Masterproef industriële ingenieurswetenschappen

Functional characterization of heavy metal transporters of the mycorrhizal fungus Suillus luteus

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Academiejaar: 2013-2014

Introduction

Since the beginning of the Industrial Revolution, human activities have led to an enormous rise of metal concentrations in certain areas. Heavy metals are toxic for living organisms, even in minor concentrations. Some organisms, however, developed specific survival strategies. Some of these strategies are exhibited by the Ycf1 and Yor1 ABC-transporters, in the yeast *Saccharomyces cerevisiae*. The Ycf1 transporter is involved in vacuolar sequestration, the Yor1 transporter in efflux of heavy metal ions.

Fig. 1: *Suillus luteus*

The research topic in this master thesis is the identification of homologues of these two transporters in the ectomycorrhizal fungus *Suillus luteus*, and to investigate if these homologues also have a role in heavy metal tolerance, more specifically in cadmium tolerance.

Phylogenetic analysis of the ATP binding cassette proteins in the Phylum Basidiomycota

For the identification of homologues in related mycorrhizal species and the potential function of these homologues, a phylogenetic tree is drafted. This was obtained with the aid of bioinformatic tools and online databases like NCBI, JGI and MEGA6.

In the resulting tree, hits of *Suillus luteus* are marked with a blue spot. Hits in species of the same order as *S. luteus*, the Boletales, are marked in green. These are closely related, so they group together. The other hits do not belong to the Boletales, but still to the same phylum, the Basidiomycota. The reference sequences of the yeasts *S. cerevisiae* and *S. pombe*, marked in red, are further related, and take a greater distance.

S. luteus appears to possess one Ycf1 homologue and two Yor1 homologues. There is a possibility that these homologues are involved in heavy metal tolerance, just as in *S. cerevisiae,* although it is not for certain.

The second part of the phylogenetic analysis is the comparison of the conserved domains of the proteins. Highly conserved regions are sequences or domains that have remained very similar in different species during the course of evolution. The more resemblance of the conserved domains in two proteins, the more confidence that their functions will match.

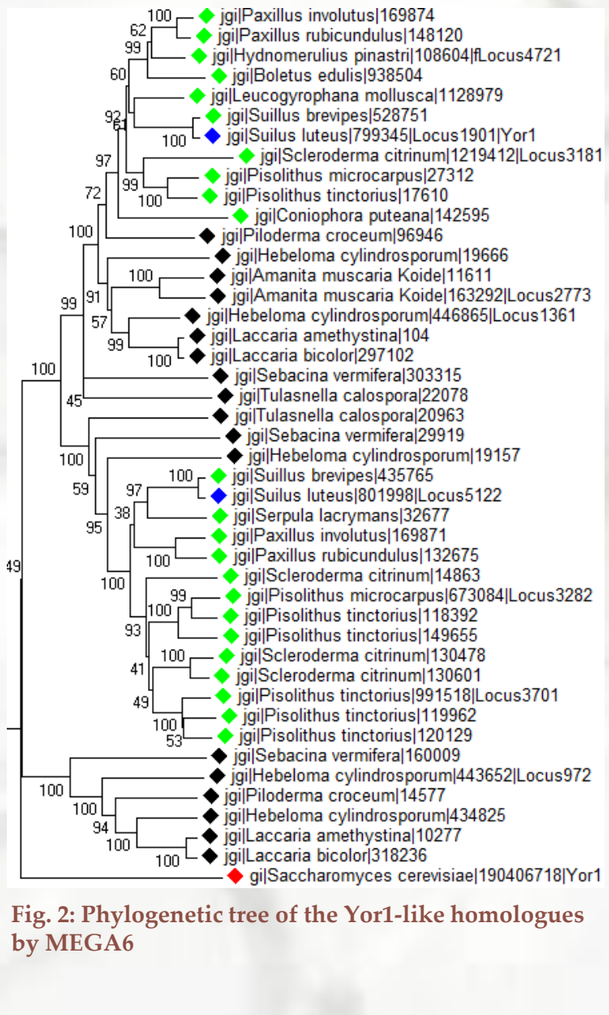

Fig. 2: Phylogenetic tree of the Yor1-like homologues by MEGA6

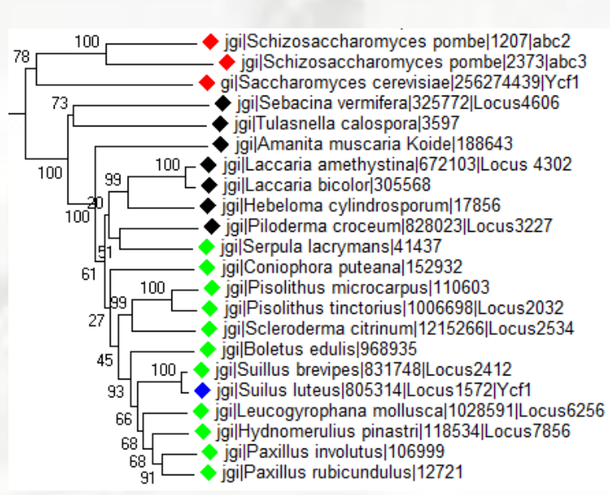

Fig. 3: Phylogenetic tree of the Ycf1-like homologues by MEGA6

To identify the conserved domains in a sequence, the Conserved Domain Database (CDD) was used.

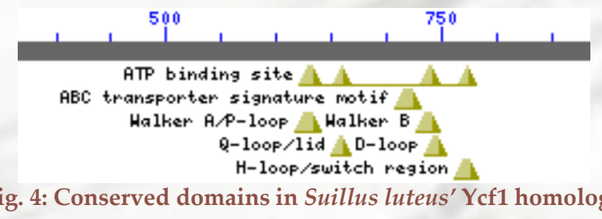

Fig. 4: Conserved domains in *Suillus luteus'* Ycf1 homologue

The conserved domains of *S. luteus* appear to be situated at the same location as Ycf1 and Yor1 in *S. cerevisiae*. This may be an indication that they exert the same function in heavy metal tolerance.

Functional test of *Suillus luteus* Ycf1-like protein (*SlYcf1)*

In order to test the influence of the Ycf1 protein on heavy metal tolerance, the gene was isolated from several *Suillus luteus* strains. With the aid of the Gateway cloning technology, the gene was imported into an expression vector, and the expression of the *Ycf1* gene was induced in a *Saccharomyces cerevisiae* strain.

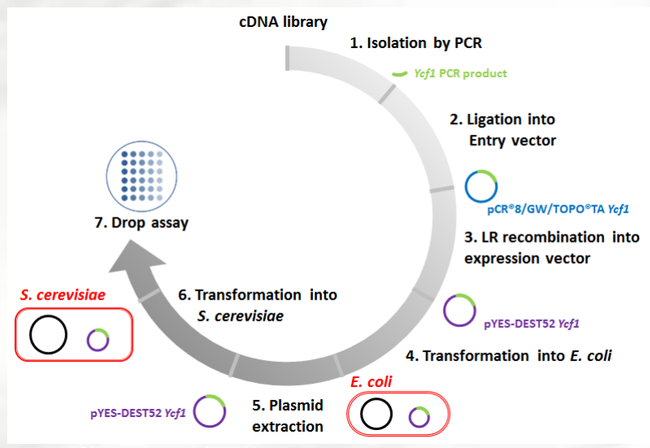

Fig. 5: Cloning of the *SlYcf1* gene into expression vector

This recombinant strain was exposed to various levels of heavy metal concentrations by drop assay, together with a wild type *Saccharomyces cerevisiae* and a mutant without the *Ycf1* gene, in order to compare.

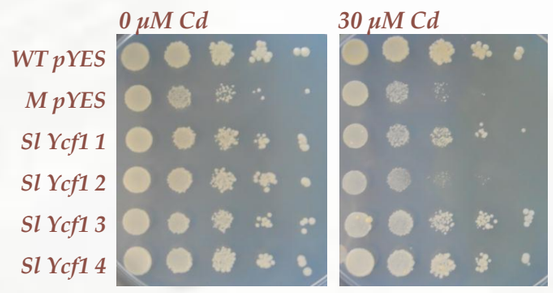

Fig. 6: Drop assay of Ycf1 pYES

Equipped with the *SlYcf1* gene, some of the recombinant strains were possibly able to survive at higher concentrations of cadmium, better than the mutant.

Conclusion

- The phylogenetic analysis has shown that *S. luteus* possesses one Ycf1 homologue and two Yor1 homologues. These homologues may also be involved in heavy metal tolerance, just as in *S. cerevisiae.*
- The complementation test indicated that the SlYcf1 protein might rescue the yeast mutants in media with high cadmium concentrations. To confirm the function of the *SlYcf1* gene, further analysis is required.
- For further research, it would also be interesting to investigate the role of the identified Yor1 homologues in heavy metal tolerance mechanisms of *S. luteus.*

References

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Promotoren / Copromotoren: Prof. Jan Colpaert, Drs. Hoai Nguyen, Ing. Liesbet Pauls

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