: heavy metal; bacteria-plant interaction; bioinoculant

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