Production of ammonia by the colonies of mutants and aging of wrinkled colonies of *Saccharomyces cerevisiae*

The aim of this diploma thesis is to observe the development, respectively the aging of cells in yeast colonies *Saccharomyces cerevisiae.* Yeast cells *S. cerevisiea* form multicellular organized structures on a solid substrate, i.e. colonies, which the intercellular interactions occur in. These interactions influence forming, morphology and aging of yeast colonies. This diploma thesis is focused partly on the changes in ammonia production by giant colonies of deletion mutants and partly on the aging of colonies with the wrinkled morphology.

I characterized mutant strains of *S. cerevisiae* with the deletion in *RTG1, RTG2, RTG3, FIS1, CIT2* genes. Their products play an important role in the colony development. The transcription of these genes changes during the transition from the acidic to alkali phase during developmental process of the colonies. I have found out that the ammonium production rate was in accordance with the results of the alkalization in giant colonies surroundings and mentioned mutants derived from the BY strain has been producing ammonia since the 15th day. The rate of the ammonia production by rtg3Δ strain was comparable to the parental strain. Compared to parental strain, lower production was discovered in fis1Δ, rtg1Δ and rtg2Δ. In contrast to it, the production of ammonia was higher in the cit2 strain.

I prepared *S. cerevisiae* ΣS^h strains where *GUT1* and *SMI1/KNR4* genes expressed in the exponential phase of the growth in the liquid culture and *SSA3* a *HSP30* genes expressed in the stationary phase of the growth was marked by the GFP (green fluorescent protein) gene. The development of the yeast colonies with the wrinkled morphology was observed in above mentioned strains. It was discovered that the gene expression occurs in the appropriate phase of the growth in the liquid culture. Based on the induction of fused fluorescent proteins in cells taken from the defined parts of the colony it was proved that older cells are located mainly on the top of the colony while the younger cells are located mainly along the border of the colony and in the middle of the bottom part of the colony. The presence of dead cells in the *S. cerevisiae* ΣS^h strain growing on a agar containing BKP indicator was also observed. Dead cells were not proved in colonies however the presence of reactive oxygen radicals was confirmed.

Keywords: *Saccharomyces cerevisiae*, ammonia production, gene expression, colony development, apoptotic features.

Klíčová slova: *Saccharomyces cerevisiae*, produkce amoniaku, genová exprese, vývoj kolonií, apoptotické znaky.