2. Purpose of the work

All eukaryotes have the ability to detect and respond to environmental signals. This adaptation to stress or to changing environmental conditions occurs by inducing specialized re-programming that promote stress protection and survival. Single-cell organisms like yeasts are particularly exposed to fluctuations in the environment during the growth at industrial level, because many simultaneous and sequential stresses could occur, like hyperosmotic, oxidative, ionic and heat stresses as well as nutrient limitation and starvation.

Knowledge of stress and stress responses is crucial to understand how cells adapt to environmental and physiological changes during their use in biotechnological applications.

Since *Kluyveromyces lactis* is an attractive host alternative to *S. cerevisiae* for heterologous proteins production [6], fundamental knowledge about its adaptative mechanisms is thus a prerequisite to optimize conditions for large-scale biomass production.

In *K. lactis*, glycolytic metabolism regulation is complex and involves the actions of several factors [49]. *RAG8* is an essential *K. lactis* gene coding for the casein kinase I isoform (CKI), involved in a wide range of cellular processes, like the transcriptional regulation of the low-affinity glucose transporter gene *RAG1* [45].

In a metabolic engineering contest, through the recombinant DNA technology, a genetic modification allows to obtain microbial mutants with improved product specific production rates, compared with the wild type parents, very useful for biotechnological applications [32].

However, even when the environmental conditions are well defined, we are far from understanding how the genomic program determines the living organism [32]. So, although the complete knowledge of *K. lactis* genome [10], it will clearly not suffice to unravel all principles governing the most complexes phenomena of cells life and adaptation.

For these reasons, given the broad physiological roles of Rag8p, to further investigate which metabolic changes took place in the *rag8* mutant (taken as a model for all *rag* mutants), to understand how the cellular responses is coordinated under growth on glucose and to globally characterize how *K. lactis* regulates metabolic responses to the lack of the casein kinase, a metabolomic approach was performed by NMR spectroscopy [53].

The use of such NMR-based metabolite profiling techniques form the basis for intelligent screening strategies to exploit the biotechnological potentials of yeasts [4] because the rational improvement of *K. lactis* strains for the production of primary and secondary metabolites requires, first of all, a quantitative understanding of their metabolism [54], allowing the development of more efficient cell factories through metabolic engineering.

However, it is important to realize that there is not always a one-to-one relationship between a gene and a metabolite, so metabolites levels are usually a complex result of the expression of many genes and the function of many enzymes.

To reveal cause and effect relationships it is important to gain deeper insight into the complex metabolic responses at the level of intracellular metabolite concentrations and fluxes [55].

Since metabolomic analysis of metabolite composition alone may be insufficient for a complete understanding of the metabolic phenotype, flux evaluation from ^{13}C-NMR labeling data is obtained to provide a useful complementary parameter for the system-wide characterization of *K. lactis* mutant strain metabolic network.