

Isolation of indigenous yeast strains from the Purcari and Trifești wine centers in the Republic of Moldova and evaluation of their impact on the quality of dry red wines

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Abstract. In the conducted research, 30 yeast strains from red grape varieties were isolated from the Purcari wine center, and 28 yeast strains from red grape varieties were isolated from the Trifești wine center in the Republic of Moldova. Morphological and cultural analysis revealed their diversity, confirming that all strains belong to the class *Ascomycotina*. Nucleotide sequencing comparisons were made with sequences deposited in the NCBI genetic bank (ncbi.nlm.nih.gov), and the yeasts were identified using the FT-IR method. Based on the biochemical and technological indices of the isolated yeast strains, the following strains were selected: from the Purcari wine center – No.21 – C-S-120-P-2, No.24 – R-NNP-2, No.29 – R-N-120-P-4, No.30 – R-N-120-P-5; from the Trifești wine center – No.27 – MTr-4, No.32 – M100Tr-1, No.35 – M100Tr-4, No.41 – C-S60Tr-2, No.43 – C-S60Tr-4. In micro-vinification conditions, it was established that the use of selected yeast strains from the Purcari wine center (No.30 – R-N-120-P-5) and Trifești wine center (No.32 – M100Tr-1, No.41 – C-S60Tr-2) allows the production of high-quality dry red wines, both by physicochemical indices and organoleptic ratings, and does not compromise the quality of wines made with imported Dry Active Yeasts. The selected indigenous yeast strains were deposited in the National Collection of Non-Pathogenic Microorganisms, and storage certificates and passports were obtained for each yeast strain, with the identification code assigned by the National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Technical University of Moldova.

1. Introduction

Currently, the isolation and selection of indigenous yeast strains with necessary fermentative activity for producing quality wines are of particular importance in the field of biotechnology and wine microbiology. This is mandated by the need for effective preservation, expansion, and fortification of the indigenous microbial gene pool specific to the wine industry, by evaluating and systematizing the morphological, cultural, and physiological-biochemical indices of selected indigenous strains.

Indigenous yeasts play a significant role in shaping the typicity and authenticity of wines in various wine centers around the world. For example, in France, in the Bordeaux region, indigenous yeasts contribute to the formation of the complex aromatic profile of Cabernet Sauvignon, Merlot, and other typical varieties of the region. In the Champagne region, indigenous yeasts are essential for secondary fermentation in bottles, which gives sparkling wines their

distinct aroma and fine bubbles. In Italy, in the Tuscany region, indigenous yeasts contribute to the formation of the unique characteristics of Chianti Classico and Brunello di Montalcino wines, reflecting the specific terroir of these areas. [1-6].

In the production of red wines, indigenous yeast strains are particularly valued not only for their fermentative capacity but also for their ability to modulate phenolic extraction, color intensity, and tannin structure—key factors in determining the final sensory profile and aging potential of the wine. During fermentation, the metabolic activity of *Saccharomyces cerevisiae* indigenous strains can influence the concentration of anthocyanins and tannins through the production of specific enzymes and metabolites [7, 8].

Several studies have demonstrated that indigenous yeasts contribute to the release of glycosylated aroma precursors and to the softening of astringency via

enzymatic activities such as β -glucosidase and protease [9,10].

This highlights the potential of selected local yeast populations to enhance the quality and typicality of dry red wines, making their isolation and characterization a crucial step for valorizing the enological biodiversity of specific wine-growing regions such as Purcari and Trifești in the Republic of Moldova.

2. Materials and methods

The research was carried out in the Laboratory of Biotechnology and Wine Microbiology at the Scientific-Practical Institute of Horticulture and Food Technologies (Republic of Moldova).

2.1. Microvinification assays

The technological operations for the production of dry red wines—crushing, sulfiting, must maceration-fermentation, wine collection by fractions, and post-fermentation—were carried out under the conditions of the microvinification unit of the Scientific-Practical Institute of Horticulture and Food Technologies. Fermentation was conducted in 10-liter tanks under specific conditions to ensure proper alcoholic fermentation. The fermentation-maceration processes were conducted following classical technology under controlled conditions. The fermentation temperature was maintained at approximately 28–30 °C.

2.2. Microbiological study

The microbiological study was carried out in accordance with specialized bibliographic sources [11, 12].

2.3. Identification of yeast strains isolated from the wine centers 'Purcari' and 'Trifești' using the PCR method

The research was carried out in the "Biotechnologies" Laboratory of the Agricultural Biotechnology Research Institute in Moscow (Russia) and in the Department of Immunology and Histocompatibility, Faculty of Medicine, University of Thessaly, CeMIA SA Company, Larissa (Greece).

For PCR amplification, the following primers were used:

ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3').

The amplification parameters were as follows: denaturation at 94°C for 1.5 minutes, annealing at 55°C for 1.5 minutes, extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes.

Species identification was performed by comparing the obtained nucleotide sequences with the data available in the NCBI genetic database (ncbi.nlm.nih.gov).

2.4. The taxonomic identification of yeast strains isolated from the 'Purcari' and 'Trifești' wine centers using FT-IR spectroscopy

The taxonomic identification of the studied yeast strains through FT-IR was conducted at the "Wine Microbiology" laboratory of the University of Geisenheim (Germany).

Preventively, the yeast strains were checked for purity by inoculating them on solid agar media, YGCB-agar and YGC-agar.

After verifying the purity of the yeast strains, each strain was taken from the Petri dish, dissolved in 0.1 ml of distilled water, and transferred to the cells of the plate (ZnSe).

The plate was then placed in a thermostat for 1 hour at 45°C. After drying the samples, the plate was introduced into the spectrometer, and the analysis program (OpusLab) was initiated.

2.5. Chemical analyses

A chemical analysis was performed to assess various parameters. The total sugars content (g/L) in the musts was determined using the areometric method according to SM GOST 27198-87 [13].

The mass concentration of non-fermented sugars (g/L) in wines was determined by the indirect titration method specified in SM GOST 13192-73 [14].

The concentration of ethanol (% vol.) was determined by distillation following SM GOST 51653:2010 [15].

Volatile acidity was measured by titration of the volatile acids separated from the wine through steam distillation and titration of the distillate, as per SM GOST 51654:2012 [16].

The total acidity was determined by titration with bromthymol blue as an indicator, in accordance with SM GOST 51621:2008 [17]. All determinations were performed in triplicate.

Determination of pH value and redox potential using the potentiometric method with the Mettler Toledo MA 235 ionometer.

Glycerol was determined using the multifunctional spectrophotometer "Bacchus 3" (France).

2.6. Sensory analysis

A sensory analysis was conducted with a panel of expert enologists in accordance with institutional regulations for tasting alcoholic beverages, using a rating scale from 0 to 10, to evaluate wines produced using *Saccharomyces* and non-*Saccharomyces* yeasts at the Scientific-Practical Institute of Horticulture and Food Technologies. The evaluation included assessments of color, taste, aroma, and typicality of the wines.

2.7. Statistical analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) to assess differences in physicochemical parameters. The analyses were performed using GraphPad Prism 5.0 software and verified with an online calculator available at [http://math.semestr.ru/\[18\]](http://math.semestr.ru/[18]).

3. Results and Discussion

3.1. Isolation and identification

To carry out microorganism identification studies, it is necessary to obtain a pure culture derived from a single cell.

The specificity of yeast strain isolation and cultivation depends on their presence in various environments (grapevine, must, wine, soil, etc.) and on the composition of the nutrient media [19, 20].

Grape samples for yeast strain isolation were collected from the vineyards of ÎM "Vinăria Purcari" SRL and "Vierul-Vin" SRL during the winemaking season.

From the vineyards of ÎM "Vinăria Purcari" SRL, grapes were collected and the following musts were obtained: Rară Neagră variety with a sugar concentration of 207 g/dm³ and titratable acidity of 5.7 g/dm³; Cabernet-Sauvignon variety with a sugar concentration of 220 g/dm³ and titratable acidity of 6.2 g/dm³.

From the vineyards of "Vierul-Vin" SRL, grapes were collected and the following musts were obtained: Merlot with a sugar concentration of 230 g/dm³ and titratable acidity of 5.4 g/dm³; Cabernet-Sauvignon with a sugar concentration of 220 g/dm³ and titratable acidity of 6.4 g/dm³.

Yeast cell isolation was carried out starting from a single cell using the method of successive dilutions and pure culture isolation by the streak plate technique.

From the class *Ascomycotina*, 30 strains were isolated from the 'Purcari' wine center and 28 strains from the 'Trifești' wine center.

To obtain pure yeast cultures and reveal qualitative properties, the studied samples were subjected to sub-culturing using the "exhausted loop" method (Figure 1).

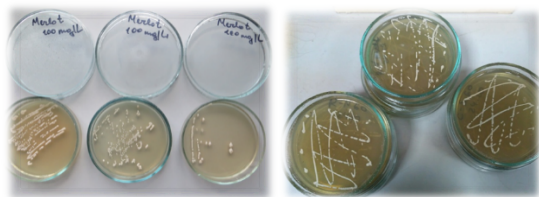


Figure 1. Cultural characteristics of yeast colonies in seeding using the exhausted loop method.

In order to determine the purity of the isolated yeasts, microscopy of the cultures was performed. For this, the yeast strains were incubated for 3 days on nutrient medium (grape must) at a temperature of 28°C.

Microscopic examination of the yeast strains allowed for visual evaluation and preliminary determination of morphological characteristics, such as: size, shape, cell grouping, and homogeneity (Figure 2).

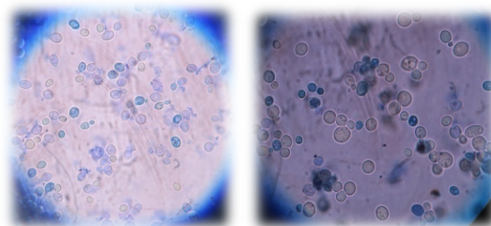


Figure 2. Microscopy of yeast strains isolated from unsulfited Cabernet-Sauvignon must, "Vinăria Purcari", vintage 2016 (examples).

Following the identification of taxonomic affiliation through PCR methods and FT-IR spectroscopy, 11 *S. cerevisiae* strains, 3 *S. pastorianus* strains, and 1 *S. bayanus* strain were identified from the Purcari wine center, and 17 *S. cerevisiae* strains, 6 *S. pastorianus* strains, and 1 *S. bayanus* strain were identified from the Trifești wine center.

The yeast strains isolated from the 'Purcari' and 'Trifești' wine centers for the production of dry red wines were tested in order to identify those strains that allow the production of wines with typical characteristics.

In the first stage, to select the yeast strains, the following technological parameters were analyzed: alcohol tolerance, SO₂ resistance, copper resistance, cold resistance, foaming ability, β-glucosidase activity, and Killer factor [11, 12].

In the qualitative characteristics assessment test, carried out under laboratory conditions, selective culture media were used to determine the ability of the selected yeast strains to produce hydrogen sulfide, acetic acid [11, 12].

As a result of the conducted research, 4 yeast strains with advanced technological properties were selected from the 'Purcari' wine center - No.21 – C-S-120-P-2, No.24 – R-NNP-2, No.29 – R-N-120-P-4, No.30 – R-N-120-P-5 and 5 yeast strains from the 'Trifești' wine center - No.27 – MTr-4, No.32 – M100Tr-1, No.35 – M100Tr-4, No.41 – C-S60Tr-2, No.43 – C-S60Tr-4.

3.2. The Study of the Influence of Different Yeast Strains Selected from the 'Purcari' Wine Center on the Quality of Dry Red Wines

For the comparative evaluation of the yeast strains: No. 21 – C-S-120-P-2, No. 24 – R-NNP-2, No. 29 – R-N-120-P-4, and No. 30 – R-N-120-P-5, selected from the 'Purcari' wine center for red wine production, they were used for the fermentation of grape must from the Cabernet-Sauvignon variety, harvested from the vineyards of ÎM "Vinăria Purcari" SRL, in comparison with the reference strain Oenoferm Be-Red for dry red wines (Germany).

The initial physicochemical characteristics of the grape must are presented in Table 1.

Table 1. Physicochemical characteristics of the grape must used for the comparative evaluation of different yeast strains (vintage 2017)

No	Grape variety	Total sugars content, g/L	Total acidity, g/L	pH	Potential OR, mV
1	Cabernet-Sauvignon	225,0±0,5	7,8±0,1	3,22±0,09	212,3±0,5

The fermentative activity of the yeast strains and their influence on the physicochemical and organoleptic parameters of the Cabernet-Sauvignon dry red wines were monitored.

The dry red wines obtained through fermentation with different yeast strains from the 'Purcari' wine center were subjected to physicochemical analyses, the results of which are presented in Table 2.

Thus, it can be mentioned that the dry red wines fermented with the use of selected yeast strains are characterized by a high ethyl alcohol concentration of 13.5% vol.

The concentration of total acidity in the wines obtained under microvinification conditions varies insignificantly within the range of 7.4–7.5 g/L. The concentration of volatile acidity in the dry red wines obtained varies between 0.46 and 0.52 g/L

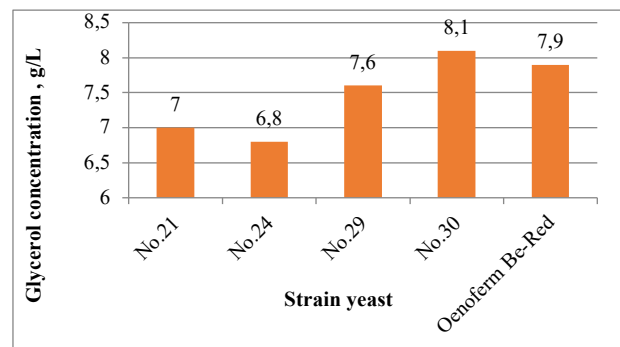
The pH index value variation in the obtained wines is within a narrow range, between 3.25 and 3.28, depending on the strain used.

Table 2. Physicochemical parameters of Cabernet-Sauvignon dry red wines obtained with different selected yeast strains (vintage 2017)

No	Strain yeast	Alcohol, % vol.	Total acidity, g/L	Volatile acidity, g/L CH ₃ COOH	pH	Potential OR, mV	Non-fermented sugars, g/L	Organoleptic assessment, 0-10
1	Oenoferm Be-Red (control)	13,5±0,1	7,4±0,2	0,46±0,03	3,28±0,09	211,1±1,1	3,2±0,1	7,95
2	Nr.21 - C-S-120-P-2	13,5±0,1	7,4±0,1	0,52±0,02	3,28±0,11	211,1±0,9	3,1±0,1	7,80
3	Nr.24 - R-NNP-2	13,4±0,1	7,5±0,1	0,46±0,03	3,25±0,08	212,0±0,8	4,2±0,1	7,95
4	Nr.29 - R-N-120-P-4	13,5±0,1	7,4±0,2	0,52±0,03	3,25±0,09	212,0±1,1	3,1±0,1	7,85
5	Nr.30 - R-N-120-P-5	13,5±0,1	7,4±0,2	0,46±0,03	3,28±0,05	211,1±1,2	1,2±0,1	8,00

Strict adherence to technological processes in grape processing allowed the production of experimental wines with low redox potential, ranging from 211.1 to 212.0 mV.

The Non-fermented sugars values in the dry wines do not exceed the permissible limit of 4 g/L, which is characteristic for this category of wines, with the exception of the red wine fermented using yeast strain No. 24 - R-NNP-2, where the mass concentration of Non-fermented sugars is 4.2 g/L.

**Figure 3.** Glycerol concentration in dry red wines obtained with different selected yeast strains.

The results regarding the glycerol content in the wines obtained with the use of selected yeast strains from the 'Purcari' wine center are presented in Figure 3. Thus, all the studied wines contain approximately 7 g/L of glycerol, with the highest value determined in the sample fermented with yeast strain No. 30 R-N-120-P-5, which is 8.1 g/L. The lowest glycerol values were determined in the dry red wines obtained with yeast strains No. 21 - C-S-120-P-2 and No. 29 - R-N-120-P-4 (Figure 3).

Therefore, we can observe a significant influence of the yeast strains on the glycerol content in the obtained wines.

These results clearly highlight the significant influence of the yeast strains on the glycerol content in dry red wines, emphasizing the importance of careful strain selection in the winemaking process to obtain the desired organoleptic characteristics [21].

In order to determine the specific aromas in red wines, characteristic of each selected yeast strain from the 'Purcari' wine center, an organoleptic evaluation of the obtained wines was carried out.

The experimental dry red wines were characterized by rich aromas of red fruits such as blackberries and black cherries. The dry red wines fermented with yeast strains No.24 – R-NNP-2, No.30 – R-N-120-P-5, and Oenoferm Be-Red stood out. The organoleptic analysis of the dry red wines obtained under microvinification conditions demonstrated that the quality of the wine samples was high, with the wine fermented using yeast strain No.30 – R-N-120-P-5 receiving the highest score of 8.0 points (out of a maximum of 10).

Thus, the research conducted under microvinification conditions during the 2017 vintage campaign demonstrated that the use of selected yeast strain No.30 – R-N-120-P-5 from the 'Purcari' wine center allows for the production of high-quality dry red wines, both in terms of physicochemical parameters and organoleptic evaluation, comparable to the quality of wines obtained using imported commercial active dry yeasts.

3.3. The Study of the Influence of Different Yeast Strains Selected from the 'Trifești' Wine Center on the Quality of Dry Red Wines

For the comparative evaluation of yeast strains No.27 – MTr-4, No.32 – M100Tr-1, No.35 – M100Tr-4, No.41 – C-S60Tr-2, and No.43 – C-S60Tr-4, selected from the 'Trifești' wine center for the production of dry red wines, these strains were used for the fermentation of Cabernet-Sauvignon must, harvested from the vineyards of SRL "Vierul-Vin", in comparison with the control strain Oenoferm Be-Red (Germany) used for dry red wines. The initial physicochemical characteristics of the grape must are presented in Table 3.

Table 3. Physicochemical characteristics of the grape must used for the comparative evaluation of different yeast strains (vintage 2018)

Nr	Grape variety	Total sugars content, g/L	Total acidity, g/L	pH	Potential OR, mV
1	Cabernet-Sauvignon	255,0±0,5	5,9±0,1	3,22±0,01	215,4±0,4

The fermentative activity of the yeast strains and their influence on the physicochemical and organoleptic parameters of the Cabernet-Sauvignon dry red wines were monitored. After the completion of alcoholic fermentation, dry white and red wines, obtained using different yeast strains selected from the 'Trifești' wine center, were subjected to physicochemical analyses, and the results are presented in Table 4.

Thus, it can be noted that the dry red wines fermented with the use of selected yeast strains are characterized by a high ethyl alcohol concentration of 15.0% vol.

Table 4. Physicochemical parameters of Cabernet-Sauvignon dry red wines obtained with different selected yeast strains (vintage 2018)

Nr	Strain yeast	Alcohol % vol.	Total acidity, g/L	Volatile acidity, g/L CH ₃ COOH	pH	Potential OR, mV	Non-fermented sugars, g/L	Organoleptic assessment, 0-10
1	Oenoferm Be-Red (control)	14,8±0,1	5,4±0,2	0,46±0,03	3,28±0,03	214,8±0,6	3,5±0,1	8,0
2	Nr.27 MTr-4	14,8±0,1	5,4±0,1	0,52±0,02	3,28±0,02	214,0±0,2	3,0±0,1	8,0
3	Nr.32 M100Tr-1	15,0±0,1	5,5±0,1	0,46±0,03	3,25±0,03	214,0±0,4	2,2±0,1	8,0
4	Nr.35 M100Tr-4	14,9±0,1	5,4±0,2	0,52±0,03	3,25±0,04	215,2±0,8	2,5±0,1	7,9
5	Nr.41 C-S60Tr-2	15,0±0,1	5,4±0,2	0,46±0,03	3,28±0,04	214,8±0,9	2,1±0,1	8,1
6	Nr.43 C-S60Tr-4	14,9±0,1	5,4±0,1	0,52±0,02	3,28±0,01	214,8±0,8	3,1±0,1	8,0

The total acidity in the wines obtained under microvinification conditions varies insignificantly within the limits of 5.4-5.5 g/L.

The volatile acidity in all the wines obtained varies between 0.46-0.52 g/L, which is typical for red wines.

The pH values in the control wine samples and those obtained using the selected yeast strains are virtually identical, ranging between 3.25-3.28.

Strict adherence to technological procedures in the processing of grapes led to the production of dry red wines with a low oxidative-reductive potential, ranging from 214.8 to 215.2 mV.

The residual sugar values in the obtained wines do not exceed the permissible limit of 4 g/dm³, which is characteristic for this category of wines.

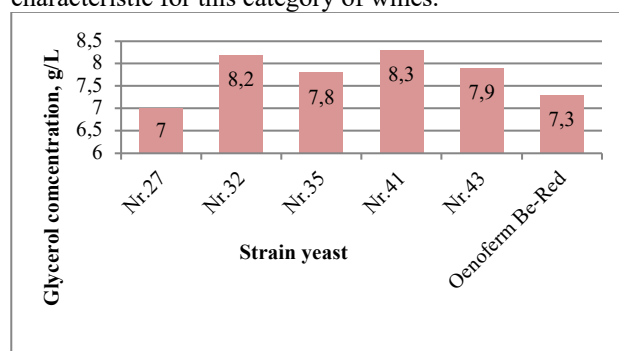


Figure 4. Mass concentration of glycerol in dry red wines obtained with different selected yeast strains.

An important component of dry wines is glycerol, and according to the data presented in Figure 4, all the wines studied have a glycerol concentration higher than 7 g/L. The highest values were determined in the samples fermented with yeast strains Nr.32 - M100Tr-1 and Nr.41 - C-S60Tr-2, ranging from 8.2 to 8.3 g/L.

The lowest glycerol values were determined in the dry red wines obtained using yeast strains Nr.27 - MTr-4 and Oenoferm Be-Red. Thus, we can conclude that the influence of selected yeast strains on the formation of glycerol in dry white and red wines is significant. In order to determine the specific aromas in dry red wines, characteristic of each selected yeast strain from the 'Trifești' wine center, an organoleptic evaluation of the obtained wines was carried out.

The dry red wines were characterized by rich aromas of red fruits, such as currants, blackberries, and black cherries. The wines fermented with yeast strains Nr.32 - M100Tr-1, Nr.41 - C-S60Tr-2, and Oenoferm Be-Red stood out. The taste was characterized by dark fruits, including plums and black strawberries, while aromas of cocoa, spices, and sometimes dark chocolate contributed to the complexity of the obtained wines. The organoleptic analysis of the dry red wines obtained under microvinification conditions showed that the quality of the wine samples is high, with the wine fermented with yeast strain Nr.41 - C-S60Tr-2 being rated the highest, with 8.1 points.

Thus, the research conducted under microvinification conditions during the 2018 wine campaign demonstrated that the use of yeast strains selected from the 'Trifești' wine center, Nr.32 - M100Tr-1 and Nr.41 - C-S60Tr-2, allows for the production of high-quality dry red wines, both in terms of physicochemical indices and organoleptic

evaluation. These wines do not fall short of the quality of those obtained using imported commercial yeasts.

4. Conclusions

It was established that the use of selected yeast strains from the wine centers of 'Purcari' (No. 30 – R-N-120-P-5) and 'Trifești' (No. 32 – M100Tr-1 and No. 41 – C-S60Tr-2) enables the production of high-quality dry red wines, both in terms of physicochemical parameters and organoleptic scores, which are comparable to the quality of wines produced using imported active dry yeasts (ADY).

It was scientifically proven that the physicochemical composition and overall quality of red dry wines largely depend on the yeast strain used during the fermentation process of the must.

It was demonstrated that all experimental wines exhibited high concentrations of glycerol (greater than 7 g/dm³), with the highest values being determined in samples fermented with strains No. 30 – R-N-120-P-5 (Purcari wine center), No. 32 – M100Tr-1, and No. 41 – C-S60Tr-2 (Trifești wine center). Thus, it can be concluded that the influence of yeast strains on glycerol formation in dry red wines is significant.

The selected indigenous yeast strains were deposited in the National Collection of Non-Pathogenic Microorganisms (CNMN) of the Institute of Microbiology and Biotechnology (IMB), which assigned the following codes: No. 30 – R-N-120-P-5 → *S. cerevisiae* CNMN-Y-31, No. 15 – S75Tr-4.4 → *S. cerevisiae* CNMN-Y-34, No. 32 – M100Tr-1 → *S. cerevisiae* CNMN-Y-36, No. 41 – C-S60Tr-2 → *S. cerevisiae* CNMN-Y-37.

Deposit certificates and passports were issued for each yeast strain, bearing the assigned CNMN codes, by the National Collection of Non-Pathogenic Microorganisms of IMB.

The selected yeast strains have been registered in the global NCBI database (National Center for Biotechnology Information, USA, Maryland).

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