



Computational Insights into Antioxidant Enzymes and Metalloproteins of *Tetmemena* sp. SeJ-2015 (Spirotrichea; Ciliophora) in Combating Heavy Metal Stress

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Abstract

Living organisms are exposed to various environmental stressors either directly or indirectly. Stressors can be natural or induced. Some of the stressors such as heavy metals are increasing in the environment due to the escalation of anthropogenic activities. Heavy metals enter the living cells and induce oxidative damage to macromolecules by the excessive generation of reactive oxygen species (ROS). Heavy metals are also known to interfere with the functioning of metalloproteins (proteins containing metal ions as cofactors) by modifying or replacing the essential divalent ions. Some of the essential metalloproteins play important role in cell defense and help to combat heavy metal stress. Thus, by studying the metal-protein interaction of metalloprotein, one can elucidate the role of metals in biological systems. This study focuses on characterization of enzymes belonging to complete glutathione cycle, i.e., superoxide dismutase (SOD1 and SOD2), catalase, glutathione peroxidase, phytochelatin synthase, and methionine sulfoxide reductase. In addition, identification, annotation, and characterization of metalloproteins (cadmium, copper, and cysteine-rich) involved in cell defense mechanisms from the whole genome data of freshwater ciliate, *Tetmemena* sp. SeJ-2015 (GenBank accession number LASU02000000) by systematic bioinformatic approach has also been carried out. In this study, *Tetmemena* sp. has been selected due to its high prevalence in freshwater bodies within the class Spirotrichea. This makes it a good model system to investigate its metal detoxification mechanism. The whole genome sequence data contained a total of 25,217 contigs out of which 17,044 contigs showed similarity with cadmium- (Cd-), copper- (Cu-) binding, and cysteine (cys) rich proteins. Further analysis of these 17,044 contigs indicated that around 7,597 were Cd-binding, 6,285 were Cu-binding, and 3,162 were Cys-rich proteins.

Keywords Antioxidant enzymes · Bioinformatics · Freshwater ciliate · Metal detoxification · Phytochelatin synthase

Introduction

Environmental stressors, both natural (like temperature, wind, moisture, wildfire, predators, and parasitism) and anthropogenic (such as pesticides, heavy metals, and e-waste), exert diverse impacts on ecosystems [1]. Heavy metals, which are natural components of the earth's crust, have been classified into essential and non-essential based on their toxicity level

and role in cell metabolism [2]. All metals are toxic to living organisms at higher concentration [2–4]. The essential heavy metals such as iron, copper, manganese, and zinc, are mainly required for various redox and metabolic processes in living organisms since they are actively involved in regulating the structure and functions of various proteins involved in crucial biological processes [5–7]. Around 25–50% of the total proteome of living organisms are predicted to use metal ions as transporters and cofactors to perform essential functions, i.e., respiration, metabolism, signal transduction, and several other critical processes [6]. These metal ions associated with proteins contribute to catalytic, regulatory, and structural roles that determine the protein function [6]. Metalloproteins are also actively involved in cell defense mechanisms and protect the cell from environmental stress [7]. In contrast, non-essential heavy metals like arsenic, cadmium, lead, and mercury

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