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REVIEW

The thesis of Lenka Přibylová deals with the osmotolerant yeast *Zygosaccharomyces rouxii*. The work was focused on osmotolerant properties of the wild strains ATCC 42981 and the type strain CBS 732, on the properties of their cell walls in connection with the response to osmotic stress, and on the investigation of Na^+/H^+ transport systems taking part in the tolerance to high osmotic pressure. The practical importance of studying osmotolerance consists in biotechnological applications making possible the fermentation during growth at high concentrations of ions and compounds. The work was accomplished in the frame of a combined postgraduate study at both the Institute of Physiology ASCR, v.v.i. and Université Louis Pasteur, Strasbourg. The thesis includes the results presented in six papers published in impacted journals, in one manuscript submitted to FEMS Yeast Research and in two chapters whose results have been prepared for publication.

As to the topics studied, the thesis can be divided into four sections interconnected by the focus on the microorganism *Z. rouxii*. The aim of the first section was to prepare a set of tools of genetic manipulation of *Z. rouxii* not available so far, comprising efficient transformation, specific episomal and centromere plasmids containing various selection genes, a system for multiple deletion using repeatedly-usable deletion casettes, the use of green fluorescence protein (GFP) for location of proteins, and the test of a possibility of using the promoter *ScGAL1* for regulated expression of genes in *Z. rouxii*. This aim was achieved, the above-mentioned set of tools for gene manipulation of *Z. rouxii* was prepared and tested. The author was first to construct centromere plasmids in this yeast. A system for multiple deletion of genes using repeatedly-usable deletion casettes *loxP-kanMX-loxP* was created and the plasmid pZGFP constructed, enabling the author to locate proteins in the cell of *Z. rouxii*. The developed methods significantly extend the possibility to study specific properties of this yeast microorganism.

The second section of the thesis, whose results were both published in the journal Yeast and their other part has been accepted for publication in Folia Microbiologica, focused on the study of osmotolerant properties of the above-mentioned wild strains of *Z. rouxii*, comparing their differences concerning the concentration of salts, production and assimilation of glycerol, and the karyotypes of both strains. The results obtained

suggested that a lower degree of osmotolerance correlated with a more rigid structure of the yeast cell wall.

The third section of the thesis concentrates on Na^+/H^+ transport proteins. The author selected from databases and further analyzed the gene sequences coding for proteins homologous to known genes and, subsequently, studied these proteins in *Z. rouxii* using the gene manipulation tools that she had developed in her previous work. A so far unknown gene coding for a transport protein was identified. Its primary structure was similar to yeast Na^+/H^+ antiporters and the author showed that the protein was able to transport Na, Li and K cations. The protein was functionally characterized in *Z. rouxii* and, after a heterologous expression, also in *Saccharomyces cerevisiae*. The expression of this protein increased osmotolerance of the two yeast microorganisms by elimination of Na, K and Li ions. Using GFP, the protein was located near the cell surface, probably in the cytoplasmic membrane. The author has proven the existence of two transport proteins able to eliminate toxic cations in *Z. rouxii*, one having a narrow and the other a broad substrate specificity. This corresponds to the situation described in other yeast species. The function of the two antiport systems was, however, not studied by the method of heterologous expression in *S. cerevisiae*, as in the above-mentioned comparative studies, but directly in the original microorganism.

The fourth section of the thesis includes the author´s contribution to the French project Génolevures 3. The author prepared genomic DNA of *Z. rouxii* for sequencing and took also part in the subsequent annotations of the genome of this yeast organism. The aim of the Génolevures 3 project was to investigate into the mechanisms of microevolution of the eukaryotic genome.

The thesis includes a concise, up-to-date introductory part that is very well written. The chapter Materials and Methods precisely describes the methodology used, maximally quoting the own papers. The thesis also includes a vast list of relevant and recent references. The scientific aims of the study have been accomplished.

Formally, the thesis seems to be perfect. The reader will appreciate the author´s capability to briefly and precisely explain the complicated theme studied. I think that the thesis fulfils all the requirements necessary for it to be accepted as a PhD thesis and I recommend it for the defence.

Prague, 4th October 2007 Dr. Čeněk NOVOTNÝ, CSc.