

## CHARACTERIZATION OF NEW YEAST STRAINS, ISOLATED FOR THE SELECTION OF THE MOST SUITABLE ONES FOR SPARKLING WINE

Alina MĂNTĂLUȚĂ<sup>\*1</sup>, D. COJOCARU<sup>2</sup>, Ancuța VASILE<sup>1</sup>, C. SAVIN<sup>1</sup>,  
Rodica PAȘA<sup>1</sup>

<sup>1</sup>Research and Development Station for Viticulture and Vinifications Iași

<sup>2</sup>Alexandru Ioan Cuza University, Iași

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**ABSTRACT** – This paper presents the provisional results of the studies on the selection of most suitable yeast strains in a lot of 84 strains from Iasi vineyard- Copou wine centre, in order to be used for sparkling wine production. For achieving this objective, we used at first an initial test for checking the fermentation features of yeast strains, with the following targets: foaming ability, evolution in time (triggering, finish) of fermentation process' stages. We selected 27 from the 84 yeast strains. In the second test for checking fermentation features, conducted in the laboratory and using fermentation tanks of 10 liters, we checked the selected strains' ability to stick or not to the walls of the fermentation tanks, the formation of granular or compact yeast deposits, the ability to completely ferment sugars from must and the property not to produce hydrogen sulphide. For this test we selected 14 strains from the 27 yeast strains, in particular: two strains MNF1 and MNC3 for producing the basic wine and 10 yeast strains MNF4, MNF8, MNF11, MNF9, MNC9, MNC12, MNC13, MNO4, MNO14 and MNO16 for the second fermentation in bottles.

**Key words:** Strains; Yeasts; Sparkling wine; Fermentations.

**REZUMAT - Caracterizarea sușelor noi de levuri, izolate pentru selecția celor performante în vederea utilizării la prepararea vinurilor spumante.** În lucrare sunt prezentate rezultatele preliminare ale cercetărilor privind selecția din lotul de 84 sușe de levuri, izolate din podgoria Iași – Centrul viticol Copou, a celor performante, în scopul utilizării în tehnologia de obținere a vinurilor spumante. Pentru realizarea acestui obiectiv, în prima etapă, s-a utilizat un test preliminar de verificare a caracteristicilor fermentative a sușelor de levuri, urmărindu-se: capacitatea de spumare, evoluția în timp (declanșare, finalizare) a etapelor proceselor fermentative. Din cele 84 sușe de levuri au fost selectate 27. În al doilea test de verificare a caracteristicilor fermentative, la nivel de laborator, folosind fermentatoare cu capacitatea de 10 litri, s-a urmărit proprietatea sușelor selectate de a adera sau nu de pereții fermentatoarelor, formarea depozitelor levuriene granulare sau compacte, capacitatea de a fermenta total

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\* E-mail: mantalutaa@yahoo.com

zaharurile din must și proprietatea de a nu produce hidrogen sulfurat. În acest test, din cele 27 sușe de levuri au fost selectate 14 sușe, și anume: două sușe MNF1 și MNC3 pentru obținerea vinului de bază și 10 sușe de levuri MNF4, MNF8, MNF11, MNF9, MNC9, MNC12, MNC13, MNO4, MNO14 și MNO16 pentru fermentația a doua în butelii.

## INTRODUCTION

The existence within a yeast species of many strains which are different from physiological point of view requires the selection of the ones that correspond to the objective aimed, in our case the strains suitable for sparkling wine.

Two categories of yeasts are involved in the technology of making sparkling wines, namely yeast strains with fermentation features for making the basic wine and the yeasts from the second fermentation in bottles, which have to provide alcohol fermentation at high pressure, at a temperature of 10 – 12°C, to form compounds which incorporate CO<sub>2</sub> into wine and to create granular yeast lees. These aspects determined specialists (Gaio, 1992; Ribereau - Gayon *et al.*, 1998; Lemasquier *et al.*, 1995; Polsinelli and Casalone, 2003) to develop in time the yeast selection criteria.

The first studies conducted in our country on basic wines for making sparkling wines were carried out by Teodorescu *et al.* (1978), Sandu-Ville *et al.* (1967), Matran (1968). The research activity was continued by approaching the issues of yeast strains used and the improvement of sparkling wine technology. We remind, therefore, the studies of

authors Babeș *et al.* (1983), Stoian *et al.* (1984), Roșu *et al.* (1997), Cotea (1997,1998), Kontec and Kontec (1976). Updated information on yeast strains used in the technologies of obtaining sparkling wines is presented in the papers of the authors Cotea (2005) and Țârdea *et al.* (2010).

The team of researchers from Research and Development Institute for Viticulture and Wine making) approached this research objective since 1967, by the studies carried out by Sandu-Ville *et al.* (1967), which were continued thus contributing to the development of the institute's yeast collection. The working protocol used in the preliminary selection test provided the suitable conditions for the fermentation properties of the 84 isolated yeast strains. The provisional selection test allows the removal of sparkling yeast strains and of those which trigger slow, insignificant fermentation processes.

The test of alcohol fermentation in fermentation tanks of 10 L allowed checking the reproducibility of parameters quantified in hours/days and characterizing the stages of alcoholic fermentation in the preliminary test, also recording new data on the quality of yeasts (adherence /non adherence to the glass walls of fermentation tanks, the type of lees).

Furthermore, the organoleptic and physical-chemical analyses on the wines obtained from this test enabled the selection of alcoholigenic yeast strains, which trigger fermentation of dry wine, that will be used as basic

wine in the technology of sparkling wine making, as well as the selection of yeast strains for the second fermentation in bottles.

### MATERIALS AND METHODS

In the preliminary test, for the selection of yeast strains we used mini-fermentation tanks of 1 L, into which was poured 0.75 L of grape must from the variety of which the yeast strains were isolated. An inoculum was prepared from each activated yeast strain and its number of cells/mL was measured to determine the volume of inoculum introduced so that the density of cells/mL should be of  $5 \cdot 10^6$  cells/mL in the grape must.

For the test using fermentation tanks of 10 L we followed the same procedure. After introducing the inoculum and the fitting of boiling tanks, the alcoholic fermentation process was monitored each day, recording, for the preliminary test, the level of foaming and the quantification of the fermentation process' stages (hours /days), and in the test performed with 10 L fermentation tanks we tracked the adherence or non-adherence to the walls of fermentation tanks, the type of yeast lees and the reproducibility of the fermentation process stages. At the end of the alcoholic fermentation, the wines were characterized from physical-chemical and organoleptic point of view, according to OIV methods.

### RESULTS AND DISCUSSION

In 2008-2009 study period, we isolated 84 yeast strains and in particular 33 from Feteasca regală vineyard, 29 strains from Cabernet Sauvignon vineyard and 22 strains from Muscat Ottonel vineyard, all

pertaining to Copou- Iași vine centre. In the preliminary test for detecting the fermentation characteristics of yeast strains, according to the foaming ability and the evolution in time (triggering, ending) of the fermentation process' stages, we selected 27 yeast strains, namely 12 from Fetească regală vineyard, eight strains from Cabernet Sauvignon vineyard and seven strains from Muscat Ottonel vineyard. The remaining strains checked, representing 66% of the total number, were removed, because they either triggered large amounts of foam, some of them even brimming over the fermentation recipients, or triggering the fermentation process after 2-3 days or, once activated it had a slow evolution, never finishing. The 27 yeasts were selected because they produced very few foam in the first 24-48 hours, five strains being even included in the non-foaming yeasts category, namely MNF4, MNF21, MNF25, MNC8 and MNO13. The evolution in time of the fermentation process' stages of the 27 yeast strains highlighted the fact that these ones triggered alcoholic fermentation under the best conditions for making high-quality wines.

The next step in the selection of potentially productive yeast strains was the laboratory test in 10 L fermentation tanks. The selection criteria in this test were: the strains' ability to stick or not to the walls of the fermentation tanks, the formation of granular or compact yeast deposits, the ability to completely ferment must sugars and the property not to produce hydrogen sulphide.

Table 1 - Characteristics of alcoholic fermentations of yeast strains (Fetească regală lot) selected in the preliminary test

Yeast strain code	Stages of the alcoholic fermentation process				Adherence + - Nonadherence	Yeast lees	Observations	Physical-chemical analyses of wine				Organoleptic analysis
	Prefermentati on stage (hours/days)	Tumultuous fermentation (days)	Slow fermentation (days)	End of fermentation (days)				H <sub>2</sub> S (quality)	Alcohol, vol. %	Total acidity, H <sub>2</sub> SO <sub>4</sub> g/L	Unfermented sugars, g/L	
MNF1	20 hours	8	4	12	+	compact	compact foam, very good clarification	-	12.6	4.2	-	fresh, very pleasant flavour, odour
MNF2	22 hours	8	4	12	-	powdery	compact foam, faulty clarification	H <sub>2</sub> S	10.5	4.4	27.3	H <sub>2</sub> S flavour and odour
MNF4	18 hours	6	5	11	+	granular	no foam, very good clarification	-	12.1	4.3	-	typical flavour, very pleasant, harmonious taste
MNF8	18 hours	7	5	12	+	granular	no foam, very good clarification	-	12.1	4.3	-	pleasant flavour, with slight aroma
MNF9	20 hours	7	4	12	+	compact	compact foam, very good clarification	-	12.1	4.2	-	fresh, very pleasant flavour, odour
MNF11	20 hours	7	4	12	+	granular	loose foam, good clarification	-	12.1	4.1	-	fresh, very pleasant flavour
MNF13	22 hours	8	5	13	-	floury	spongy foam, faulty clarification	H <sub>2</sub> S	11.0	4.4	19.0	very fresh, very pleasant flavour
MNF19	20 hours	6	5	11	-	granular	loose foam, average clarification	H <sub>2</sub> S	12.1	4.1	-	H <sub>2</sub> S flavour and odour
MNF20	22 hours	7	5	12	-	granular	spongy foam, very good clarification	-	12.1	4.3	-	very pleasant odour and flavour
MNF22	21 hours	8	5	13	-	compact	compact foam, good clarification	H <sub>2</sub> S	9.7	4.1	41.0	H <sub>2</sub> S flavour and odour
MNF23	20 hours	7	5	12	-	compact	no foam, good clarification	H <sub>2</sub> S	10.3	4.2	30.9	H <sub>2</sub> S flavour and odour
MNF24	18 hours	9	4	13	-	compact	loose foam, average clarification	H <sub>2</sub> S	10.1	4.4	34.2	H <sub>2</sub> S flavour and odour

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Table 2 - Characteristics of alcoholic fermentations of yeast strains (Muscat Ottonel lot) selected in the preliminary test

Yeast strain code	Stages of the alcoholic fermentation process					Adherence - +	Yeast lees	Observations	Physical-chemical analyses of wine				Organoleptic analysis
	Prefermentati on stage (hours/days)	Turbiduous fermentation (days)	Slow fermentation (days)	End of fermentation (days)	Nonadherence +				H <sub>2</sub> S (quality) %	Alcohol, vol. %	Total acidity, H <sub>2</sub> SO <sub>4</sub> g/L	Unfermented sugars, g/L	
BO3	24 hours	8	5	13	+	compact	loose foam, average clarification	H <sub>2</sub> S	9.2	4.0	31.3	pleasant, full, balanced flavour	
MNO2	24 hours	8	6	14	-	dusty	loose foam, faulty clarification	H <sub>2</sub> S	8.9	3.7	36.6	H <sub>2</sub> S flavour and odour	
MNO3	18 hours	8	5	13	+	floury	foam disappears after 5 days, average clarification	-	11.0	3.9	-	pleasant, fresh flavour	
MNO4	22 hours	7	5	12	+	compact	spongy foam, average clarification	-	11.1	4.0	-	fresh flavour, fruity odour	
MNO7	20 hours	7	5	12	+	compact	spongy foam, average clarification	H <sub>2</sub> S	9.0	4.1	35.0	fresh, pleasant flavour, odour	
MNO14	20 hours	7	5	12	+	granular	loose foam, good clarification	-	11.0	3.5	-	very pleasant, fruity, full flavour and odour	
MNO16	18 hours	7	5	12	+	granular	loose foam, very good clarification	-	11.0	3.4	-	fresh flavour, pleasant, harmonious odour	
MNO17	18 hours	7	7	14	+	compact	spongy foam, average clarification	-	11.0	3.7	-	pleasant, fresh flavour and odour	

Table 3 - Characteristics of alcoholic fermentations of yeast strains (Cabernet Sauvignon lot) selected in the preliminary test

Yeast strain code	Stages of the alcoholic fermentation process					Adherence - + Nonadherence	Yeast lees	Observations	Physical-chemical analyses of wine				
	Prefermentati on stage (hours/days)	Tumultuous fermentation (days)	Slow fermentation (days)	End of fermentation (days)					H <sub>2</sub> S (quality)	Alcohol, vol. %	Total acidity, H <sub>2</sub> SO <sub>4</sub> g/L	Unfermented sugars, g/L	Organoleptic analyses
MNC3	18 hours	7	5	12	+	floury, compressed	Loose foam, clarification from the 7 <sup>th</sup> day	-	12.0	4.8	-	very pleasant flavour, odour	
MNC5	18 hours	8	5	13	-	compact	compact foam, good clarification	H <sub>2</sub> S	10.1	4.5	32.1	H <sub>2</sub> S flavour and odour	
MNC7	18 hours	8	5	13	+	floury, compressed	loose foam, average clarification	-	12.0	4.6	-	pleasant, fresh flavour	
MNC9	18 hours	8	5	13	+	granular	no foam, fast clarification	-	12.0	4.4	-	harmonious, very pleasant flavour	
MNC12	23 hours	8	4	12	+	granular	spongy foam, very good clarification	-	12.0	4.5	-	very pleasant flavour, fresh odour	
MNC13	18 hours	7	6	12	+	granular	spongy foam, very good clarification	-	12.0	4.6	-	very pleasant, full flavour, odour	
MNC18	18 hours	7	6	13	-	floury	loose foam, very slow clarification	H <sub>2</sub> S	10.3	4.7	28.6	H <sub>2</sub> S flavour and odour	
MNC19	23 hours	5	7	12	+	granular	loose foam, very good clarification	-	12.0	4.4	-	harmonious, pleasant flavour and odour	

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The data collected in this test are showed in *Tables 1-3*.

In *Table 1* we illustrate the results achieved in the assay conducted on the 12 yeast strains, selected from Fetească regală vineyard. From this yeast lot we selected MNF1 strain for making the basic wine for sparkling wines, because of the high alcohol content which determines the alcoholic fermentation process and the production of a dry wine, very appreciated from organoleptic point of view. For the second fermentation in bottles, we selected strains MNF4, MNF8, MNF11 and MNF9, because the first three form granular lees and the last one because the yeast lees are compact, stable, and easily removable. Moreover, these strains do not stick to the walls of the fermenting tanks and produce dry wines, with no hydrogen sulphide odour, also having very good organoleptic features.

We removed six strains from this lot as they did not meet the selection criteria from the perspective of yeast lees' type, had stuck to the fermentation tanks' walls, and the wines made had dregs of unfermented sugars and hydrogen sulphide odour.

From the lot of eight strains selected in the preliminary test and isolated from the Cabernet Sauvignon vineyard (*Table 2*), only five yeast strains complied with the selection criteria for sparkling wines. The strain MNC3 was selected for making the basic wine for sparkling wines. This strain, thanks to its fermentation characteristics was included in the

category of productive yeasts for high quality dry wines.

For the second fermentation in bottles, we selected the yeast strains MNC9, MNC12 and MNC13, which, besides their alcoholigen character of complete sugar fermentation, do not stick to the glass walls of the fermentation tanks and form granular dregs.

In the lot of eight yeast strains isolated from Muscat Ottonel vineyard and tested in the laboratory in fermenting tanks of 10 litres, only the granular strains MNO4, MNO14 and MNO16 were noticed for their fermentation features. These strains did not stick to the glass walls of fermentation tanks and were alcoholigen causing fermentation to dry wines.

## CONCLUSIONS

Following the research activity on the selection of yeast strains suitable for sparkling wines we isolated 84 new yeast strains from Copou – Iași vine centre.

In the preliminary selection test, 66% of the strains checked in the alcoholic fermentation process were removed. The 27 yeast strains selected were assessed in the process of alcoholic fermentation in tanks of 10 L, analyzing the physical-chemical and organoleptic features of the wines obtained.

For this test we selected 14 yeast strains among which two strains with suitable features for producing the basic dry wines and 12 yeast strains

for testing the second fermentation in bottles.

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