P-V-56 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 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. Mutagenic effects of Nitrosoguanidine and U.V. light were compared and density Percoll gradient centrifugations were optimized as a technique of mutant enrichment, separating cells grown in the presence of high NaCl concentrations or adapted to salt stress from cells in some way affected in osmoregulation mechanisms.