

Gene expression analysis in the model yeast *S. cerevisiae* by Real Time PCR to assess the toxicity of cadmium at environmental level

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Introduction

The heavy metal cadmium is considered a serious environmental health threat. Recently, DNA Microarray and Proteomic assays, implemented on the budding yeast *Saccharomyces cerevisiae*, identified high regulated genes in response to cadmium exposure (see references below). However, those studies mainly used high concentrations of cadmium (more than 10 times the EC50) and low exposure times (1 to 2 hours) far from field conditions. On the other hand, the Real Time PCR (RT PCR) is a very sensitive tool which allow to detect slight changes in gene expression. In this context this study propose to use the RT PCR to examine the potential of known regulated genes as molecular biomarkers for detecting cadmium pollution in the field.

Study Protocol

Two biological experiments in duplicate have been carried out on growing yeasts. We used 2 concentrations of CdCl₂ (1) 0.2μM corresponding to the No Observed Effect Concentration (NOEC) of algae species and (2) 20nM corresponding to the Predicted No Effect Concentration (PNEC) at five incubation times (from 2h to 32h). After RNA extraction and reverse transcription using random primers, the gene expression of ten target genes involved in different detoxification pathways (Figure 1) and two references genes have been analyzed by RT PCR. In parallel, cadmium concentrations have been measured in the media by ICP-MS.

Normalization and quality control

RNA quality assessment and quantity normalization have been performed by Electrophoresis and Spectrophotometry. References genes were selected among 10 candidates using the GeNorm[®] software. Relative Expression (RE) of the target genes and quality control of the data's have been carried out with the qBase[®] software. The RE of the different experiments were pooled for each gene after no significant difference (P>0.05) was found by One way ANOVA test. A Two way ANOVA test was used to assess the effect of the exposure time and the cadmium concentration on the RE of each gene. When the variances were not homogeneous and/or the residuals deviated from the norm, the data were transformed prior to statistical analysis.

References

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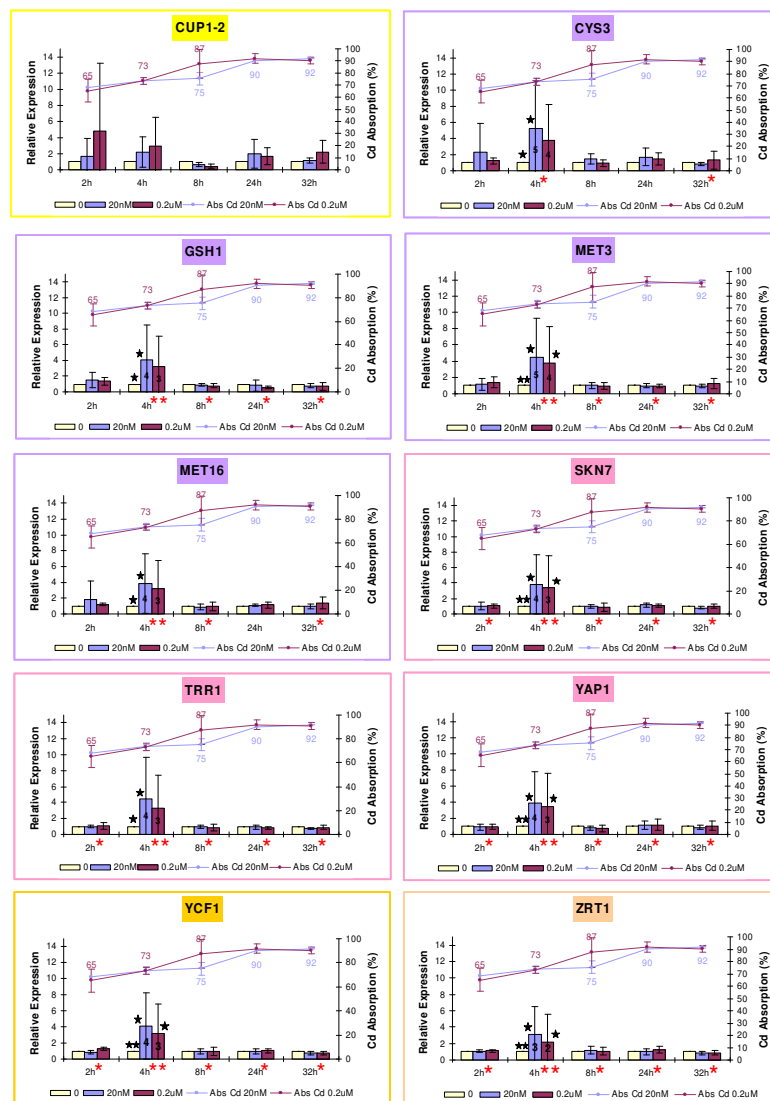


Fig.2: Relative Expression (RE) of the genes for two cadmium concentrations with corresponding cadmium (Cd) absorption by the cells at different exposure times. The Average and the Standard Deviation of the two biological experiments in duplicate are represented. For each exposure time the RE is calibrated by the not treated sample (0). * or **, significant differences between exposure times (P<0.05); * or **, significant differences between the Cd concentrations (P<0.05)

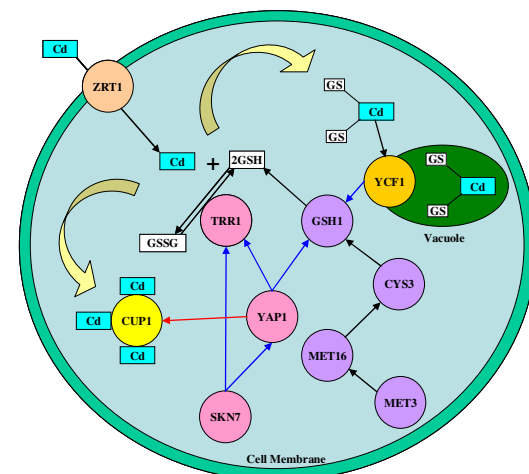


Fig.1. Model of Yeast showing the Target Genes selected in this study and involved in different detoxification pathways of Cadmium: **○** Sulfur amino acid biosynthesis pathway; **○** Oxidative stress response; **○** Cell membrane transporter; **○** Vacuolar sequestration; **○** Metallothionein binding protein and known **→** positive **→** negative regulation between them
Abbreviations: Cd, cadmium; GSH, glutathione; GSSG, oxidized GSH; CdGS2, bis(glutathionato)cadmium

Gene Expression by RT PCR and Chemical Analysis

The results are showed Figure 2. The chemical analysis reveals that around 65% of cadmium was absorbed by the cells after only 2h and around 92% after 32h for both concentrations. All the selected genes, except CUP1-2, were significantly over expressed (2 to 5 times) at 4h exposure time. However, among those genes, two groups could be distinguished (1) the genes involved in oxidative stress response and in membrane/vacuole transports, (2) the genes involved in the sulfur amino acid biosynthesis pathway. For the first group the up regulation is short (only at 4h) and observed for both concentrations (except for TRR1). In the second group it seems that the up regulation already start at 2h and is still present at 24h (CYS3 and MET16) and 8h (CYS3) and is mainly detectable for the lowest concentration. Moreover, the gene expression level is never correlated with the cadmium concentration probably because they were similar (a multiple factor of 10).

To conclude, we found that nine on the ten tested genes could detect very low cadmium concentrations such as PNEC level. Firstly, this result demonstrated the importance of those genes in the cadmium detoxification at environmental level. Secondly, it confirm the sensibility of the RT-PCR tool to assess the impact of field contamination. Finally, those genes are promising early warning indicators of exposure to and effects of cadmium on eukaryote organisms in the environment.