Investigation of yeast community of "Grillo" grapes and musts from Marsala wine production area

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The oenological interest in the autochthonous yeast applications has increased since they represents an important supplement to wine quality (Martinez *et al*., 1989; Moreno *et al*., 1991). Yeast populations harboured onto the surface of berries and in musts of "Grillo" grape variety were isolated and analyzed. In order to obtain a first blastomycetic mapping of Marsala wine production area, eight vineyards were chosen on the basis of different climatic and agronomic parameters, including altitude, exposure,vineyard age, grape biotype, grape cultivation system, vegetative vigour, pruning, green pruning, yield per plant, phytosanity state, irrigation and closeness to wood areas. Analysis of blastomycetic populations was performed by cell counts on specific culture media for yeasts. Cell concentrations were evaluated on grapes and unfermented musts and during spontaneous must micro-fermentations at different times (after 3 and 13 days). Furthermore, during micro-fermentations non-*Saccharomyces* populations were distinguished from presumptive *Saccharomyces* based on the appearance of colonies after growth onto Wallerstein Laboratory (WL) nutrient agar (Pallman *et al*., 2001). Non-*Saccharomyces* reached about 106-107 CFU/ml both at the third and at the thirteenth day, while *Saccharomyces* exhibited variable cell concentrations, in particular, the majority of experiments showed level around 104 CFU/ml at the third day and almost all samples reached level of about 106 CFU/ml at the thirteen day. *Saccharomyces* cell concentrations positively correlated with weight loss registered during fermentations, since higher weight loss values were found in samples with their higher levels. After colony morphology inspection, 54 isolates were collected from grapes and unfermented musts and 60 during must micro-fermentations after 3 and 13 days, forming a total of 114 isolates. They were clustered into eight groups and into nine groups by optical microscopic observation of cell morphologies. Strain typing and differentiation was carried out by randomly amplified polymorphic DNA (RAPD-PCR) (Moschetti *et al*., 1998) and the band patterns were analyzed by means of the unweighted pair group method using arithmetic average clustering algorithm (UPGMA). The representative strains of each group are being genetically identified. So far, analysis of D1/D2 region of the 26S rRNA gene revealed the presence of *Candida zampliinina*, *Hanseniaspora uvarum*, *Issatchenkia terricola*, *Metschnikovia pulcherrima*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. Moreover, the study was specifically oriented to the *Saccharomyces* spp. strains, which were isolated from musts fermented for 21 days using a "modified ethanol sulphite agar" (MESA), prepared from ESY medium. A total of 42 cultures were collected and 28 presumptive *Saccharomyces* yeasts, as selected by microscopic observation, were confirmed to be *Saccharomyces* spp. through amplification of ITS-5.8S rRNA region (Esteve-Zarzoso *et al*., 1999). All the isolates were typed as above reported. The representative strains of each group were characterized for technological traits with interest in wine production such as hydrogen sulphide production, ethanol tolerance and potassium metabisulphide resistance. Strains showing the best performance were used to carry out "Grillo" must micro-fermentations lasting 13 days to select yeast starter cultures. The work is still in progress.

Keywords: autochthonous yeast, Grillo grape, Marsala wine, *Saccharomyces.*

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