ENOLOGICAL POTENTIAL OF NATIVE YEASTS ISOLATED FROM GRAPES IN IASI WINE DISTRICT, ROMANIA

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Abstract

Spontaneous alcoholic fermentation and the quality of a wines depends on the microbial communities present of the grapes and the external physical variables. Grapevine cultivar, viticultural practices, macro- and microclimatic conditions, and the vineyards geographic location all have an impact on the biological activities of fermenting microorganisms which prevail on the surface of grape berries. The taste and organoleptic features of wines are heavily influenced by the microbial communities present during grape must fermentation. The goal of this study was to isolate and select yeast strains with good enological traits for use as regional starter cultures and, as a result, to generate wines with specific sensory characteristics that can be connected to terroir of Iasi vineyards. After isolation and purification from different grape varieties, in order to determine their ecologically important properties, 9 indigenous yeasts strains were selected and have been tested in the laboratory for rate of fermentation, foam production, capacity to consume sugars from must and alcoholic capacity. After the testing procedures (micro-fermentations at 25°C), 4 yeasts strains (SCZ, SCH, CHC3 and GB3) were retained and could be used as future starters after further tests in large scale fermentations, in order to optimize the fermentation processes and to obtain quality wines from Iași viticultural area.

Key words: yeast strains, yeast isolation, enological properties, vineyard Iasi

Romania is a significant European wine producer with a long history of oenology and viticulture. Knowledge of the indigenous yeast community present on grape berries, bunches and must is essential for understanding the winemaking process and yeasts role in forming the characteristics of a wine independently of other factors Among other technological aspects, the selection of a suitable yeast strain is required in order to obtain quality wines with a local flavor, allowing for the development of modern winemaking practices and the diversification of wine products (Lipsa F.D. *et al*, 2013).

Prior strain selection, the enological properties of yeast must be established and for this purpose are many different selection criteria, which can be divided into positive properties (for example, ethanol tolerance, good performance in the transformation of sugars into ethanol, ability to grow in high sugar concentrations) and unfavorable properties (such as H2S production, foam production or volatile acidity) (Degré R., 1993; Fernández-González M., Briones A.I., 2013). Furthermore, some aspects that are commonly regarded as favorable properties, such as the killer phenotype and malic acid degradation, can be classified as neutral properties (Esteve-Zarzoso B. *et al*, 2000). Although commercial yeasts used as starter cultures are available for fermentation, using pure yeast cultures from the region where they will be used for wine production, also known as local yeast selection, may be more effective. Local yeasts are thought to be more competitive because they are better adapted to the environmental conditions (Regodon J.A. *et al*, 1997), and thus will be able to dominate the fermentation (Rodriguez M.E. *et al*, 2010).

The use of commercial yeasts as starter cultures allows a more rapid and complete fermentation of grape must, as well as a higher level of reproducibility in the atmosphere of specific wines (Suarez-Lepe J.A., Morata A., 2012), but the obtained commercial wine yeasts lack some desirable characteristics provided by natural or spontaneous fermentation (Pretorius I.S., 2000). Additionally, the wine industry's continued use of a small number of strains as commercial starter cultures is eroding microbial diversity (Aponte M, Blaiotta G., 2016).

The sensory and organoleptic characteristics of wines are heavily influenced by the microbial communities present during grape must

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fermentation. The inoculated yeast starters coexist during fermentation with surviving indigenous non-Saccharomyces and Saccharomyces yeast, fungi, and bacteria species, shaping the final sensory and organoleptic profile of the produced beverages, even in regular sulfur dioxide treated must (i.e., used to limit and/or kill the endogenous microbiota and as a protective antioxidant agent). The entire indigenous microbial community present in the must conducts the alcoholic fermentation in the case of spontaneous fermentation, a challenging and risky winemaking process with potentially unpredictable outputs. The study of indigenous microbial communities in grapes is a major research area in oenology due to its scientific and industrial importance (Eder M.L.R., Rosa A.L., 2019).

The aim of this study was to identify yeast strains from the indigenous yeast community present on grape berry surfaces, bunches and must. 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.

MATERIAL AND METHOD

Grape samples were collected from the viticultural center in Copou, part of the vine-growing district in Romania's eastern region, during harvest in September 2020. Aligoté, Chardonnay, and Golia were the grape varieties used to isolate yeast. Around 1–2 kg of grapes per variety were collected at random and aseptically in plastic autoclavable bags. Only healthy and undamaged grapes were harvested from the selected viticultural center and immediately transported to the microbiological laboratory in Iasi in cold boxes. Within 24 hours of the vineyard's harvest, samples were analyzed.

Reproducible methods were used to isolate and purify yeast strains from a variety of habitats, including the plant (berries and grape bunches), raw compounds (grape must), and intermediate products (must at various stages of alcoholic fermentation triggered by spontaneous yeast microflora).

100 berries were taken from these samples and placed in an Erlenmeyer flask containing sterile physiological saline solution. The grape bunches obtained after the berries were removed were treated exactly the same. The water resulted from the berry and bunch washing process were thus developed as two sources of yeast strain isolation.

Aliquots (0.1 ml each) of several dilutions (from 10^{-1} to 10^{-6}) were spread in triplicates on different nutritive media in order to isolate all yeast: glucose peptone agar (GPCA), yeast malt agar (YMA), and malt extract agar (MEA). After the incubation at 28°C for 3–5 days the colonies were counted out and differentiated on the basis of their morphology (size, shape, colors). Yeast isolates were then stored at -20°C for further analysis.

The grape must used in the fermentation tests had a sugar content of 240 g l^{-1} and was sterilized at 100°C for 10 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 liquid 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 for 9 yeast strains. Each strain was evaluated in three parallel fermentations.

RESULTS AND DISCUSSIONS

To conduct this research, yeasts were isolated from a variety of habitats, including berries, the bunch, and the musts of the grape varieties Aligoté, Chardonnay, and Golia grown in the Copou viticultural center (N-E Romania). From all of the grape samples, fifty-four yeast colonies were isolated and purified. Following the yeast isolation process, the colonies were examined visually and morphologically, and then the yeasts were examined microscopically. The yeasts were then subjected to microscopic measurements in order to determine the size of the yeasts in the selected strains by averaging the measurements.

Table 1 contains information on the characterization of yeast strains isolated from the indigenous flora of the Iasi vineyard, more specifically the strains' coded names, as well as aspects of the colour and shape of the colonies on nutritive media.

500 mL graduated cylinders were used to determine the degree of foaming of yeast strains, into which 300 mL of sterile grape must was introduced. A quantity of active inoculum was added to the sterile must distributed in graduated cylinders. The yeast cell density per ml for inoculation was the same ($1x10^6$ CFU/ml) The cylinders were kept in an incubator at 25°C after inoculation. For 72 hours, the fermentation process was monitored every 24 hours. Each strain was given a score based on the amount of foam it produced (in cm^3). Four strains (GC3, AC3, CHC3, and SCH) produced no foam. All the information about Foam capacity and appearance of produced foam are presented in *table 2* according to the recorded volume density to show the volume differences between strains with foaming capacity. Because no large amounts of foam were recorded, no foam spilled from the

graduated cylinders. The ability of yeast to foam during the fermentation process is not a desirable characteristic.

Table 1

Morphological characteristics of the isolated yeasts

Yeast strain code	Grape variety (source of isolation)	Yeast colony color	Macroscopic characteristics (colony)
GB3	Golia (grape berries)	white	circular form, flat profile, smooth and shiny surface, creamy texture
GC3	Golia (grape bunches)	white	circular form, flat profile, smooth surface, opaque, creamy texture
G4	Golia (grape must)	white	circular form, convex profile, smooth and shiny surface, creamy texture
AB3	Aligoté (grape berries)	creamy-white	circular form, flat profile, smooth and shiny surface, mucilaginous texture
AC3	Aligoté (grape bunches)	red	circular form, flat profile, smooth and shiny surface, mucilaginous texture
A2	Aligoté (grape must)	creamy-white	circular form, convex profile, smooth surface, opaque, creamy texture
SCZ	Aligoté (grape must)	creamy-white	circular form, flat profile, smooth and shiny surface, creamy texture
CHC3	Chardonnay (grape bunches)	orange	circular form, flat profile, smooth surface, opaque, creamy texture
SCH	Chardonnay (grape must)	gray-white	circular form, convex profile, smooth and shiny surface, creamy texture

Foam formation by yeast is a method of detecting poor and improper hygiene in the winemaking process, and the foam formed provides an ideal condition for the development of acetic bacteria.

Table 2

Foam capacity and appearance of produced foam

Yeast strain code	Foam volume (cm³/L)				Appearance of produced foam
	24h	48h	72h	80h	
GB3	-	2	5	8	Low-volume foam
GC3	-	1	1	1.5	No foam produced
G4	-	1	2.5	4.5	Low-volume foam
AB3	-	-	3	6	Compact foam, adhering to the walls
AC3	-	-	-	2	No foam produced
A2	-	1	4	7	Compact foam, adhering to the walls
SCZ	-	-	28.5	10	Spongy foam, decrease in volume after tumultuous stage
CHC3	-	1	1	1	No foam produced
SCH	-	-	-	1	No foam produced

Grape must that had previously been sterilised was used to determine the alcohol capacity. It was then divided into graduated cylinders, inoculated, and the alcoholic concentration (% vol. alcohol) was determined using densimetric methods after the fermentation stage was completed. The obtained wines were characterized by different alcohol content, amounting from 4.48 to 12.31% vol. The lowest amount of ethanol was observed in sets fermented with AC3 yeast strain code (*Rhodorula rubra*) (*Table 3*). Perhaps it was a result of too high sugars concentration in the fermentation medium.

Table 3

Alcohol capacity of the isolated yeasts

Yeast strain code	Alcohol content (% vol.)
GB3	**11.00**
GC3	10.06
G4	9.54
AB3	5.58
AC3	4.48
A2	7.23
SCZ	**11.04**
CHC3	**11.30**
SCH	**12.31**

These compounds are necessary for the proper functioning of microorganisms and the production of ethanol; however, their excess adversely effects on the cells (Cioch-Skoneczny *et al*, 2020).

This study confirms that yeasts with superior oenological characteristics can be isolated from vineyards and, after further studies, can eventually be used in winemaking to produce wines with distinct flavours.

CONCLUSIONS

Grape berries, bunches and must provide an ideal environment for a variety of yeast species. Understanding the impact of these microorganisms on wine quality begins with understanding the kinetics of their growth and metabolism.

From Copou viticultural centre in autumn 2020 were isolated in pure cultures through inoculums dissemination and loop exhaustion techniques on solid nutrient media nine yeast strains.

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

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 four are non-foaming.

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

After the testing procedures 4 yeasts strains (SCZ, SCH, CHC3 and GB3) were retained and could be used as future starters after further tests in large scale fermentations, in order to optimize the fermentation processes and to obtain quality wines from Copou viticultural area.

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