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MOLECULAR MECHANISMS INVOLVED IN METAL BIOACCUMULATION

Ph. D. THESIS ABSTRACT

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The thesis consists of two main parts: ***Theoretical consideration,*** which describes the data from the literature on research topics addressed and ***Original contributions,*** presenting the personal contributions.

The first subchapter presents the general aspects related to the eukaryotic model *Saccharomyces cerevisiae* used as a potential biosorbent. In this part the external and internal factors that affect the ability of *Saccharomyces cerevisiae* to uptake metals from contaminated effluents, particularly aquatic environment are presented.

In the second subchapter I have discussed upon data and information about heavy metals, highlighting the sources of heavy metals in the environment, the impact of heavy metals upon the environment, removal of metals from the environment, heavy metal toxicity and effect of heavy metals on cells of *S. cerevisiae*.

In the third subchapter, ways to engineer *S. cerevisiae* cells to increase capacity to accumulate heavy metals are presented.

The main objective of this thesis was to obtain strains of *Saccharomyces cerevisiae* capable to accumulate significant amounts of potentially polluting metal ions and to develop cellular systems involved in bioremediation of waste water contaminated with heavy metals. This was achieved by studying the molecular mechanisms involved in the bioaccumulation of metal ions.

The work presented in this thesis was focused on five directions of research.

Metal remediation through common physico-chemical techniques is expensive and unsuitable in case of voluminous effluents containing complex organic matter and low metal contamination. Alternative biotechnological approaches received great deal of attention in the recent years. Engineering cell lines that would hyperaccumulate heavy metals can be an invaluable tool in removing such ions from aqueous environments.

In the first direction of research, the production of mutant strains of *S. cerevisiae* resistant to high concentrations of heavy metals that would accumulate heavy metals in a non-toxic manner was attempted. As a result of the studies we obtained 2 strains tolerant to high concentration of Ni^{2+} and one strain tolerant to high concentrations of Co^{2+}. These strains had the ability to accumulate metals in vacuoles. An important aspect was the fact that the strains were able to reduce the amount of Me^{2+} from media in a single cycle of growth, which made them good candidates for use in bioremediation processes.

The second direction of research focused on the possibility of using “kamikaze” strains with high potential for bioaccumulation of heavy metals, but killed in the process of bioremediation. The most interesting was the strain defective in the ATP-ase pump Pmr1p (responsible for detoxification of metals by excluding them by cellular secretory pathway). It was shown that null-mutant strain *pmr1Δ* had an increased capacity to remove Mn^{2+}, Cu^{2+}, Co^{2+} or Cd^{2+} from synthetic effluents due to the ability to hyperaccumulate these cations. Due to increased metal accumulation, the mutant strains was more efficient than the wild type in removing heavy metals containing 1-2 mM cations, with a selectivity $Mn^{2+} > Co^{2+} > Cu^{2+}$ and also in removing Mn^{2+} and Cd^{2+} from synthetic effluents containing 20-50 µM cations, with a selectivity $Mn^{2+} > Cd^{2+}$.

It was also found that the *pmr1Δ* cells had a tendency to accumulate large amounts of metal ions as compared to cells that normally express the *PMR1* gene.

The third direction sought to obtain strains that hyperaccumulate heavy metals, not by deletion of genes involved in cellular detoxification, but rather by overexpression of genes encoding transporters for metal ions. In addition, we studied the effects of the gene overexpression on the ability of cells to bioaccumulate metal ions. Pho84p, the protein responsible for the high-affinity uptake and transport of inorganic phosphate across the plasma membrane, is also involved in the low-affinity uptake of heavy metals in the *Saccharomyces cerevisiae* cells.

This part of the thesis demonstrated that under metal ions excess, yeast cells overexpressing *PHO84* gene acquire an increased capacity to bioaccumulate Mn^{2+}, Cu^{2+} or Co^{2+} and in some genetic background cells becomes hyperaccumulators. As *PHO84* overexpression triggered the Ire1p-dependent unfolded protein response, abundant plasma membrane Pho84p could be achieved only in *ire1Δ* cells (lacking the gene that encodes a transmembranare kinase which transmit the signal about unfolded proteins to RE). Under environmental surplus, *PHO84* overexpression augmented the metal accumulation by the wild type, accumulation that was exacerbated by the *IRE1* deletion. The *pmr1Δ* cells (lacking the gene that encodes the P-type ATPase ion pump that transports Ca^{2+} and Mn^{2+} into the Golgi), hyperaccumulated Mn^{2+} even from normal medium when overexpressing *PHO84*, a phenotype which is rather restricted to metal-hyperaccumulating plants.

For a better understanding of the mechanisms involved in cell survival and adaptation to stress caused by heavy metals, in the fourth direction of research the involvement of Ca^{2+} in signaling the cell exposure to high concentrations of metals was studied. Using the *S. cerevisiae* cells which expressed a transgenic Ca^{2+}-sensitive photoprotein it was found that among the metals tested, only Cd^{2+} surplus was signaled by calcium.

The yeast cells responded through a sharp increase in cytosolic Ca^{2+} when exposed to Cd^{2+}, and to a lesser extent to Cu^{2+}, but not to Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, or Hg^{2+}. The response to high Cd^{2+} depended mainly on external Ca^{2+} (transported through the Cch1p/Mid1p channel), but also on vacuolar Ca^{2+} (released into the cytosol through the Yvc1p channel). The adaptation to high Cd^{2+} was influenced by perturbations in Ca^{2+} homeostasis. The data obtained in this part of the thesis indicate that the presence of high concentrations of Cd^{2+} in the environment is signaled through immediate and sudden pulses of cytosolic Ca^{2+}. Apparently, sudden and sharp pulses of Ca^{2+} allow the adaptation to high Cd^{2+}, while the absence of $[Ca^{2+}]_{cyt}$ signaling or broad pulses and lingering $[Ca^{2+}]_{cyt}$ are responsible for Cd^{2+} hypersensitivity.

Since some cell strains that were shown to be hyperaccumulators of heavy metals die due to the toxicity of those metals, in a fifth direction of research, we studied the using of plant antioxidants to protect cells from the stress induced by heavy metals.

Vaccinium corymbosum L. are a rich source of antioxidants and their consumption is believed to contribute to food-related protection against oxidative stress. Four varieties of blueberries were used in the study, and it was found that the extracts with high content of total anthocyanidins exhibited significant protective effect against the toxicity of cadmium and H_2O_2. Both the blueberry extracts and pure cyanidin exhibited protective effects against cadmium in a dose-dependent manner, but without significantly interfering with the cadmium accumulation by the yeast cells. Thus, it was proved that the extract of *Vaccinium corymbosum* berries has a protective effect against *S. cerevisiae* cells exposed to Cd^{2+} ions, one of the most toxic metals studied. The results imply that the blueberry extracts might be a potentially valuable food supplement for individuals exposed to high cadmium.

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