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The accumulation of manganese by *Chlamydomonas acidophila* strains isolated from acid mine drainage

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Acid mine drainage ponds represents a specific artificial ecosystem that gives advantage to extremophilic microalgae. The mechanisms of adaptaton of such strains to excess metal concentrations that are common for their habitat, are poorly understood. Herein, we analyzed two strains of the green microalga *Chlamydomonas acidophila* - 137 and PM01, which have been isolated from different mining sites, for their interactions with Mn ions. The effects of different concentrations of Mn²⁺ were investigated in the late exponential/early stationary phase of culture growth (15 days). Viability was determined by Evans blue assay. No toxic effects were observed at concentrations as high as 2 mM Mn²⁺. The the time dynamics of Mn accumulation in the biomass was determined using ICP. It was shown that the maximum accumulation of Mn in strain 137 (3.79 ± 0.68 µg/mg) was reached at 24h, while for PM01 the highest uptake (3.23 ± 0.17 µg/mg) was observed at 72 h. Next we analyzed redox settings by measuring the levels of reduced thiols using *in vivo* EPR spin probing. The treatment with 2 mM Mn²⁺ induced a rapid and irreversible decrease in the level of thiols which indicates that Mn²⁺ activated prooxidative processes. The maximum drop of thios levels were: from 4.80 ± 0.05 to 2.70 ± 0.05nmol/mg fresh weight after 1h for 137; and from 4.70 ± 0.05 to 3.40 ± 0.05 nmol/mg fresh weight after 30 min for PM01. Further, the level of reactive oxygen species was evaluated using fluorescent probe DCFH-DA assay. The changes were in agreement with thiol levels. Both tested algal strains show resistance to Mn and are therefore good candidates for application in water bioremediation.

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