6. Conclusions

In the present work, the complex metabolic effects of the genetic mutation performed on *Kluyveromyces lactis* yeast cells is investigated with a specific, 'information-rich' analytical approach: differential NMR metabolomics.

Wild-type and mutant cell lines metabolomes are compared each other: through the application of multivariate statistical models a metabolic network is built on statistical basis, describing the metabolite phenotype of the *rag8* mutant *K. lactis* strain.

However, the measurement and interpretation of such in vivo metabolite dynamics at a systems level is inherently difficult. Indeed, decipher the intricate web of metabolic networks of a complex system and, particularly, infer something about gene functions only based on metabolite profiling [4], is one of the greatest challenges in molecular biology which cannot be resolved fully by any metabolomic tool [83].

To resolve and improve the metabolic network description, an extension of the ^{13}C labeling protocol for investigating eukaryotic cellular systems is applied in this work. The resulting labeling pattern of each metabolite reflects the relative importance of the alternative pathways within the metabolic network [32]. This observation underscores the need for acquiring ^{13}C-isotopomer data, instead of just steady-state concentrations, to deduce meaningful relationships between metabolites in related pathways [89].

It was displayed that differential ^{13}C-labeled isotopomer profiles and abundance can serve as a fingerprint of the metabolic networks activity and could reflects both qualitative and quantitative differences in the metabolic pathways that lead to the synthesis of each metabolite [104].

The key challenge is quite evidently the compartmentalization of the eukaryotic cell, which leads to a dissection of central carbon metabolism in sub-networks localized in either the cytosol or in organelles, like mitochondria or peroxisomes.

It was also showed that NMR is ideal for discerning the fractional enrichment at specific carbon atoms in particular metabolites. These fractional enrichments provide primary data from which metabolic fluxes can be deduced, making possible to construct an appropriate flux model that matches the underlying metabolic network [57], providing useful information about the metabolic phenotype of mutant cells respect to the wild type strain.

In this way, the role of several metabolic processes could be defined, allowing the exploration of metabolic pathways, leading to qualitative information on the links between labeled precursors and their products and quantitative information on metabolic fluxes [86].

It was demonstrated that in yeasts it has been possible to make significant progress in the analysis of carbon metabolism by using ^{13}C NMR to measure metabolic fluxes in genetically modified cells [57,182]. This approach, so, can be used for functional genomic analysis of yeast mutants providing detailed quantitative information for the understanding of a biological network useful to identify the key genes for strain improvement [58].

Moreover, an intelligent screening of the large unexploited fungal biodiversity opens the possibility to the development and use of directed genetic modifications of cell factories for the production of novel compounds, that are otherwise difficult to produce by chemical synthesis, and also of new, efficient and environmentally friendly bioprocesses. These possibilities open the way to many comparative functional studies and will certainly change the respective importance of the different yeasts, building up new model yeasts for specific studies [10].