MICROBIOLOGICAL RESEARCH ON SOME YEASTS ISOLATED FROM THE MOLDAVIA VINE-GROWING REGION, ROMANIA

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Abstract

Yeasts are the most important microorganisms in wine production, and their number and diversity on grape berry surfaces and stalk are influenced by grape variety, degree of grape maturity at harvest, climatic conditions, geographic location, physical damage of grapes, the intensity of pest management etc.
This paper pursues two lines of research: isolation and purification of yeast strains responsible with the alcoholic fermentation from vineyards Focşani and Bucium-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, because they influence fermentation speed, wine flavor and other wine qualities. These informations are important for wine-makers to produce wine with high quality and typical attributes. 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 a total of 15 yeasts strains were selected and further studied in the laboratory regarding degree of foam production and alcoholic capacity. After the testing procedures, three yeasts strains were retained for future research to optimize the fermentation processes and to obtain quality white wines from both vineyards. Two yeasts strains (V0101, V2101) were originally from viticultural center Bucium Iaşi and one strain (AC2) originates from viticultural center Focşani.

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

Romania represents an important wine producer from Europe with history in oenology and viticulture. Development of knowledge about indigenous yeast community present on grape berries is essential to understand the winemaking process and to generate products with a local character allowing the development of modern winemaking practices and the diversification of wine products.

The diversity of yeasts population that colonise the skin of grapes, 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, Lipşa et al., 2013). Furthermore, the type and amount of aroma in wines are influenced by climatic conditions, geographical location, application of antifungals, soil type, vineyard age, grape variety, harvest technique, vinification process, pH, amount of sulphur dioxide, amino acids present in the musts and malolactic fermentation (Chavan et al., 2009, Lilly et al., 2000, Longo et al. 1991, Monteil et al. 1986, Farris et al. 1990, Pretorius et al. 1999). All recent research agrees that the apiculates yeasts *Hanseniaspora* (anamorph *Kloeckera*) and the oxidative *Candida*, *Pichia*, *Rhodotorula, Kluyveromyces* are the predominant genera in freshly crushed grape must. 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 study was to identify new strains from the indigenous yeast community present on grape berry surfaces and stalk. After isolation and selection these yeast could be used to obtain wines that reflect the personality and potential of the grape variety that are specific to both vineyards.

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MATERIAL AND METHOD

Grape samples were collected during harvest in September 2013 from viticultural centers Focşani and Bucium-Iaşi, members of vine-growing district in the eastern part of Romania. Five grape varieties were analyzed: Feteasca regala and Aligoté from Bucium-Iaşi vineyard and Feteasca alba, Merlot and Aligoté from Focşani viticultural center. 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 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 both vine cultivars situated in Iaşi vine-growing region were isolated nine different yeast species and from Focşani viticultural center other six species.

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)
V0101	**Aligoté**	**white**	**circular form, convex profile, smooth shiny surface, straight edge**
V0102	Aligoté	white	circular form, flat profile, smooth surface, opaque, straight edge
V0106	Aligoté	gray-white	circular form, convex profile, smooth surface, opaque, straight edge
V0109	Aligoté	white	circular form, flat profile, smooth surface, opaque, fine ramifications at the edge of culture
V0110	Aligoté	red	circular form, convex profile, smooth shiny surface, straight edge
V2101	**Fetească regală**	**gray**	**circular form, flat profile, smooth surface, opaque, straight edge**
V2103	Fetească regală	gray-white	circular form, flat profile, smooth surface, opaque, lobated edge
V2104	Fetească regală	white	circular form, convex profile, smooth shiny surface, fine ramifications at the edge of culture
V2105	Fetească regală	white	circular form, convex profile, smooth shiny surface, with less lobes marked and fine ramifications at the edge of culture
AC1	Aligoté	red	circular form, convex profile, smooth shiny surface, straight edge
AC2	**Aligoté**	**white**	**circular form, convex profile, smooth opaque surface, straight edge**
AC3	Aligoté	cream	circular form, convex profile, smooth shiny surface, straight edge
FA1	Fetească albă	gray-white	circular form, convex profile, smooth surface, shiny surface, straight edge
FA2	Fetească albă	white	circular form, convex profile, smooth shiny surface, straight edge
M2	Merlot	white	circular form, flat profile, smooth surface, opaque, lobated edge

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 Focşani and Bucium-Iaşi viticultural centers.

From the point of view of the foaming degree, the data obtained after the application of the tests show that five yeast are averagely foaming, three yeast are minimum foaming (3 – 5 cm^3/L foam) and seven 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	
V0101	**-**	**-**	**-**	**1**	**No foam produced**
V0102	-	1	1	1.5	Compact foam, adhering to the walls
V0106	-	-	2.3	1.5	No foam produced
V0109	-	-	1	5	Compact foam, adhering to the walls
V0110	-	-	-	4	Compact foam, adhering to the walls
V2101	**-**	**1**	**2**	**2**	**No foam produced**
V2103	-	-	27.7	15	Spongy foam, decrease in volume after tumultuous stage
V2104	-	1	3	9	Compact foam, adhering to the walls
V2105	-	1	1	2	No foam produced
AC1	-	-	2.5	10	Compact foam, adhering to the walls
AC2	**-**	**-**	**-**	**1**	**No foam produced**
AC3	-	10	13	7	Spongy foam, decrease in volume after tumultuous stage
FA1	-	-	2	3	No foam produced
FA2	-	-	2	1	No foam produced
M2	1	7	23	13	Spongy foam, decrease in volume after tumultuous stage

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.)
V0101	**11.3**
V0102	3.9
V0106	7.3
V0109	6.8
V0110	5.7
V2101	**10.8**
V2103	6.5
V2104	3.6
V2105	4.9
AC1	8.0
AC2	**11.9**
AC3	3.9
FA1	7.1
FA2	7.0
M2	6.6

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 three yeasts strains (V0101, V2101, and AC2) and to eliminate the other.

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 Ancuța (2009).

The main objective of the present work was to identify and to select native *Saccharomyces* populations associated to the viticultural centers Focşani and Bucium-Iaşi in order to elucidate the participation of these *Saccharomyces* populations in spontaneous fermentations of musts. The obtained results suggest that white vine varieties maybe offer an advantage for yeast diversity in comparacy to the red varieties. 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 both 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 Focşani and Bucium-Iaşi vineyards in autumn 2013 were isolated in pure cultures through inoculums dissemination and loop exhaustion techniques on solid nutrient media 15 yeast strains.

After the testing procedures, three yeasts strains were retained for future research to optimize the fermentation processes and to obtain quality white wines from both vineyards.

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

From the point of view of alcoholic capacity three yeast strains produced a alcoholic concentration over 10.5% vol. alcohol.

ACKNOWLEGMENTS

„Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine Iaşi (Project No. 5527/25.04.2013) financed this research.

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