

CONTRIBUTIONS REGARDING SOME SOIL YEAST STRAINS FROM VINEYARD IAȘI, ROMANIA

Costinela PATROLEA (ATODIRESEI)¹, Florin-Daniel LIPȘA¹, Eugen ULEA¹

e-mail: costinela.patrolea@gmail.com

Abstract

The aim of this research was to isolating and selection soil yeast strains from the indigenous flora of Iasi vineyard. In the research activity, were chosen four grape varieties Fetească Neagră, Fetească Albă, Busuioacă de Bohotin and Fetească Neagră from the ampelographic collection of "Vasile Adamachi" Farm, from viticultural center Copou. The experimental protocol provided for the sampling of soil samples in two different phenophases of plant development: the end of flowering and ripeness stage. The soil samples were taken at 3 depths: 5-10 cm, 10-15 cm, 15-20 cm. Isolation of yeast strains and obtaining pure cultures was accomplished by successive replicates using the GPCA solid medium seeding technique. Thus, 18 yeast strains were selected from the soil and analyzed morphologically regarding the shape, profile, color and surface of the colony.

Key words: isolation, soil yeast strains, vineyard Iași

The soil microbiota exhibits fluctuations directly proportional to climate changes and the dynamics of vine phenophases.

The analysis of the phenological stages of grapevine is also of particular importance in viticulture technology as it provides information on the application of treatments to combat diseases and pests, the application of the fertilization and also the optimal execution of the work required in the grapevine.

Yeasts are defined as unicellular fungi belonging to the suborders Ascomycotina and Deuteromycotina that reproduce mainly vegetatively by budding or fusion (Kurtzman C.P. and Fell J.W., 1998). Currently there are approximately 1500 yeast species identified and their size can vary depending on the species.

From an oenological point of view, non-sporogenic yeasts such as *Kloeckera*, *Candida*, *Torulospora*, *Kluyveromyces* start and lead the process of alcoholic fermentation until close to the alcoholic threshold of 5-7% vol. alc., at which point *Saccharomyces* species become dominant, thus taking over directing the fermentation. (Fleet G.H., Heard G.M., 1993; Romancino D. *et al*, 2008; Sabate J. *et al*, 1998; Demuyter C. *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 research was to isolating and selection soil yeast strains from the indigenous flora correlated with the pedoclimatic conditions of the vineyard Iasi.

Were chosen four grape varieties Fetească Neagră, Fetească Albă, Busuioacă de Bohotin and Fetească Neagră from the ampelographic collection of "Vasile Adamachi" Farm, from viticultural center Copou.

The sampling process was carried out in the summer of 2021 and 2022 from vineyard Copou, in two different phenophases: the end of flowering and ripeness stage.

For each sampling depth for each variety, 3 partial samples of the same weight (approximately 100g) were made. The sampling points of the partial samples were made by sampling by the zig-zag method. The partial samples were mixed to obtain the average sample. The soil sampling scheme excluded marginal areas of plots/rows.

In order to inoculate, was used the method of successive dilutions. Seedings were performed using dilutions diluțiile 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ in various replicates. It was used as a solid culture medium: Glucose peptone chloramphenicol agar (GPCA) containing: 2% yeast extract, 5% peptone, 1% potassium phosphate, 0,05% chloramphenicol, 20% dextrose, 15% agar.

¹ Iasi University of Life Sciences, Romania

The Petri plates were incubated at 28°C for 4 days and after this period the number of colony-forming units (CFU) was calculated by the quantification method. The method involves the actual counting of the colonies developed on the Petri plates counted and morphologically examined

RESULTS AND DISCUSSIONS

In *figure 1* is presented the yeast load in the soil at the end of flowering and at full maturity in the two years of the study.

Comparing the samples from the two phenophases of sampling, a constant increase in the number of colonies in the superficial layer of the soil (5-10 cm deep) can be observed up to a maximum value reached at the ripeness stage of

the grapes. This trend was evident both in the first year of study and in the second year.

Regarding the yeast load of the lower layers of the soil, a constancy of the number of CFU between the end of flowering and the full maturity of the grapes was highlighted.

In the phenophase of ripeness in the superficial layers of the soil, the maximum UFC threshold was reached by the Fetească Neagră Colecția variety, with an average of approx. 16.5×10^6 UFC/g soil, closely followed by the Fetească Neagră variety with an average of 15×10^6 UFC/g soil. The Fetească Albă and Busuioacă de Bohotin varieties at ripeness grapes stage recorded an average of 14.5×10^6 CFU/g soil in the superficial layer.

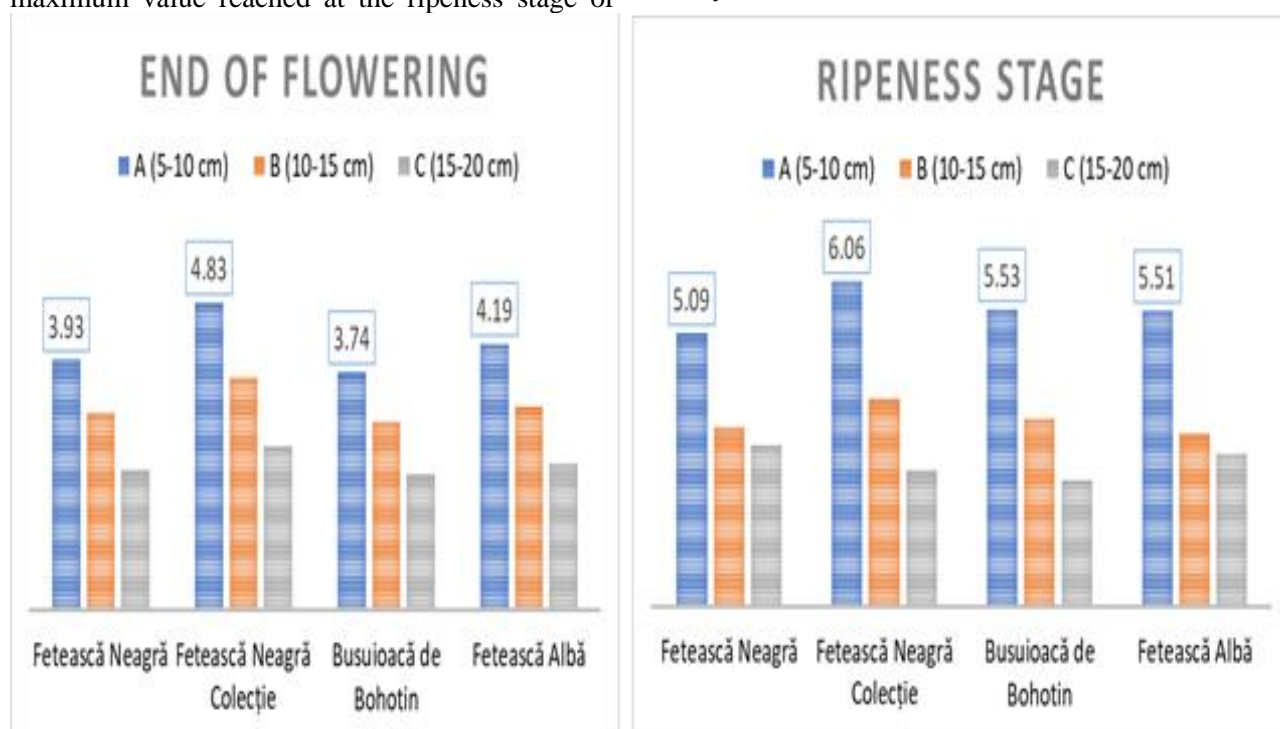


Figure 1 Microbial load of the studied colony

Therefore, 15 yeast strains colonies were morphological analyzed, establishing the specific characteristics (shape, profile, surface and color). The colonies of the isolated strains showed convex

and flat profile, shiny, opaque surface and in some cases mucoid appearance, colors ranging from white to gray and red (*tabel 1*).

Table 1

Colony characteristics

Yeast strain code	Isolation source	Colony form	Colony profile	Colony surface	Colony color
A5	Fetească Albă	circular	convex	opaque	white
B6	Busuioacă de Bohotin	circular	convex	opaque	white
N7	Fetească Neagră	circular	convex	opaque-mucoid	gray-white
C8	Fetească Neagră Colecție	circular	convex	opaque-mucoid	white
B9	Busuioacă de Bohotin	circular	convex	shiny	white
B10	Busuioacă de Bohotin	circular	convex	opaque-mucoid	white
B11	Busuioacă de Bohotin	circular	convex	opaque-mucoid	white
B12	Busuioacă de Bohotin	circular	convex	opaque	gray-white
C14	Fetească Neagră Colecție	circular	convex	opaque	white
C15	Fetească Neagră Colecție	circular	convex	opaque-mucoid	yellowish
C20	Fetească Neagră Colecție	circular	convex	shiny	gray-white

N21	Fetească Neagră	circular	convex	opaque	white
A22	Fetească Albă	circular	convex	opaque	white
A23	Fetească Albă	circular	convex	opaque	white
A24	Fetească Albă	circular	convex	opaque	white
C29	Fetească Neagră Colecție	circular	convex	shiny	red
A32	Fetească Albă	circular	flat	opaque	white-light pink
B34	Busuioacă de Bohotin	circular	convex	shiny	white

Thus 4 yeast strains presented shiny surface, 9 yeast strains opaque surface and 5 yeast strains opaque-mucoid surface.

Analyzing the yeast strains according to the sampling depth, the yeasts taken from the depth of 5-10 cm showed white-gray coloration, variable surface from shiny to opaque and convex profile.

In the case of the strains taken from the lower depths of the soil, respectively 10-15 cm and 15-20 cm, their morphological characteristics showed circular shape, white color and opaque-mucoid surface.

According to the presented results, it can be concluded that the yeast microbiota in the superficial layers of the soil shows high diversity compared to the lower layers.

CONCLUSIONS

Various strains of yeast were isolated from the soil of the Iași vineyard and analyzed quantitatively and morphologically. Following successive replications and obtaining pure cultures, 18 strains were isolated that presented complex morphological characteristics.

In the vertical division of the yeasts in the soil, it is found that the number of microorganisms shows a downward slope proportional to the sampling depth, thus it can be deduced that the constant decrease in the activity of the microbiota in the lower layers is characterized by the lack of oxygen, the decrease in the amount of nutrients.

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

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