

Potential Application of Modified *Saccharomyces Cerevisiae* for Removing Lead and Cadmium

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Abstract

Recently, heavy metal pollution has become a worldwide concern. *Saccharomyces cerevisiae* have been used for bioremediation process for heavy metal uptake. In this study, *S. cerevisiae* was genetically modified with the gene EC20 (synthetic phytochelatin) with the purpose to enhance the accumulation capability for Lead (Pb²⁺) and Cadmium (Cd²⁺). *S. cerevisiae* has showed high biosorption capability of Pb²⁺, followed by Cd²⁺. Indeed, the transformed cells exhibited even higher capacity to accumulate lead. TEM (transmission electronic microscopy) images of transformed *S. cerevisiae* showed accumulation of heavy metals on cell wall, which was also verified by EDS (energy dispersive spectrum). There are many mechanisms involved in metal uptake and for some unknown reason. The results of yeast expressing EC20 were better detected when yeast cells were grown in the presence of Pb²⁺ than with Cd²⁺, as clearly observed with the improvement in Pb²⁺ retention by yeast cell wall.

Keywords: Yeast; Genetic modification; Transformation; Heavy metals; Cadmium; Lead uptake

Introduction

In recent years, pollution of wastewater has increased and heavy metals (such as lead and cadmium) are one of the heavy metals in the environment, causing health effects [1-4] and serious environmental problems [5-8]. Mining, industrial and volcanic activities are the main source of heavy metals in environment [9]. In the aquatic system, heavy metal pollution has become an environmental concern, as it is extremely recalcitrant and non-degradable. Even at low concentrations, heavy metals can cause toxicity to humans and other forms of life [5]. It is necessary to uptake these heavy metals from wastewater before its disposal. The toxicity of heavy metals could be transformed from relevant low toxic species into more toxic forms. Moreover, most of the heavy metal salts are soluble in water, hindering some methods of separation (such as, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, chemical precipitation, ion exchange, membrane technologies, adsorption on activated carbon) [10]. As a result, microbial biomass is an option for developing economic and eco-friendly wastewater treatment process [11,8]. An alternative process is called biosorption that can be defined as the removal of metal from solution by biological material (biosorbents) [12]. These biosorbents can effectively sequester dissolved metal ions of solutions with high efficiency and speed. It also offers several advantages over conventional treatment methods including cost effectiveness, minimization of chemical/biological sludge and regeneration of biosorbent with possibility of metal recovery [13]. Metal polluted environment contains fungi, which has adapted to toxic concentration of heavy metals and become metal resistant [14]. Metals retention by living fungi depends on several factors such as culture conditions and metal concentration [15]. Especially, fungi used for biosorption can be fulfilled by simple physical methods without any damaging the fungi structural integrity [16]. *Saccharomyces cerevisiae* can be easily cultivated using unsophisticated fermentation techniques and inexpensive growth media [17] at large scale or collected from waste of food industries and have become a benchmark in the study of biosorption. Moreover, it is an excellent model microorganism in biological research and its biomass is also considered as safe [18-20]. Therefore, biosorbents made from *S. cerevisiae* can be easily accepted by the public when applied especially to investigate the

interactions of metal-microbe at molecular level [21]. The use of yeasts in model systems is particularly attractive because of the easy genetic manipulation and the availability of the complete genomic sequence knowledge on the molecular biology of the yeast is helpful to identify the molecular mechanism of biosorption in metal ion removal [22-24]. Transformation technique is an important tool in which exogenous DNA is introduced into a cell, resulting in genetic modification. To improve the biosorption capacity of the potential yeast biosorbent, a short metal-binding can be displayed in the cell's surface with purpose to increase its heavy metal biosorption capacity from solutions. Among the heavy metal-binding in the cells, the phytochelatin (PCs) is one of the best studied so far [25]. The EC20 gene encodes a synthetic phytochelatin [25-27]. These peptides bind to metal ions, keeping them on their cell surface [28,29]. To verify the capacity of binding heavy metal on the fungal cell, progress has been made in exploring the cell surface ultra-structure of yeasts owing to the development of techniques such as the transmission electron microscopy with energy-dispersive spectrum analysis (TEM-EDS) [18].

The aim of this study was to modify by genetic transformation a strain of *S. cerevisiae* to improve the biosorption of toxic heavy metals (Lead and Cadmium) and to compare the strain's capacity of retaining heavy metals before the genetic transformation and after that.

Materials and Methods

Saccharomyces cerevisiae and *Escherichia coli* strains and growth media

S. cerevisiae W303-1A (*MATa ade2-1 trp1-1 his3-1,15 leu2-*

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3,112 *ura3-1 ρ+can^R*) strain (R. Rothstein-Columbia University) was cultivated on rich glucose media (YPD - 1% yeast extract, 2% peptone, 2% dextrose) and minimal glucose media (0,67% yeast nitrogen bases, 2% glucose) supplemented with the appropriate auxotrophic supplements. The *E. coli* strain RR1 ($\Delta(gpt-proA)62, leuB6, thi-1, lacY1, hsd_{B20}, rpsL20 (Str^r), ara-14, galK2, xyl-5, mtl-1, supE44, mcrB_B$) (3183) was cultivated on LB plate (Luria Bertani) supplemented or not with ampicilin for plasmid selection.

EC20 synthesis, vectors construction and yeast transformation

EC20 was assembled from the oligonucleotides pairs EC20-1 and EC20-2 [30-32]. The complementary oligonucleotides were heated at 90°C for 2 min and hybridized, first at 55°C for 30 min followed by 37°C for 30 min. The double stranded fragment was digested, respectively, with BamHI and HindIII and inserted into YIp351 [33] and YCp22 containing the *TRP1* marker [34] previously cut with the same restriction enzymes. YIp351-EC20 and YCp22-EC20 recombinant plasmids transformed W303-1A yeast strain following the procedure of Schiestl and Gietz [35]. YIp351-EC20 was linearized with BstXI and integrated into chromosomal *LEU2* locus [30].

Recombinant DNA manipulation

Standard methods were used for recombinant DNA manipulations, *E. coli* growth and transformation [36]. The recombinant plasmids YIp351-EC20 and YCp22-EC20 were isolated from *E. coli* in small and medium scale procedures [37,38]. For plasmid sequencing was used the Pure Yeild TM Plasmid Mini prep System kit (Promega Co., Madison, WI, USA). The obtained sequences were analyzed using Bio Edit program.

Metal ion solutions and biomass preparation

Two kinds of metal ion solution were used: solutions containing lead and cadmium. Metal ion solutions were made by dissolving analytical $Pb(CH_3COO)_2 \cdot 3H_2O$ (1g/L) and $CdCl_2 \cdot 2,5H_2O$ (0,5g/L) in deionized water. The fungal biomass was prepared in Sabouraud broth (Sb) (Oxoid, England), and incubated at 25°C for 5 days, and shaken at 150 rpm. After incubation, the pellets were harvested and washed with double-deionized water, corresponding to live biomass. The pH value of the solutions was adjusted. The conditions were optimized during the preliminary experiments to have all metals in a dissolved aqueous form and to achieve high biosorption capacity of all metal ions.

Transmission electronic microscopy

From the growth of fungi, broth was filtered to obtain biomass that was sent to the microtome sector to obtain the ultrathin sections. The ultrathin sections were examined by electron microscopy (JEOL-1010) at IPEN and ICB department at the University of Sao Paulo. Transmission electron microscopy was performed with the strain of *S. cerevisiae* modified genetically and non-modified in this study with the purpose to reveal the presence of heavy metals in the fungal cell.

Results

Transformation of *Saccharomyces cerevisiae*

With regard to transformation of yeast, *S. cerevisiae* strain was transformed with YCp22-EC20 and YIp351-EC20 recombinant. YCp22-EC20 was chosen for yeast plasmid rescue and it was again sequenced confirming the stability of YCp22-EC20 in yeast. The transformed cells were tested for increased ability to chelate heavy metals into their cell surface, which showed an improvement in

its capability to grow in medium amended with different lead and cadmium concentrations.

Transmission electronic microscopy

Transmission electron microscopy images and EDS analysis of the cells of *S. cerevisiae* showed that heavy metals uptake have increased by transformed yeast cells and also revealed the presence of heavy metals mainly in the cell wall. Comparing to the control (Figure 1), the cells showed a high increase in their Pb^{2+} binding capacity (Figure 2a and b). The micrograph Figure 2a showed a transformed *S. cerevisiae* after genetic transformation and the lead accumulated in the wall cell. The micrograph Figure 2b showed a non-transformed *S. cerevisiae* and the wall cell with less lead accumulated. As indicated above, we observe that *S. cerevisiae* cells have a lower capacity of Cd^{2+} binding than Pb^{2+} binding (Figure 3a and 3b). TEM images and EDS analysis of *S. cerevisiae* grown in the presence of Cadmium after genetic transformation. The micrographs (Figure 2a) showed a transformed *S. cerevisiae* with cadmium accumulated in the wall cell. The micrograph (Figure 2b) showed a non-transformed *S. cerevisiae* and cell wall without cadmium accumulated. No heavy metals were detected in the control. As a result, during biosorption, the cells of *S. cerevisiae* released some substances without strongly damaging the cells integrity. The presence of electron-dense deposits was observed by transmission electron microscopy.

Discussion

Yeast cells have been used in the removal of heavy metal ions from wastewater [39-41]. *S.cerevisiae* could treat all major toxic heavy metals [1]. Metals are frequently internalized by the fungal cell and are accumulated into the vacuoles or can also be bound to low molecular weight proteins such as phytochelatins [41,42]. To improve metal uptake capacity, some techniques of biomass processing have been studied. Most biomass modification techniques described in the literature have shown to improve biosorption ability [43,44]. Metabolism-independent binding of metal ions in yeast cell is usually fast and large amounts of heavy metals can be bound to the cell [1]. In our study, *S. cerevisiae* cells were genetically transformed. Synthetic genes encoding for EC20 were synthesized as described previously [25,32]. According to our study, modified and non-modified strains were used to uptake lead and cadmium from broth. In studies of Bae [25], the EC20 gene was used to

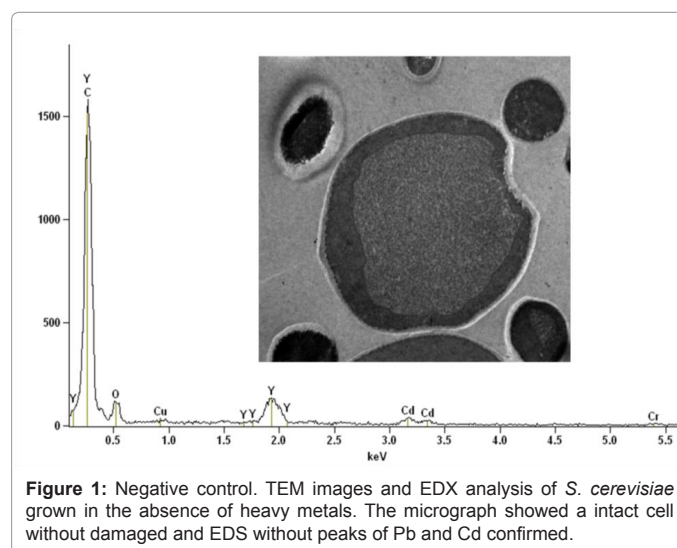


Figure 1: Negative control. TEM images and EDX analysis of *S. cerevisiae* grown in the absence of heavy metals. The micrograph showed an intact cell without damaged and EDS without peaks of Pb and Cd confirmed.

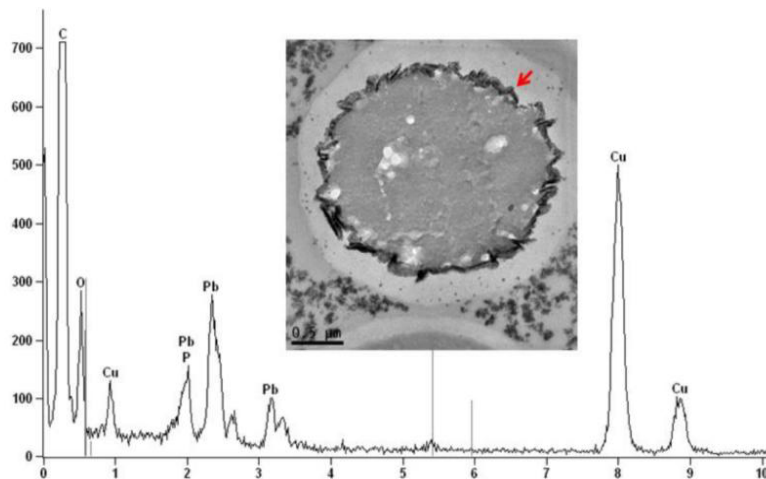


Figure 2a

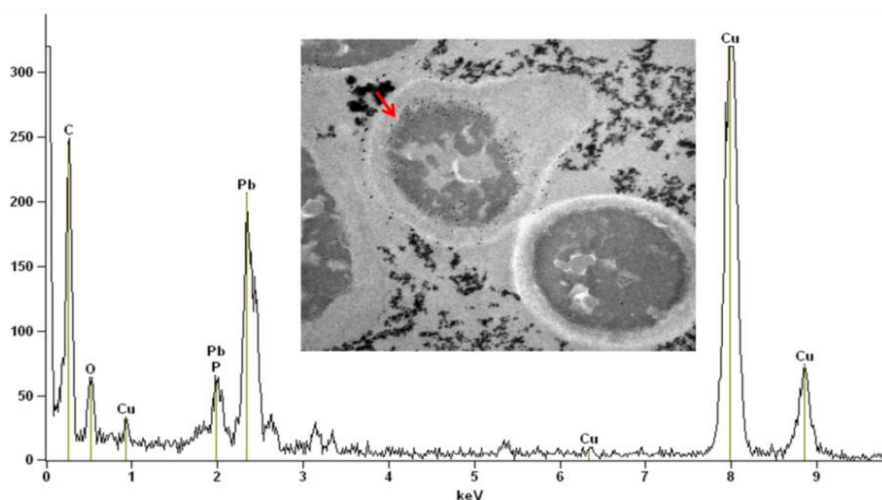


Figure 2b

Figure 2: TEM images and EDS analysis of *S. cerevisiae* grown in the presence of Lead. Lead in *Saccharomyces cerevisiae* after (a) and before (b) genetic transformation. The micrographs showed (2a) a transformed *S. cerevisiae*. On figure 2a, the arrow shows the lead accumulated in the wall. The micrograph (2b) showed a non-transformed *S. cerevisiae*. On figure 2b, the arrow shows the wall cell with less lead accumulated. On both figures, EDS analysis confirms the presence of lead on the local showed by the arrow.

anchor EC20 on the surface of the cells, providing an increase in their heavy metal binding capacity. Furthermore, Biondo et al. [32] described the effects of the cell surface display of a synthetic phytochelatin in *Cupriavidus metallidurans* for enhanced metals bioremediation. Great progress has been made in exploring the cell surface ultra-structure of yeasts owing to the development of techniques such as the transmission electron microscopy with energy-dispersive spectrum analysis (TEM-EDS). In our experiments the majority of accumulated Pb^{2+} was found in the cell wall, indicating the involvement of functional groups of cell wall [45-47], in sequestering the Pb^{2+} ions. Moreover, Brady and Duncan [47] also found that biosorption can be achieved through complexation by cell wall and membrane hydroxylated components. Kunst and Roomans [48] found polyphosphate granules localized in

or close to the cell vacuoles, which are the major storage vesicles for heavy metal ions in *S. cerevisiae*. Orlovich and Ashford [49] followed up on aforementioned studies and determined the exact vacuolar location for polyphosphate granules incorporation. Favero et al. [50] found that 80% of Cd^{2+} was bound to the hyphal cell walls in their study of *Pleurotus ostreatus*. In summary, the potential advantages in use of fungal biomass especially *S. cerevisiae* certainly could be applied for treatment of large scale heavy metals pollutants without the generation of toxic sludge. In the same context, genetic manipulation of *S. cerevisiae* could to increase the biosorption of heavy metals.

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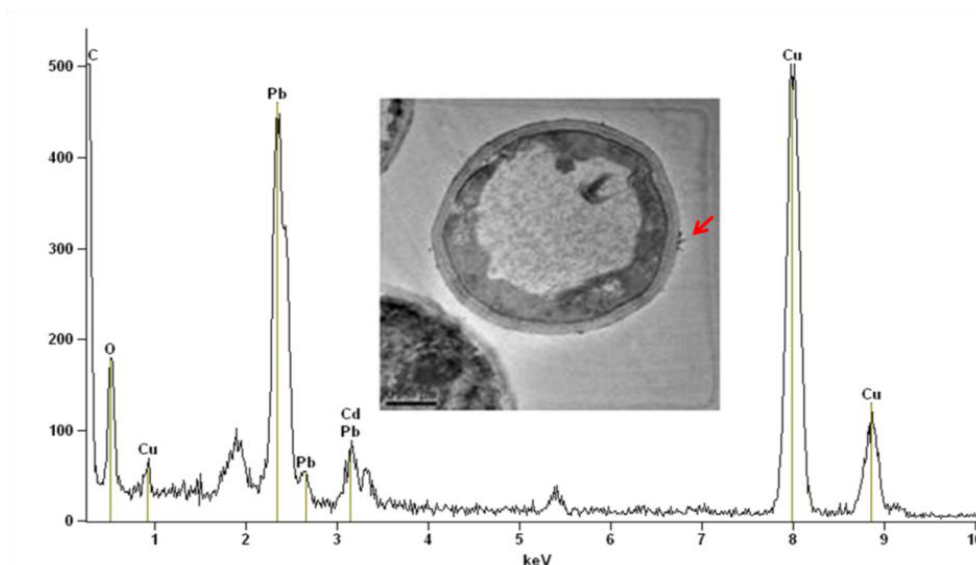


Figure 3a

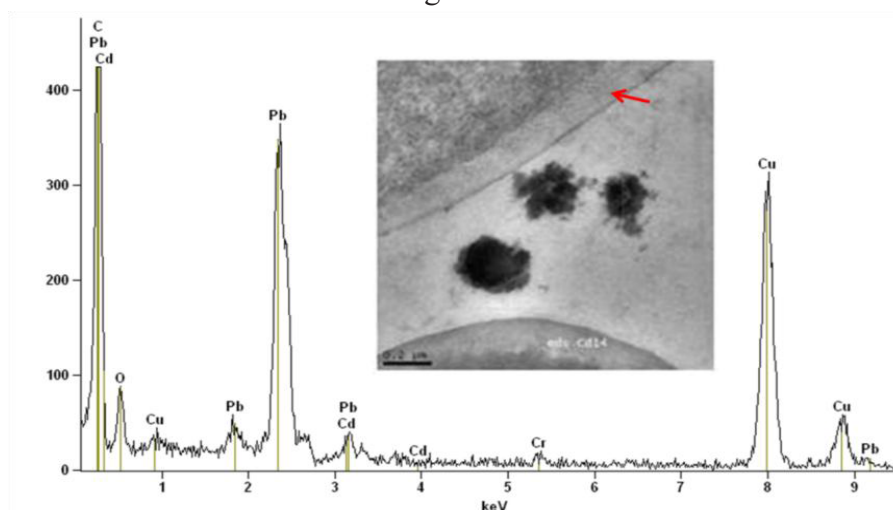


Figure 3b

Figure 3: TEM images and EDS analysis of *S. cerevisiae* grown in the presence of Cadmium. Cadmium in *Saccharomyces cerevisiae* after (A) and Before (B) Genetic transformation. The micrographs showed (2a) a transformed *S. cerevisiae*. On figure 2a, the arrow shows the cadmium accumulated in the wall cell. The micrograph (2b) showed a non-transformed *S. cerevisiae*. On figure 2b, the arrow shows the wall cell without cadmium accumulated. On both figures, EDS analysis confirms the presence/absence of cadmium on the local showed by the arrow.

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