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CLASSICAL GENETIC STUDIES ON THE HALOTOLERANT YEAST *PICHIA SORBITOPHILA* AS BACKGROUND FOR MUTAGENESIS STRATEGY.

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Pichia sorbitophila, is an halotolerant yeast, presenting a very high pattern of resistance to salt *stress*. This capacity is tied up to, at least, two already characterized phenomena: the accumulation of high intracellular concentration of glycerol and a membrane H^+/glycerol symport system.
In order to be able to establish a mutagenesis strategy to obtain mutants deficient on either of these phenomena, classical genetics studies were performed. Sporulation was obtained and tested in a wide variety of conventional and non conventional media, including salt *stress* media, and the obtained tetrad separated by micro-manipulation. Each ascospore was then tested by itself and crossed to test homo or heterothalic behaviour. Evidence was found pointing to a life cycle composed of an haploid vegetative phase intercalated by a transient diploid phase, obtained, most probably, by mother-bud conjugation prior to the formation of asci. So, to assure haploidy, an ascospore culture was used in the subsequent mutagenesis assays.
Mutation was performed utilizing 90% death on Nitrosoguanidine and subsequent density Percoll gradient centrifugations, optimized as a technique of mutant enrichment, separating cells grown in the presence of high NaCl concentrations or adapted to salt *stress*, from cells potentially affected in osmoregulation mechanisms. Selection media were designed for mutant selection. All the mutants thus found proved to be affected in salt resistance pattern and defective in glycerol transport. On the other way around, all the cells tested, presenting an untouched salt resistance pattern, presented no changes in H^+/glycerol symport uptake kinetics. Nevertheless, resistance was reduced, not entirely eliminated, what constitutes strong indication that other mechanisms, besides transport, must be involved in osmoregulation in *Pichia sorbitophila*.
Results reinforce the idea that constitutive active transport systems for compatible solutes, are indispensable mechanisms contributing to high salt tolerance in yeasts.