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Evaluation of PhD. thesis

Mgr. Lenka Přibylová defends her PhD Thesis: "Osmotolerant yeast *Zygosaccharomyces rouxii* - construction of tools and characterization of specific features". Form of the work differs from the most common one only in the chapter 5: "Results and discussion", where autor replaced normal text for reprints of her original papers. Individual papers are interconnected by brief introductory chapters. In several cases the text is substituted by paper, which is ready for press or which was submitted for publication. The thesis contains four already published papers, two papers accepted for publication, one paper, which is ready for publication and two chapters containing so far unpublished results.

To make this point clear the following list is summarizing the papers, which take part in the thesis [publications are in the order of their appearance in the thesis]:

1. 5.1.1. Efficient transformation of the osmotolerant yeast *Zygosascharomyses rouxii* by electroporation [2003] J Microbiol Methods 55[2]: 481-484
2. 5.1.2. Expterssion of the *Saccharomyces cerevisiae* MPR1 gene encoding acetyl transferase in *Zygosaccharomzces rouxii* conferes resistence to L-Azetidine-2-carboxylate [2006] Folia Microbiol 51[3]: 203-207
3. 5.1.3. Characterization of *Zygosascharomyces rouxii* centromeres and construction of the first *Z.rouxii* centromeric vectors [2007] Chromosome Res in press DOI 10.1007/s10577-007-1136-z
4. 5.1.4. Tools for the genetic manipulation of *Zygosaccharomyces rouxii* [2007] submitted to FEMS Yeast Res
5. 5.2.1. Osmoresistant yeast *Z.rouxii*: the two most studied wild-type strains differ in osmotolerance and glycerol metabolism [2007] Yeast 24[3] 171-180
6. 5.2.2. Differences in osmotolerant and cell wall properties of two *Z.rouxii* strains [2007] Folia Microbiol in press
7. 5.3.1. Exploration of yeast alkali metal cation/H+ antiporters: Sequence and structure comparison [2006] Folia Microbiol 51[5] 413-424
8. 5.3.2.-9. Characterization of transport proteins ZrNha1 and ZrSod2-22[2007] prepared for publication
9. 5.4. In the frame of the project Genolevures 3 L.Přibylová prepared genomic DNA library for sequencing and annotation of genome *Z.rouxii*. In addition she took a part in processing of the annotation of gene families according to their function. In preparation

The scientific results summarized in the thesis of Lenka Přibylová were collected approximately five years during her post-gradual study in Prague (ASCR) and in Strasbourg (ULP/CNRS).

According to the scientific content the obtained results may be classified to the following groups:

1. Tools for genetic engineering of *Z. rouxii* (4 papers)
2. Osmotolerance of *Z.rouxii* (2 papers)
3. Metal cation/ H+ antiporters (2 papers)
4. Participation in analysis of four yeast genomes in the frame of the project Génolevures 3 (probably at least 1 publication)

Aims of the thesis were clearly formulated (page 41) and author succeeded to reach all of them.

In the "Introduction" author reasonably explained, why the thesis is subdivided to four main parts and what were the main reasons to select these directions.

In the next chapter (2) she described in detail what is known so far about osmotolerance and metal cation/ H+ antiporters, which tools are used in genetic engineering of yeast and which of them are missing in genetic ingeneering of Z.rouxii, and what is the aim of the project Génolevures, which is focused on *Hemiascomycetes* as a model group for the study of evolution in eukaryotic genomes. It can be concluded, that author was perfectly theoretically prepared for her experimental work and that she has broad overview in the field of her study.

I am just missing in this part a note about possibility of transformation of yeast cell by linear DNA molecules with help of homologous recombination and about possibilities of rebuilding yeast chromosomes and any sequences of nucleotides in chromosomal or plasmid DNA (chapter 2.3.). It seems to me that the role of PEG in DNA-transformation of protoplasts is not described exactly enough (chapter 2.3.1.1). Function of PEG is according to my knowledge particularly the destruction of water shell around cell membrane.

L.Přibylová learned large number of methods and some of them modified or evolved. Some methods are described only in included reprints of publications.

General comment to the chapter "Results and discussion":

I am appreciating the way of introducing the papers. **However, I am missing the common discussion to the groups of individual papers. To what extent participated author of thesis on the discussions in individual publications.** The total number of papers published or ready for publication is impressive.

Comments to individual papers:

5.2.1. I am missing the characteristic of dependence in frequency of transformation on the size of DNA-molecules. Knowledge of this dependence is very important particularly in the case of electroporation technique, because in the range of small molecules the frequency is size-

independent, while above this range the frequency is usually decreasing with size of DNA-molecules.

5.1.2. It was shown that MPR1 from *S.cerevisiae* confers resistance on *Z.rouxii* to L-azetidine-2-carboxylate. It is a pity that the frequency of spontaneous formation of resistance is so high, that it is not possible to use this gene as dominant selection marker in *Z.rouxii*. Why further resistance markers, which were successfully used in *S.cerevisiae* were not tested?

5.1.3. As the centromeres from *S.cerevisiae* do not function in *Z.rouxii* and *vice versa,* it was important to isolate centromeres from *Z.rouxii* and construct the first set of centromeric plasmids for manipulating this yeast.

5.1.4.* Construction of new auxotrophic strains with different combination of ura3, leu2 and ade2 is very important for manipulating *Z.rouxii*, however, I do not understand why the combination with deleted ura3 were not constructed, because particularly these strains are very important tools due to the following features: Both, URA3 and ura3 are positively selectable markers and cassette with URA3 can be exploited for multiple deletion of various genes even without loxP/cre system (but only in ura3 zero mutant strains or alternatively when URA3 from different species-nonhomological sequence- is used). I am missing this point also in the chapter 2. In addition, I would like to mention, that the exploitation of tandem loxP-FRT leads to more efficient removing the genes from the integrated cassette by FLP- or cre-recombinase in coparisson with the cassette with loxP only. According to my experience pGAL1 can not be completely repressed even in *S.cerevisiae.* Which promoter will be the next candidate for this role in *Z.rouxii.

I have no critical comments to further chapters.

Conclusion: The PhD thesis of Mgr. L.Přibylová proves that she is able to study critically scientific literature, use broad spectrum of methods, accumulate many scientifically interesting and quite original results and write high quality scientific papers. According to my proposal the scientific level of PhD. thesis of Lenka Přibylová is **exceptional** and for this reason I can fully recommend this work as a base for the graduation of its author.

Prague October 4, 2007 Doc.RNDr. Vladimír Vondrejs, CSc.