STRESS, TRANSPORT AND METABOLISM OF α-GLUCOSIDES IN *Saccharomyces cerevisiae*

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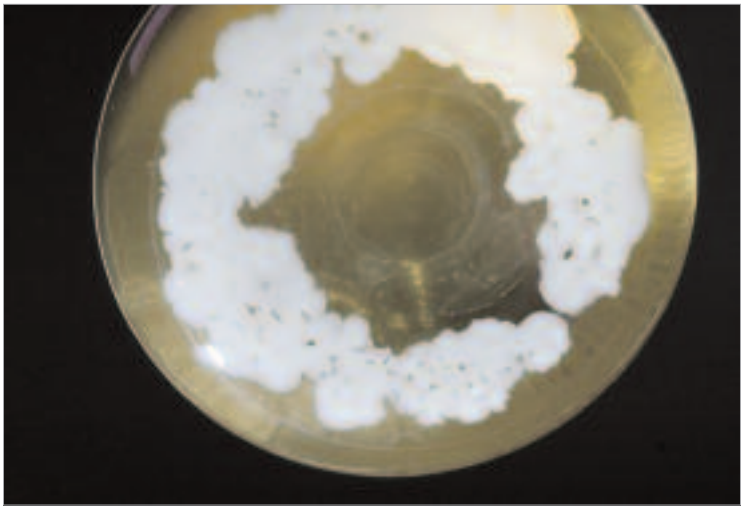


Colonies of S. cerevisiae

In this thematic project we intend to study cellular responses to stress conditions using the yeast *Saccharomyces cerevisiae* as a model organism for eukaryotic cells. We intend to explore the trehalose protective effects under stress conditions, focusing in oxidative stress. The tolerance acquisition mechanism under stress conditions that disrupt the cellular redox equilibrium will be our main interest in this research line. Since plasma membrane trehalose protection depends on the presence of the disaccharide on both leaflets of the membrane, the manipulation of the trehalose transporter transmembrane channel structure could lead us to dissociate the glucoside transport from the proton co-transport. Thus, the mutated gene could be expressed in other eukaryotic cell types, which would allow us to understand the conditions for dehydration resistance in those cell types. The study of the metabolic regulation for other α-glucosides besides trehalose could provide interesting results about the mechanism of his regulation, and eventually, led us to obtain strains that utilize these carbon sources more effectively. This could have some biotechnological interest. The mechanism of action of the purified and reconstituted into liposome's trehalose transporter will be studied both with the native as well as with the mutant proteins.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A new metabolic pathway for the metabolism of α-methyl glucoside was described and characterized which involves the hydrolysis of the glucoside in the yeast cell periplasmic space.

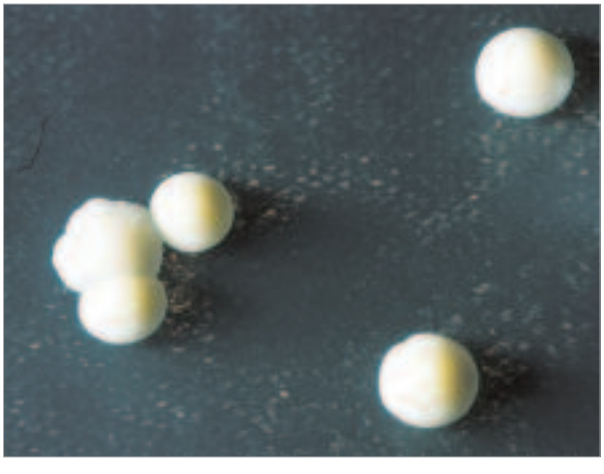
The negatively charged residues (Asp and Glu), which face the interior of the trehalose transporter transmembrane channel, have been replaced by alanines. In two mutant proteins, the glucoside transport became dissociated from the proton transport.

The asparagine residue from the same region was also mutated and this residue proved to be essential for the transport activity. An isoleucine, close to the asparagine, was mutated to threonine following an indication which appeared recently, that this substitution leads to an increase in the transport of maltotriose. However, no conclusive results were obtained in our case.

A construction linking gluthatione-S-transferase to the carboxyl end of the trehalose transporter was successful. This chimera is currently under study to obtain an efficient method for the purification of the trehalose permease.

The protective effect of trehalose was established for diverse stresses including those generating active oxygen species and cadmium yeast accumulation.

Saccharomyces cerevisiae *cells*



MAIN PUBLICATIONS

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