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A Comparative Study on the Bioaccumulation of Copper, Cadmium, Chromium and zinc by Saccharomyces Cerevisiae

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ABSTRACT

The bioaccumulation of heavy metals in *Saccharomyces cerevisiae*, has gathered considerable attention. *Saccharomyces cerevisiae* is the simplest and convenient to cultivate under standard laboratory conditions, making it an ideal organism for studying the uptake and transport mechanisms of various heavy metals. This study investigates the differential bioaccumulation tendencies of Cu^{2+},Cd^{2+},Cr^{3+} and Zn^{2+} by *Saccharomyces cerevisiae*. The comparative analysis involves exposing *S.cerevisiae* cultures to synthetic growth medium containing each metal ion separately in varying concentrations, followed by measuring dry mass and accumulation levels within the cells and then compared with alterations in cellular protein content. The findings shed light on the metal-specific accumulation patterns and provide insights into the potential applications of *S. cerevisiae* in bioremediation processes targeting heavy metal pollutants.

Keywords: Bioaccumulation, Cu^{2+}, Cd^{2+}, Cr^{3+}, Zn^{2+}, *Saccharomyces cerevisiae*, Proteins.

I. INTRODUCTION

Bioremediation using microbes is a cost effective and an efficient way of removing heavy metals[1]. Micro-organisms accumulate metals by a number of different processes, which results in different types of biochemical effects. *Saccharomyces cerevisiae* is quite useful biosorbent for the removal of heavy metal ions and to investigate the metal-microbe interaction[2-8]. It has been reported that *S.cerevisiae* accumulates not only Cd^{2+} cation but also Cu^{2+}, Pb^{2+}, Zn^{2+} and Co^{2+} ions[9]. The plasma membrane of the yeast cells selectively allows certain molecules to pass through, permitting vital elements and nutrients to enter while facilitating the removal of waste materials[10-13].

Membrane-bound proteins are involved in actively transporting a variety of nutrients[14-17]. Metallothionein (Metal binding proteins) serves a crucial function in both storing and transferring specific metal ions. Acting as a rapid and effective homeostatic mediator, it controls the flow of metal ions through the cells. Ligand binding properties of essential nutrients or competitive interactions between different metals may alter their uptake and metabolism which may ultimately have serious implications.

Heavy metals are chemical species that bear resemblance to important nutrients. It's crucial to recognize that they can function as both toxins and nutrients for living organisms.Their incorporation via transport mechanisms can significantly impact cellular metabolism. A comparative analysis of toxicity for a large set of heavy metal and metalloids in yeast has been reported[18] and concluded that yeast cadmium factor-1 (YCF-1) transporter actively participates in the detoxification of several heavy metals. A family of metal ion transporter proteins (The CDF family) has been identified in both prokaryotes and eukaryotes[19]. This family is the first transport protein family so far, which is specific and exclusive for heavy metal ions[20]. Lutsenko & Kaplan have reported that P-type ATPases enzymes transport cations into or out of cells or intracellular compartments[21]. Disruption of divalent soft metal P-type ATPases leads to sensitivity for Zn^{2+}, Cd^{2+} and Pb^{2+} ions[22-25]. Zinc metabolism is transport metabolism and members of a variety of protein families transport zinc. Paulsen I.T. and Saier M.H. have reported that ZRT-1p high affinity and ZRT-2p low affinity transporters of the ZIP family are involved in uptake of zinc into *S.cerevisiae*[20]. Cor-A transporter which transports zinc in *S.cerevisiae*[26]. In *S.cerevisiae*, cadmium is bound by glutathione and the resulting cadmium-bi-glutathionato complex is transported by the YCF-1p transporter, an ABC transporter, into the vaculoe[27,28]. The periplasmic Cop-A Protein shows conservation of copper binding sites. The Cop-C and Cop-D Proteins seem to catalyze copper uptake into the cytoplasm. Before accumulating into the yeast cells, Cu(II) is first reduced by the Iron/Copper specific Reductases FRE-1p and FRE-2P to Cu(I)[29], which is transported into the cell by the CTR-1p[30,31] transporter, a novel protein with two related possible copper transporters (CTR-2p,CTR-3p) in yeast[20].

The present study aims to elucidate the correlation between metal concentrations in the environment and their accumulation by *S.cerevisiae*, while also exploring the role of metal binding proteins in this process. Our results will shed light on how varying concentrations of selected metals within cells affect biologically significant molecules such as proteins. Through the characterization and quantification of total proteins, we will assess the involvement of different proteins in the accumulation of heavy metals.Variation in protein contents therefore will indicate the change in metabolic activities in-vivo.

Experimental

Preparation of inoculums

Stocks of *Saccharomyces cerevisiae* were preserved on YEPD rich medium. It comprises 1% Yeast Extract, 2% Peptone, 2% Dextrose, and 2% Agar-Agar, respectively. Yeast cells were cultured in a synthetic growth medium (SGM) through agitation on a horizontal shaker for 15 hours at room temperature. SGM was prepared by dissolving 0.5% Glucose, 0.3% $(NH_4)_2SO_4$,

0.3% KH_2PO_4, 0.025% $CaCl_2$, 0.025% $MgSO_4$ and 0.001% Biotin in double-distilled water, then autoclaved at 1 $lb/inch^2$ pressure.

Characteristics of growth

The optical density of the synthetic growth medium was measured hourly using a UV-Visible spectrophotometer at a wavelength of 570 nm and a temperature of 25ºC for a duration of 15 hours. A plot was created correlating time with optical density to identify the mid-log phase (Fig-1), during which cellular metabolic activities and accumulation of essential nutrients reach their peak. The mid-log phase occurred at the 7 hour mark, indicating the period when cellular growth was most rapid.

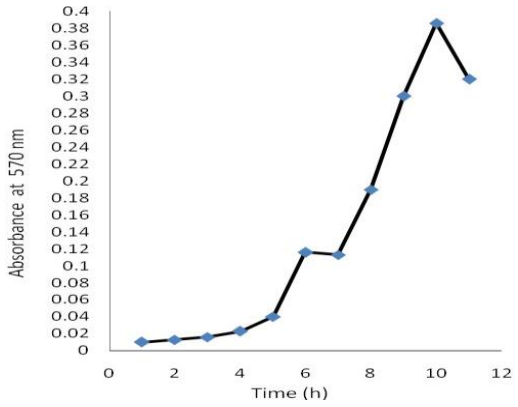

Figure 1: Growth curve of *S.cerevisiae* at 25 ^0C.

Assessment of accumulated Cu^{2+},Cd^{2+},Cr^{3+} and Zn^{2+}

Yeast cells were cultured for 7 hours in synthetic growth medium (SGM) containing concentrations of Cr^{3+} and Cu^{2+} (1, 2, 5, 7, 10 µg/ml) as well as Cd^{2+} and Zn^{2+} (5, 10, 20, 50, 100 µg/ml)respectively. Following the 7-hour growth period, the yeast cells underwent centrifugation, after which they were harvested and washed using a citrate buffer with a pH of 4.8. Subsequently, these cells were dried and digested using a 1% HNO_3 solution. The accumulated copper, cadmium, chromium and zinc within the cells were analyzed using Atomic Absorption Spectrophotometry, and the results were compared. Furthermore, the accumulated copper, cadmium, chromium and zinc were quantified in terms of µg/ng of total proteins(Fig.-2).

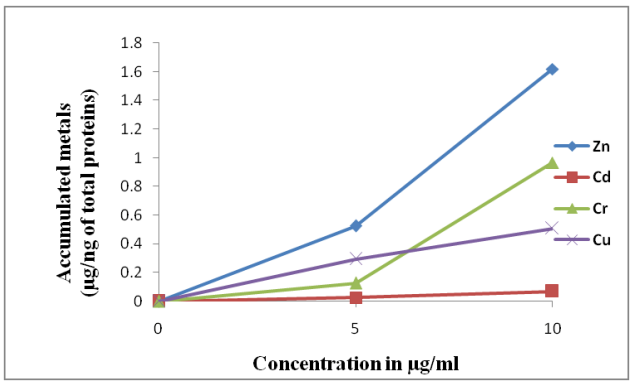

Figure 2: Accumulated Cu^{2+},Cd^{2+},Cr^{3+},Zn^{2+} ions by *S.cerevisiae* indicated as µg/ng of total proteins.

Measuring the dry mass of *S.cerevisiae* across different concentrations of Cu^{2+},Cd^{2+},Cr^{3+}, and Zn^{2+}

Yeast cells were dispersed in synthetic growth medium (SGM) containing varying concentrations of Cr^{3+} and Cu^{2+} (1, 2, 5, 7, 10 µg/ml) as well as Cd^{2+} and Zn^{2+} (5, 10, 20, 50, 100 µg/ml) respectively, and were grown for 7 hours at 25ºC under aerobic conditions. Upon reaching the mid-log phase of yeast growth, the SGM was centrifuged, and the cells were collected and harvested. Subsequently, these cells underwent washing with a citrate buffer (pH 4.8) followed by distilled water, and then were dried. The dried mass of yeast cells grown in various concentrations of Cu^{2+},Cd^{2+},Cr^{3+} and Zn^{2+} was measured, and the results were compared (Fig.-3).

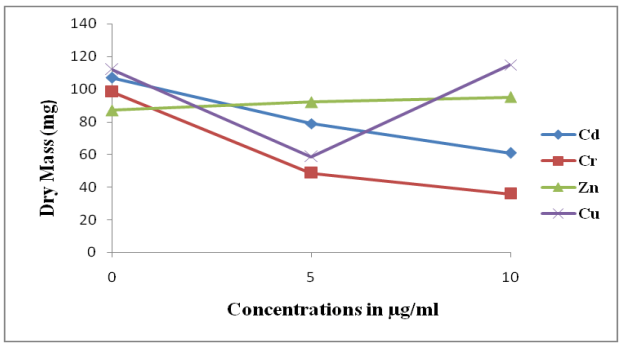

Figure 3: Assessment of *S.cerevisiae* dry mass in the presence of 5µg/ml and 10µg/ml concentrations of Cu^{2+}, Cd^{2+}, Cr^{3+}, and Zn^{2+} ions.

Evaluation of total protein extraction in the presence of various concentrations of Cu^{2+},Cd^{2+},Cr^{3+} and Zn^{2+}

Yeast was cultivated in the presence of varying concentrations of Cr^{3+} and Cu^{2+} (1, 2, 5, 7, 10 µg/ml) as well as Cd^{2+} and Zn^{2+} (5, 10, 20, 50, 100 µg/ml) respectively for a duration of 7 hours. Subsequently, centrifugation was carried out to settle the cells, which were then dried. The dried cells underwent treatment with 10% trichloroacetic acid (5ml) and an ethanol-ether mixture (5ml), followed by the addition of tris glycine buffer (0.2M, pH 8.6; 10ml) and boiling. The resulting supernatants were utilized for total protein determination using Lowry's method[32]. The obtained results were compared (Fig.-4).

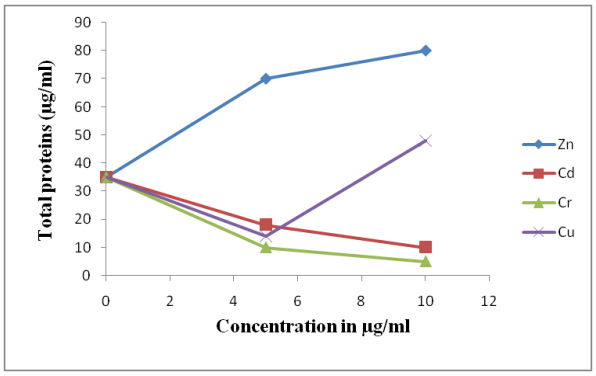

Figure 4: Total protein levels in *S.cerevisiae* assayed with 5µg/ml and 10µg/ml of Cu^{2+}, Cd^{2+}, Cr^{3+}, and Zn^{2+} ions.

Result and discussion

Growth characteristics of *S.cerevisiae* in terms of dry mass and accumulation were studied in control as well as in the presence of different concentrations of Cu^{2+}, Cd^{2+}, Cr^{3+} and Zn^{2+} ions. Copper accumulation exhibited a direct increase as concentrations rose, indicating a concentration-dependent relationship. It was noted that the dry mass of cells decreased at lower concentrations[33]. Interestingly, at a concentration of 10µg/ml, increased growth of cells was observed. This suggests that the reduction of Cu^{2+} ions may activate specific systems, resulting in higher dry mass production.It has been studied that excessive supply of Cu^{2+} ions, resulting in reduction of Cu^{2+} inside the cell[34]. Accumulation of Cu^{2+} ions leads to the production of free radicals in tissues. In the presence of copper, production of peroxide radicals and interaction with the cell membrane causes cell poisoning[35]. It was supported by decrease in total proteins with increasing concentrations, which reflects the harmful effect of copper.

Cadmium showed least accumulation among these heavy metals, which suggests that cadmium is not a preferred metal inside the cell. The detrimental impact of cadmium on the growth of yeast cells was evident, as cell growth was notably suppressed by Cd^{2+} ions compared to the control. Yeast cells exhibited reduced cadmium accumulation at lower concentrations. At 100µg/ml, cadmium accumulated more than zinc. Cd^{2+} ions, identified as soft acids, exhibit a preference for binding with the nucleophilic –SH group (a soft base) of enzymes like glutathione (GSH)[36,37], consequently hindering the activity of this sensitive enzyme. Total protein content decreased with varying cadmium concentrations. Due to larger size and more atomic weight in comparison to zinc, cadmium may cause the protein molecules to disintegrate[38] and therefore get denatured as evidenced by the observed minimum total proteins in the presence of different concentrations of cadmium. At 20µg/ml, yeast cells may develop an efflux system, leading to increased protein content. Cd-MTs are important Cd-binding complexes that function in the sequestration of cadmium. Additionally, it has been demonstrated that metal-binding proteins are synthesized in response to cadmium as a protective detoxification mechanism[39]. Cadmium binding with proteins induces aggregation and the generation of new molecules with greater molecular mass.

The estimation of accumulated chromium and protein content of yeast is important because GTF in yeast has chromium ion binding on a protein molecule[40]. Chromium is required by living organisms in trace amounts, it plays a significant role in carbohydrate metabolism[27] in yeast cells. Increased growth of yeast in the presence of 1µg/ml Cr^{3+} was observed. Zetic et al. has also shown that addition of Cr(III) into the medium containing yeast *S.cerevisiae* stimulates the yeast's growth and ethanol production due to chromium binding on protein molecule[28]. However, at higher concentrations, a decline in growth occurred, indicating the detrimental effects of chromium on yeast. Inorganic Cr^{3+} ions, when bound to amino acids or biomolecules, are more readily absorbed through plasma membranes via diffusion. Upon absorption, Cr^{3+} attaches to β-globulin fractions of serum proteins, notably transferrin. Within the cytoplasm, chromium undergoes reduction, generating free radicals that inhibit metabolic activities. At concentrations ranging from 2µg/ml to 10µg/ml of Cr^{3+} ions, decreased protein contents were observed. It has been proved that the affinity of Cr^{3+} for transferrin is similar to that of Fe^{3+}, thus competing with iron for the same binding sites. It may participate in crucial metabolic reactions, replacing iron from important biomolecules and consequently inducing protein degradation[41-43]. Although the cells accumulated chromium much more than cadmium, the lowest total protein contents were estimated in the presence of chromium. Existing proteins may become involved in efflux mechanisms for accumulated Cr^{3+} ions, leading to a decrease in protein contents.

Among these heavy metals, Zn^{2+} exhibited the highest accumulation, implying its crucial role in the proper functioning of numerous proteins. Its properties make it an effective trigger or controller for many enzymatic reactions due to its strong binding affinity. At elevated concentrations, zinc can be taken up by nonspecific uptake systems and transported into cells. Research suggests that Zn^{2+} ions have a greater affinity for

imidazole groups compared to Mg^{2+} or Ca^{2+} ions, resulting in efficient binding and potential displacement of Mg^{2+} ions from their binding sites[44]. Consequently, higher concentrations of zinc may lead to decreased growth due to these interactions and disruptions in cellular processes. The enhanced accumulation of Zn^{2+} up to a concentration of 20µg/ml, along with the increase in total protein content, suggests its involvement in the synthesis of new proteins. However, in the present study, decreased protein contents were observed when the Zn^{2+} concentration supplied exceeded 20µg/ml. Zinc influences proteins because it is a component of a large number of enzymes and proteins, participating in various enzymatic reactions, nucleic acid metabolism, protein synthesis, and the maintenance of membrane structure and function. The maximum total proteins observed in the presence of lower concentrations of Zn^{2+} ions can be attributed to its essential role as a trace element for living organisms. Zinc, cadmium and copper may induce metallothionein synthesis in cultured cells or in vivo.

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