CHARACTERIZATION OF THE CHLORITE DISMUTASE FROM *PSEUDOMONAS SP:* A SUSTAINABLE TOOL FOR THE REMEDIATION OF CHLORITE

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ABSTRACT

The widespread use of chemicals and the inadequate waste disposal cause contamination of soil, water, and air. In Europe there are 34,000 sites that should be treated from pollutants that are hazardous for humans and the environment. There are a lot of traditional methods for cleaning up pollutants, however the various disadvantages of these methods turned the interest to the enzymes that are considered the most efficient and cost-effective tools for the bioremediation of pollutants *ex situ* or *in situ*[1]. Protein engineering is a prominent method for the design and creation of new enzymes with innovative catalytic and structural features, with enhanced stability and effictiveness under special conditions or particular substrates[2]. In addition, metagenomics technologies can contribute to the discovery of new enzymes with specific physicochemical characteristics. The anthropogenic pollutant chlorite (ClO_2^-) is detected in rising concentrations in groundwater, surface waters, and soils and according to World Health Organization, chlorite is extremely toxic, causing oxidative damage to red blood cells[3]. Therefore its removal from the environment is considered crucial. For this purpose, the chlorite dismutase enzyme can be used since it catalyses the decomposition of chlorite into harmless chloride (Cl^-) and dioxygen (O_2)[4]. In the present work, a whole genomic DNA from soil, sampled from a Hospital in Athens, was used for the isolation of chlorite dismutase gene. Sequencing analysis showed that the Cld gene encodes for 187 amino acids with high identity to the Cld from *Pseudomonas sp*. The expression of the cloned gene was studied in different *E. coli* strains and optimized under a range of different culture conditions. Furthermore, molecular modelling studies allowed the prediction of potential amino acids that can contribute to its structural and functional features. Based on the analysis of the crystal structure of Cld from *Cyanothece* sp., specific residues were selected as potential hot-spots for site-saturation mutagenesis. The wild type and mutant enzymes were purified using metal affinity chromatography and their chlorite dismutase activity was evaluated using a Clark-type oxygen electrode. In addition, kinetic analysis was carried out to gain further insights into the catalytic and biochemical properties of the mutant enzymes. The results of the present work suggest that chlorite dismutase may represent a promising tool for water treatment and ClO_2^- detoxification.

KEYWORDS: chlorite dismutase, enzymatic bioremediation, oxygen production, biocatalysis, heme enzyme

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