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Impact of heavy metals in the microalga *Chlorella sorokiniana* and assessment of its potential use in cadmium bioremediation

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ABSTRACT

The chlorophyte microalga *Chlorella sorokiniana* was tested for the bioremediation of heavy metals pollution. It was cultured with different concentrations of Cu^{2+}, Cd^{2+}, As (III) and As (V), showing a significant inhibition on its growth at concentrations of 500 μM Cu^{2+}, 250 μM Cd^{2+}, 750 μM AsO_3^{3-} and 5 mM AsO_4^{3-} or higher. Moreover, the consumption of ammonium was also studied, showing significant differences for concentrations higher than 1 mM of Cu^{2+} and As (III), and 5 mM of As (V). The determination of intracellular heavy metals concentration revealed that *Chlorella sorokiniana* is an outstanding Cd accumulator organism, able to accumulate 11,232 mg kg^{-1} of Cd, and removing 65% of initial concentration of this heavy metal. Finally, antioxidant enzymes, such as catalase (CAT) and ascorbate peroxidase (APX), and enzymes involved in the production of glutamate and cysteine, such as glutamine syntethase (GS), glutamate dehydrogenase (GDH), O-acetylserine (thiol) lyase (OASTL) and NAD-isocitrate dehydrogenase (NAD-IDH) were studied both at gene expression and enzymatic activity levels. These enzymes exhibited different grades of upregulation, especially in response to Cd and As stress. However, GS expression was downregulated when *Chlorella sorokiniana* was cultured in the presence of these heavy metals.

1. Introduction

Heavy metals are hazardous pollutants especially dangerous for aquatic ecosystems. The mining and mineral treatment activities, which produce wastewater with Cu, Cd or As, among others, are the main cause of pollution by heavy metals of the aquatic ecosystems. These heavy metals have a high impact in all the organisms present in the polluted environments, especially in microalgae (Salama et al., 2019). It has been described that metal stress can induce the increase of reactive oxygen species (ROS) and the expression of enzymes involved in the elimination of these molecules, such as catalase (CAT), glutathione reductase (GR) or ascorbate peroxidase (APX) (Sabatini et al., 2009). Moreover, other cellular pathways involved in nutrients assimilation or metabolism are also affected by heavy metals (Devriese et al., 2001; León-Vaz et al., 2021). Additionally, alterations in the DNA, mutagenesis and other toxic effects have also been described in microalgae and other aquatic organisms exposed to heavy metals (Salama et al., 2019). Thus, understanding these responses and the metabolic modifications produced under metal stress conditions is essential for the development of heavy metals phycoremediation procedures.

Different heavy metal remediation methods, such as chemical precipitation, ion-exchange, flocculation or membrane filtration, have been developed with different results (Salama et al., 2019). On the other hand, an increasing number of studies have shown the potential of bacteria and microalgae, in order to remove heavy metals from aquatic environments (Li et al., 2020). Microalgae have been reported to have several advantages for bioremediation of heavy metal compared to bacteria and fungi, because of their tolerance and high accumulation capacity. Although exposure to heavy metals may affect growth and metabolism of microalgae, many species are able to deal with high concentrations of these compounds and remove them from wastewater (Debelius et al., 2009). As a consequence, microalgae could be a cost-effective and ecologically safe alternative for remediation of heavy metals in aquatic environments and an excellent model, providing important information about the physiological impact of these contaminants in plant cells.

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