CONTRIBUTIONS REGARDING SOME OENOLOGICAL YEAST STRAINS ISOLATED FROM VINEYARD IAŞI, ROMANIA

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Abstract

This paper pursues two lines of research: isolation and purification of yeast strains responsible with the alcoholic fermentation from vineyard Iaşi, and testing of the oenological characteristics of the isolated yeast strains. Presented results represents a part of a project aiming selection of new yeasts strains from indigenous flora, thus obtaining wines that reflect the personality and potential of the grape variety that are specific to the Iaşi vineyard. Yeast isolation and obtaining of the pure cultures was done mainly through inoculums dissemination and loop exhaustion techniques on solid nutrient media. Following isolation and purification 10 yeasts strains were selected and have been tested in the laboratory for the degree of foam production and the alcoholic capacity. After the testing procedures, 5 yeasts strains were retained for future research to optimize the fermentation processes and to obtain quality white wines from Iaşi vineyard. Four yeasts strains (SC3, SC5, SC7, SC8) were originally from viticultural center Copou and one strain (SCt9) originates from viticultural center Bucium.

Key words: vineyard Iaşi, yeast strains, isolation, foam production, alcoholic capacity

Grape must fermentation into wine is a complex microbiological process involving the presence of different yeast species. The diversity of yeasts population present on grape berries, vine leaves and soil is one of the important factors responsible for the sensory quality and organoleptic characteristics of wine (Fleet et al., 2002; Clemente-Jimenez et al., 2004). Other factors important for type and amount of aroma are climatological conditions, geographic location, grape variety, fruit condition, fungicide application, vineyard age, vinification process, pH, amount of sulphur dioxide, amino acids present in the must and malolactic fermentation (Lilly et al., 2000; Combina et al., 2005; Raspor et al., 2006; Nisiotou and Nychas, 2007; Chavan et al., 2009).

The recent research agrees that in freshly crushed grape must a diversity of yeast species is present, principally within the genera *Hanseniaspora* (anamorph *Kloeckera*), *Candida*, *Pichia*, *Rhodotorula* and *Kluyveromyces*. Several studies report that fermentative species from genera *Saccharomyces* (e.g. *Sacch. cerevisiae*) have a low occurrence among the natural yeast populations found on grape berries and vineyard soils (Fleet, 2003; Martini, 1993; Pretorius, 2000; Jolly et al., 2006). The natural fermentation of grape must is usually started by low-alcohol-tolerant apiculate yeasts (*Kloeckera* spp.) that dominate the first stages of fermentation. After 3–4 days, they are replaced by elliptical yeasts (*Sacch. cerevisiae*) that continue and finish the fermentation process (Amerine et al., 1980; Martini, 1993).

The aim of this work was to obtain through isolation and selection new yeasts strains from indigenous flora which could allow us to obtain wines that reflect the personality and potential of the grape variety that are specific to the Iaşi vineyard.

MATERIAL AND METHOD

Grape samples were collected during harvest in September 2011 from viticultural centers Copou and Bucium, members of the Iaşi vine-growing district in the southeastern part of Romania. Two grape varieties were analyzed: Aligoté and Băbească neagră. For each grape variety and from at least five different vines, around 1–2 kg grapes per sample were randomly and aseptically collected in plastic autoclavable bags. Only healthy and undamaged grapes were harvested from the selected viticultural centers and transported immediately in cold boxes to the microbiological laboratory in Iaşi. Samples were analyzed within 24 h of harvest from the vine.

From each sample 1000 g grapes were aseptically homogenized for 15 min. Aliquots (0.1 ml each) of several dilutions (from 10^{-1} to 10^{-6}) were spread in duplicates on yeast–malt agar (YM), containing 3% yeast extract, 3% malt extract, 5% bacteriological peptone, 10% glucose, 20% agar. After the incubation at 28 °C for 3–5 days the

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colonies were counted out in duplicates and differentiated on the basis of their morphology. Thirty yeast colonies were randomly isolated and purified from each sample.The isolates were preserved at −20°C for further identification.

The grape must used in the fermentation tests had a sugar content of 27% (w/v) and was pasteurized at 90°C for 15 min. Alcoholic capacity was evaluated in duplicate experiments carried out in 500 mL Erlenmeyer flasks containing 300 mL of grape must at 20°C by inoculation with 48-h pre-cultures (grown in the must medium at 20°C) to obtain an initial inoculation level of 10^6 cells mL^{-1}. In order to appreciate the foam capacity, glass recipients with a volume of 1000 mL were used, monitoring the foaming degree and the time intervals (hours/days) of the fermenting phases.

RESULTS AND DISCUSSION

From vine cultivars Aligoté and Băbească neagră situated in Iaşi vine-growing region ten different yeast species have been isolated.

Ten yeast strains were isolated from grape must and were characterized macro- and microscopic. The main macroscopic characteristics are presented in Table 1.

Table 1

Morphological characteristics of the isolated yeasts

Yeast strain code	Source of isolation	Yeast colony color	Macroscopic characteristics (colony)
SC1	Aligoté	white	circular form, convex profile, smooth shiny surface
SC2	Aligoté	white	circular form, flat profile, smooth surface, opaque
SC3	Aligoté	gray-white	circular form, convex profile, smooth surface, opaque
SC4	Aligoté	white	circular form, flat profile, smooth surface, opaque
SC5	Aligoté	white	circular form, flat profile, smooth surface, opaque
SC6	Aligoté	gray	circular form, flat profile, smooth surface, opaque
SC7	Aligoté	gray-white	circular form, flat profile, smooth surface, opaque
SC8	Aligoté	white	circular form, convex profile, smooth shiny surface
SCt9	Băbească neagră	white	circular form, convex profile, smooth shiny surface
SC10	Aligoté	red	circular form, convex profile, smooth shiny surface

In order to select the most valuable yeast strains, from the point of view of the fermentation characteristics, the isolated yeasts strains have been preliminarily tested in the laboratory.

In order to appreciate the fermentation capacity, during the first stage, glass recipients with a volume of 1000 mL were used, monitoring the foaming degree and the time intervals (hours/days) of the pre-fermenting phase, the tumultuous fermentation phase and the post-fermenting phase of the ten yeasts strains selected from the indigenous flora of the Copou and Bucium viticultural centers.

From the point of view of the foaming degree, the data obtained after the application of the tests show that three yeast are averagely foaming, two yeast are minimum foaming (3 – 5 cm^3/L foam) and five are non-foaming (Table 2).

Table 2

Foam capacity and appearance of produced foam

Yeast strain code	Foam volume (cm^3/L)				Appearance of produced foam
	24h	48h	72h	80h	
SC1	-	-	-	1	No foam produced
SC2	-	1	1	1.5	No foam produced
SC3	-	-	2.3	1.5	No foam produced
SC4	-	-	1	5	Compact foam, adhering to the walls
SC5	-	-	-	4	Compact foam, adhering to the walls
SC6	-	1	2	2	No foam produced
SC7	-	-	27.7	15	Spongy foam, decrease in volume after tumultuous stage
SC8	-	1	3	9	Compact foam, adhering to the walls
SCt9	-	1	1	2	No foam produced
SC10	-	-	2.5	10	Compact foam, adhering to the walls

Alcoholic capacity (Table 3) was tested in identical conditions, through the determination of the alcoholic concentration (% vol.) at the end of alcoholic fermentation processes. It was noticed that all the tested yeasts strains have alcoholigenous power, and were able to start the fermentation process in a must with high sugar content (27% w/v).

Table 3

Alcohol capacity of the isolated yeasts

Yeast strain code	Alcohol content (% vol.)
SC1	3,21
SC2	4,35
SC3	**11,54**
SC4	6,49
SC5	**11,72**
SC6	4,33
SC7	**11,41**
SC8	**11,98**
SCt9	**12,08**
SC10	9,23

Not all ten yeasts strains selected from the indigenous flora present a real interest for wine-making, so that the laboratory testing was decisive in choosing those yeasts that correspond to the practical needs from the oenological area. Analysis of the dates obtained in the laboratory tests allowed us to select five yeasts strains (SC3, SC5, SC7, SC8 and SCt9) and to eliminate the other five.

The selected yeasts strains start the alcoholic fermentation after 18–20 h from the introduction of the leavens in the must. The stage of the tumultuous fermentation started after 48 h and continued for 8–10 days, which is the optimum time interval for the producing of a moderate metabolization of the sugars. This observation is similar to previous report of Vasile Ancuţa (2009).

Among the five indigenous yeast isolates, the largest diversity of yeast species was found on the vine variety Aligoté (four species), followed by Băbească neagră (one specie). These results suggest that white vine varieties maybe offer an advantage for yeast diversity. Varietal factors, e.g. thickness of grape skins, can play a role (Bisson and Kunkee, 1991). The obtained results confer a image over the oenological value of the new selected yeasts strains, which allows their recommendation in the technology of production of the dry white wines in the Iaşi vineyard.

However, further experiments containing ecological data reports, identification of yeast species and their population counts from the same vine variety, vine plant, vineyard during several years should be performed.

CONCLUSIONS

From Iaşi vineyard were isolated in pure cultures through inoculums dissemination and loop exhaustion techniques on solid nutrient media ten yeast strains.

After the testing procedures, 5 yeasts strains were retained for future research to optimize the fermentation processes and to obtain quality white wines from Iaşi vineyard. Four yeasts strains (SC3, SC5, SC7, SC8) were originally from viticultural center Copou and one strain (SCt9) originates from viticultural center Bucium.

From the point of view of foaming capacity three yeast strains are averagely foaming, two are minimum foaming (3 – 5 cm^3/L foam) and five are non-foaming.

From the point of view of alcoholic capacity five yeast strains produced a alcoholic concentration over 11% vol. alcohol.

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