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GLUTATHIONE ACCUMULATION IN MUTANT AND WILD-TYPE STRAINS OF *S. CEREVISIAE* UNDER CONDITIONS OF SMOOTH CYSTEINE ADDITION

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It is a common knowledge that the regulation of glutathione synthesis can occur both on enzyme and transcriptional level and its excessive over-accumulation is restricted by feedback inhibition of Gsh1p enzyme activity and *GSH1* expression. In the present work we investigated the molecular mechanisms allowing glutathione over-accumulation in yeast *S. cerevisiae* strain selected via random mutagenesis. The comparative sequencing of *GSH1* in glutathione over-accumulating mutant and its wild-type parent strain did not reveal any mutations in the ORFs, which could lead to a disruption or release of the feedback inhibition of GSH on Gsh1p in the mutant. Ethanol-stat fed-batch cultivation of mutant with the continuous addition of cysteine, the analysis of glutathione accumulation kinetics and *GSH1* expression suggested that the feedback inhibition of Gsh1p activity by glutathione was functional in the mutant and that the mutation(s) must be in other locations instead of *GSH1* to cause glutathione over-accumulation. The results of gene expression analysis revealed that the expression levels of the genes leading to cysteine biosynthesis (*CYS3* and *CYS4*), but also *GSH1* and its transcription factor *YAPI* were several fold higher in the mutant than those of the wild-type strain. The higher expression levels of *CYS3*, *CYS4*, *GSH1* and *YAPI* together with an increased intracellular cysteine concentration are the most likely reasons for glutathione over-accumulation in the mutant.