THE IDENTIFICATION OF SOME YEAST STRAINS FROM VINEYARD IAȘI, ROMANIA

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Abstract

The aim of this study was to isolate and select yeast strains from the indigenous flora of Iasi vineyard. The experiment study included 4 local grape varieties: Busuioacă de Bohotin, Fetească Albă, Fetească Neagră and Fetească Neagră (from Collection of grape varieties) from viticultural center Copou. The experiment was carried out using randomized block design with three replications. The samples were taken in two different phenophases: bunch compaction and ripeness stage. As source of isolation was used the washing water from bunches and grapes. The yeast strains isolation and obtaining of the pure cultures was completed by successive replications using sowing on solid nutrient media technique. Therefore, 15 yeast strains were selected and morphological analyzed by colony shape, profile, structure and color.

Key words: isolation, yeast strains, vineyard Iasi

Nowadays, obtaining alcoholic beverages on a large scale as well as improving their quality represents a continuous concern. Considered as essential, the alcoholic fermentation of the must is the basic link of wine-making technology.

The alcoholic fermentation process is dependent on the quality of the yeast microflora.

Yeasts are unicellular fungi, found both in soil and on plants (leaves, grapes) where they can reach because of various factors (raindrops, insects, etc.). The diversity of yeasts found on leaves, grapes and soil is the main factor which is influencing the wine organoleptic characteristics and quality. (Clemente-Jimenez *et. al*., 2004).

Oenological indigenous yeast strains with particular characteristics are representative for a certain vineyard and their presence can increase the wines typicality (Barrera Cardenas S.M., 2011).

MATERIAL AND METHOD

The aim of this study was the isolation and selection of new yeast strains from the indigenous flora correlated with the pedoclimatic conditions of the vineyard Iasi.

The experiment study included 4 local grape varieties: Busuioacă de Bohotin, Fetească Albă, Fetească Neagră and Fetească Neagră (from Collection of grape varieties) from viticultural center Copou. The samples were coded as follows: B.B., F.A., F.N. and F.N.c.

The sampling process took place in summer 2021 from vineyard Copou, in two different phenophases: bunch compaction and ripeness stage. For this experiment was used only the undamaged and healthy beans. For each grape variety the samples were collected in aseptic plastic bags and transported to the microbiological laboratory of Iasi University of Life Sciences.

Figure 1 **Isolation source – washing water grapes**

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Figure 2 **Isolation source – washing water clusters**

The samples were prepared aseptically with dilution technique (10^{-1} – 10^{-6}) in duplicates using two distinct solid nutrient media:
-Glucose peptone chloramphenicol agar (GPCA) containing: 2% yeast extract, 5% peptone, 1% potassium phosphate, 0,05% chloramphenicol, 20% dextrose, 15% agar
-Malt extract agar (MEA) containing: 20% glucose, 1% peptone, 20% malt extract, 20% agar.

The Petri plates were incubated at 28°C for 4 days and after this period the colonies were counted and morphologically examined. The yeast strains isolation and obtaining of the pure cultures was completed by successive replications using sowing on solid nutrient media technique.

The colonies were examined in base of microbial load using a semi-automatic colony counter.

RESULTS AND DISCUSSION

Quantitative examination results

The microbial load of the studied colony is shown in *table 1*. Regarding the yeast load after 72h of incubation, the data results did not show significant differences between the samples seeded on two solid nutrient media: GPCA and MEA.

Regarding the phenophases the data showed increased values of levurian load after 72h of incubation, for the samples collected at ripeness stage compared to the bunch compaction phenophase. The values increased in all four varietes of grapes.

Table 1

Microbial load of the studied colony

Source of isolation	Dilution	Bunch compaction		Ripeness stage	
		CFU/ml		CFU/ml	
		GPCA	MEA	GPCA	MEA
F.N.c	10^{-3}	100	108	230	185
	10^{-4}	54	56	95	80
	10^{-5}	25	19	58	45
	10^{-6}	10	12	43	32
F.N.	10^{-3}	90	100	150	160
	10^{-4}	49	45	75	60
	10^{-5}	22	31	40	52
	10^{-6}	13	20	25	30
F.A.	10^{-3}	95	98	200	140
	10^{-4}	45	32	90	75
	10^{-5}	25	26	42	36
	10^{-6}	13	6	25	34
B.B.	10^{-3}	89	95	194	151
	10^{-4}	56	47	96	94
	10^{-5}	29	31	45	25
	10^{-6}	18	9	34	21

Furthermore, the data showed increased values for F.N.c. (Fetească Neagră colecție) on both nutrient media 100 CFU for GPCA and 108 CFU for MEA, for bunch compaction, and 230 CFU and 185 CFU in case of ripeness stage.

Qualitative examination results

Therefore, 15 yeast strains were selected and were characterized micro- and macroscopic. The yeast stains were morphological analyzed by colony shape, profile, structure and color (Table 2).

(Table 2)

Colony characteristics

Yeast strain code	Grape varieties	Colony characteristics	Yeast colony color
A4P	Fetească Albă	convex profile, shiny surface, circular form	white-yellow
A5C	Fetească Albă	convex profile, shiny surface, circular form	red
A4C	Fetească Albă	convex profile, shiny surface, circular form	gray-white
A5P	Fetească Albă	convex profile, opaque surface, circular form	white-yellow
A3C	Fetească Albă	convex profile, opaque surface, circular form	white
A3P	Fetească Albă	convex profile, opaque surface, circular form	yellowish
N4P	Fetească Neagră	convex profile, shiny surface, circular form	white
N3P	Fetească Neagră	convex profile, opaque surface, circular form	gray
N3C	Fetească Neagră	convex profile, opaque surface, circular form	gray-white
C4P	Fetească Neagră colecție	convex profile, shiny surface, circular form	white
C3C	Fetească Neagră colecție	convex profile, opaque surface, circular form	white
C5P	Fetească Neagră colecție	convex profile, opaque surface, circular form	white
B3P	Busuioacă de Bohotin	convex profile, shiny surface, circular form	red
B4C	Busuioacă de Bohotin	convex profile, opaque surface, circular form	Gray
B5P	Busuioacă de Bohotin	convex profile, opaque surface, circular form	white

Analyzing the source of isolation, it is noticed that most yeast strains were found on the grape samples of Fetească Albă variety (six strains), the other grape varieties showed each three indigenous yeast strains.

Regarding the colony characteristics, all 15 yeast strains presented convex profile and circular form. Also 6 yeast strains were caracterized by shiny surface and 9 yeast strains opaque surface.
The yeast colonies presented various colours, from white to gray and yellow, and 2 strains showed red color.

Acording to the results obtained in this study, the white grape varieties can offer an advantage in the indigenous yeast distribution and diversity. Clearly, the most important thing is to explore and preserve the biodiversity of oenological indigenous yeast strains in vineyard.

Based on these results, future experiments will be performed in order to select the most valuable yeast strains in terms of fermentation characteristics.

CONCLUSIONS

Various yeast strains were isolated from indigenous flora of Iasi vineyard and analyzed quantitatively and qualitatively (morphological characteristics). After the isolation in pure culture, 15 yeast strains were selected and will be analyzed on matter of fermentation characteristics.

Concerning yeast load, Fetească Neagră Colecție variety presented the highest values of CFU both on GPCA and MEA solid nutrient media.

Based on morphological characteristics, the yeast strains analyzed showed an important diversity in terms of the colony color, structure and surface.

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