Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Oral Communication Abstract - 2.09

DO ADH1 GENE MUTATIONS AFFECT THE *SACCHAROMYCES CEREVISIAE* COLONY DEVELOPMENT?

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allilyc alcohol tolerance, yeast, ADH1 gene, insertion/deletion mutation

Spontaneous mutants, resistant to allylic alcohol, have been isolated from the wine yeast, *Saccharomyces cerevisiae* 1014. It is known that *S. cerevisiae* strains, resistant to allylic alcohol, can carry mutations in the ADH1 gene. Cells of the mutants, when grown on a medium containing glucose as energy source, originate colony of different size, "*large*" and "*small*", whereas, when grown on a medium with an energy source not fermentable, only *small* colony are produced. Cells from only *small* colonies, grown on a medium with glucose, produce *large* and *small* colonies. Cells from the hybrid when grown on glucose supplied medium originated only large colony, suggesting that the mutation is recessive. Analysis of tetrads, derived from crosses between mutant strains and the parental strain, has shown that the phenotypes, *large* colony and *small* colony, segregate in the ratio 2:2, suggesting that allylic alcohol resistance, in the analysed strains, is due to single gene mutations.

With the aim of localizing the allylic alcohol resistance mutations, specific primers have been designed on the ADH1 gene sequence and on that of its promoter region sequence, both sequences already present in GenBank. The primers have been used for the isolation of one fragment (430 bp) of the N-terminal region of the transcript, and one (780 bp) of C-terminal region, and one fragment (480 bp) of the promoter. PCR analysis has been carried out to verify the presence of mutations as deletions or insertion. An insertion mutation has been observed in the longer fragment of the transcript. No difference has been found in the dimension of the N-terminal fragment and in that of the promoter. The dimension of the amplified fragment of DNA from *large* and *small* colonies cells has been found about 30 bp longer respect to the amplified fragment of DNA from wild type colony cells.

Two are the DNA fragments formed in the hybrid, as expected, 780 bp, from the wild type, and 810 bp, from the mutant. PCR analysis on DNA from the tetrad progenies has shown that presence/absence of the mutation on C-terminal region of the ADH1 gene segregate in a 2:2 ratio. All the obtained data indicate that the trait "*colony dimension*" is associate with the mutation on ADH1 gene.

In our laboratory work is currently done to highlight the role of the mutation and its role in the expression of ADH1 gene and its functionality in the colony growth. Moreover, mitochondria analysis will be also done to evaluate the capacity to growth on medium supplied with not fermentable energy source.