THE PRELIMINARY SELECTION OF ISOLATED YEAST STRAINS FROM THE INDIGENOUS FLORA OF IASI VINEYARD

SELECȚIA PRELIMINARĂ A UNOR SUȘE DE LEVURI IZOLATE DIN FLORA INDIGENĂ A PODGORIEI IAȘI

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Abstract: *Knowing the importance of the yeasts with specific fermentation properties, capable to contribute to the creation of some quality wines, the isolation and selection activity is a must. It is well known that most of the wines, typically characterised, are achieved when the used yeasts are isolated and selected form the wine microbiota of the vineyard. Between 2005 and 2006 a number of 86 yeast strains was isolated in pure culture from wine-growing plantations of Fetească albă, Sauvignon blanc and Chardonnay, in the vineyard of Iasi – wine/growing centre in Copou. In order to make the selection of the biological material, from the point of view of the fermentation characteristics, the yeast strains isolated from the indigenous flora of the vineyard were preliminarily tested in laboratory in two phases, by monitoring the main parameters of the alcoholic fermentation process as well as the physical – chemical and organoleptical characteristics of wines. The data analysis shows that only the 20 % from the tested yeast strains can be considered as high-performance ones, given the stable characters when realising the fermentation processes and the quality of the achieved wines.*

Key words: yeasts, isolation, Iasi, microbiota, organoleptical characteristics

Rezumat: *Cunoscând importanța levurilor cu proprietăți fermentative specifice, capabile să contribuie la realizarea unor vinuri de calitate, activitatea de izolare şi selecție se impune ca o necesitate. Este ştiut faptul că cele mai bune vinuri, caracterizate prin tipicitate se obțin atunci când levurile utilizate sunt izolate şi selecționate din microbiota vinicolă a podgoriei respective. În perioada 2005 – 2006 s-au izolat în cultură pură din plantațiile viticole de Fetească albă, Sauvignon blanc şi Chardonnay din Podgoria Iaşi - centrul viticol Copou un număr de 86 de suşe de levuri. Pentru a realiza selecția materialului biologic, din punct de vedere al caracteristicilor fermentative, suşele izolate din flora indigenă a podgoriei au fost testate preliminar la nivel de laborator în două etape, monitorizându-se principalii parametrii ai procesului de fermentație alcoolică precum şi caracteristicile fizico-chimice şi organoleptice ale vinurilor. Din analiza datelor obținute reiese că doar 20 % din suşele testate pot fi considerate performante, datorită caracterelor stabile în realizarea proceselor de fermentație şi a calității vinurilor obținute.*

Cuvinte cheie: levuri, izolare, Iasi, microbiota, caracteristici organoleptice

INTRODUCTION

The wine was obtained a long time from the spontaneous fermentation of the must sugars by the yeasts present on the grapes without the deliberate inoculation at the beginning of the process (Di Maro Elena et co., 2007). The spontaneous alcoholic fermentation of the grape must is a complex process in which a great number of genders and species of yeast take part. During the first 2-4 days of the alcoholic fermentation process the apiculate yeasts are active, with a low tolerance against the ethanol concentrations, which partially contribute to the wine character (Efstratios Nikolaou et co., 2006). Once the ethanol concentration increases, the species from the gender *Saccharomyces* become dominant, affecting thus the development of the non – *Saccharomyces* species and they finish the must fermentation (Fleet G.H., 1999).

The species from the *Saccharomyces* gender selected as starter are preferable, as they have a good adaptation to the conditions of the wine-production microarea, and they can easily dominate the spontaneous flora. Moreover, these yeasts can assure the conservation of the typical sensitive properties and the characteristic profile of the wine in each area.

Therefore, the variability, the adaptation and the wide spreading of the yeasts in various biotopes allow the isolation of new yeast strains with properties which may influence the fermentation processes. From this point of view the activity of isolation and selection of the yeasts from the wine microbiota of the vineyards is a necessity in the activity of research and production.

MATERIAL AND METHOD

The isolation of the yeast strains from the Feteasca alba, Sauvignon blanc and Chardonnay varieties was made after the harvesting of the berries and rahis from five grapes, separately, which were introduced in jars with sterile serum. The wash waters were collected after shaking. Also, from the rest of the samples of aseptically harvested grapes the musts were obtained, distributed in sterile bottles. One bottle of must without sulphur was kept for each variety. The rest of the musts were distributed into sterile bottles and sulphited with doses 50, 75, 100, 150 and 200 mg/L SO2. From the waters resulted after the washing of the berries, of the rahis and also from the unsulphited or sulphited musts with doses between 50/200 mg/L SO2 the yeast strains isolation was performed by using the Domercq and Lindner methods (Anghel I, et co., 1991). The preliminary selection, from the point of view of the fermentation characteristics, of the isolated yeast strains was made by using glass bottles of 1000 mL where 750 mL of sterilized grape must were introduced. After the introduction of the inoculation from each yeast sources and the attachment of the boilers, the mini-fermentation containers were incubated at 20-22°C. From this moment daily observations were made monitoring the foam degree and the duration of the alcoholic fermentation phases. The yeast strains selected during the preliminary test were then verified in 10 L fermentation containers, checking the reproductibility of the fermentation characteristics.

RESULTS AND DISCUSSIONS

During the study period 2005-2006 from the vineyard of Iasi – Copou vineyard, 86 yeast strains were isolated, that is: 24 yeast strains from the

plantation of Feteasca albă, 33 yeast strains from the plantation of Sauvignon blanc and 26 yeast strains from the plantation of Chardonnay.

According to the isolation sources (the water after washing the grape berry, the water after washing the steam, the unsulphited musts and the sulphited musts), the percentual representation of the isolated sources (fig.1) shows that the lowest percentage is represented by the yeasts isolated from the washing waters, that is 16%. The percentage of isolated yeasts grew to 27% and to 56% in the case of the unsulphited and sulphited musts, respectively, incubated at 20-22°C.

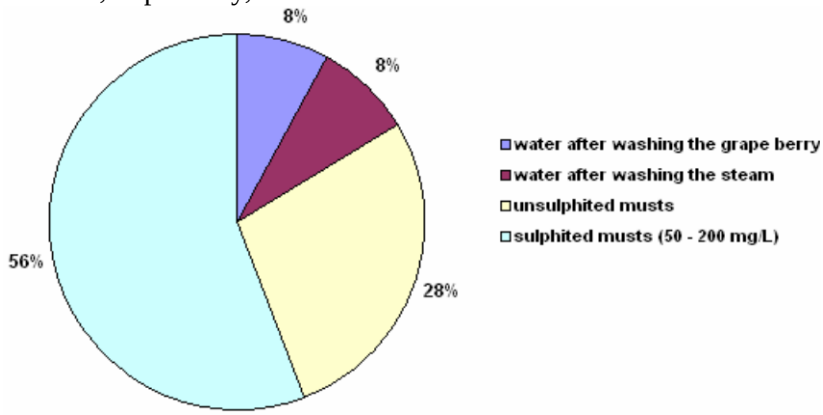

Fig. 1. Yeast strains isolated in the indigenous flora of Iasi vineyard between 2005 – 2006.

In the isolation activity the influence of the SO_2 doses was underlined, used for the treatment of the must, most of the sources being isolated during the alcoholic fermentation process in concentrations between 50-100 mg SO_2/L, the number of isolated yeasts decreases while the SO_2 doses increases.

In order to make the selection of the biologic material, from the point o view of the fermentation characteristics, the yeast strains were preliminarily tested in two phases. In the first phase, the 86 yeast strains were verified during the alcoholic fermentation process, monitoring the foaming degree, and the time intervals (hours/days) of the pre-fermentation, tumultuous fermentation and post-fermentation (calm fermentation) phases. From the analysis of the data obtained from the 86 yeast strains of isolated, 64% were eliminated. This percentage includes the yeast strains with abundant foam, with late start and finishing of the alcoholic fermentation processes or did not start the fermentation process.

Thus, in the preliminary test 31 yeast strains were selected: 11 yeast strains isolated in the plantation of Feteasca alba, 9 yeast strains isolated in the plantation of Sauvignon blanc and 11 yeast strains isolated in the plantation of Chardonnay. In continuation, the yeast strains isolated were studied in the second phase of the selection, verifying the reproducibility of the fermentation characteristics on the musts obtained from these varieties. In order to verify the fermentation capacity of the 11 yeast strains isolated from the plantation of Feteasca alba, fresh must was used with a sugar concentration of 198 g/L and the total acidity of 4,1 g/L H_2SO_4. The data obtained are presented in table I. By analysing the achieved results, they showed that foaming, also

monitored in the preliminary selection phase, remained at the same parameters for each selected yeast strains.

Table 1

Preliminary testing in the alcoholic fermentation of the yeast strains isolates on the plantation of Feteasca alba

Code of the yeast strains	Pre-fermentation phase (hours)	End of fermentation process (days)	Adherence – Non-adherence +	Alcohol Vol. %	Rest of sugar g/l	H_2S producers (qualitatively)
MNF1	20	14	+	11,6	-	-
MNF4	23	14	-	11,0	8	H_2S
MNF5	20	12	+	11,6	-	-
MNF6	18	12	+	11,6	-	-
F2(50)	23	11	-	11,5	2,5	H_2S
F1(75)	18	12	+	11,6	-	-
F1(100)	18	13	+	11,4	4,2	-
F2(100)	22	15	+	11,5	2	-
F3(100)	21	13	-	11,6	-	H_2S
F1(150)	20	12	-	10,2	24	-
F1(200)	18	13	+	11,6	-	-

From this point of view the investigations must continue for all the yeast strains selected in order to find the high-performance yeast strains. But, if we take into consideration the rapidity of growth, evaluated in hours/days at the beginning of the pre/fermentation phase and termination of the alcoholic fermentation, the sources MNF5, MNF6, F2(50), F1(75), F1(100) and F1(200) made themselves noticed. They started the alcoholic fermentation 18-21 hours after the inoculation and finished the fermentation between 11-13 days. It was also seen that they form compact, dense, stabile sedimentations, which is an important property in the case of exploitation at industrial level. The MNF1 and F2(100) yeast strains, although they finish the alcoholic fermentation process in a longer time (14 – 15 days), they will be studied together with the above mentioned at pilot level, because they are quality compatible, they have the right foaming degree, the right alcohol concentration (11.5 – 11.6%), and the correspondent organoleptical characteristics of the obtained wines. From the total of 11 yeast strains, four were excluded: MNF4, F2(50), F3 (100) and F1(150), because during processes they stick to the walls of the fermentation containers, and the obtained wine are not organoleptical right, emanating a smell of sulphured hydrogen.

The yeast strains isolates form the plantation of Sauvignon blanc, selected during the preliminary test, were submitted to the second selection phase, using fresh must from the grapes of the same variety. The sugar concentration and the total must acidity was of 230 g/L and 5.2 g/L H_2SO_4, respectively.

The analysis of the results mentioned in table 2 shows that the yeast strains selected in the preliminary test does not modify the foaming capacity and the duration of the alcoholic fermentation processes. Regarding the multiplication rapidity, 7 yeast strains started the alcoholic fermentation process 18-20 hours from the inoculation, and they finished fermentation after 11-13 days. When analyzed from the point of view of the clearing process, the yeast MNS9, S1(75), S3(75) and S4(200) did not

prove to be favourable, being evaluated as average and reduced, being also characterized by dusty, detachable sedimentations.

Table 2

Preliminary testing in the alcoholic fermentation of the yeast strains isolates on the plantation of Sauvignon blanc

Code of the yeast strains	Pre-fermentation phase (hours)	End of fermentation process (days)	Adherence – Non-adherence +	Alcohol Vol. %	Rest of sugar g/l	H_2S producers (qualitatively)
MNS6	19	13	+	13,5	-	-
MNS9	20	13	+	12,9	10	H_2S
S1(75)	20	12	+	13,5	-	H_2S
S3(75)	23	12	-	12,8	12	H_2S
S4(75)	22	12	+	11,6	32	-
S5(75)	20	14	+	13,3	8	-
S6(75)	18	13	+	13,5	-	-
S3(150)	20	11	+	13,5	-	-
S4(200)	20	14	-	12,4	19	H_2S

The yeast strains MNS6, S1(75), S5(75) and S3(150) made wines with an alcoholic concentration of 13.5% vol. of alcohol, and the yeast MNS9, S3(75), S4(200) made wines with the alcoholic concentration between 12.4 – 12.9% vol. alcohol. One syeast strains, S4(75), finished the fermentation with 11.6% volume of alcohol. The selection of the potentially high-performance yeast strains from this lot was possible after the organoleptical analysis. Thus, the yeast MNS9, S1(75), S3(75) and S4(200), although they presented advantageous characteristics from the point of view of the non-adherence on the walls of the fermentation containers and of the wines alcoholic concentration, were not selected because they produce sulphured hydrogen. From the 9 yeast selected in the preliminary test, the following are still of interest: MNS6, S5(75), S6(75) and S3(150). Moreover, the yeast S4(75) deserves attention when testing at pilot level, as the wine produced by it has a rest of sugar, and this aspect may be exploited at industrial level in order to obtain semi-sweet wines without interrupting the fermentation with SO_2. The verification of the fermentation capacity of the 11 yeast strains of potential high-performance, isolated from the plantation of Chardonnay and selected during the preliminary test, was also made on must from the same variety with a sugar concentration of 235 g/L and a total acidity of 4.9 g/L H_2SO_4 (table 3).

The yeast sources selected during the preliminary test and verified during the alcoholic fermentation process in 10 L fermentation containers do not modify their foaming capacity, being it a stable feature which has already evolved within the known parameters. Eight sources from this lot started the alcoholic fermentation process after 18-22 hours, and three of them after 23 hours, C3(50), C4(50) and C2(50) respectively, the finalization times of the fermentation were constant. After the organoleptical analysis, the sulphured hydrogen-producing sources were eliminated, that is MNC1, MNC7, C1(50), C3(50) and C2(100). Given the stable and reproductive properties, five sources were selected: BC1, MNC2, MNC4, C1(100) and C4(100).

Table 3

Preliminary testing in the alcoholic fermentation of the yeast strains isolates on the plantation of Chardonnay

Code of the yeast strains	Pre-fermentation phase (hours)	End of fermentation process (days)	Adherence – Non-adherence +	Alcohol Vol. %	Rest of sugar g/l	H_2S producers (qualitatively)
BC1	22	12	+	13,8	-	-
MNC1	20	14	-	12,2	27	H_2S
MNC2	18	13	+	13,8	-	-
MNC4	20	11	+	13,6	3,8	-
MNC7	22	14	-	12,8	17	H_2S
C1(50)	20	14	-	13,8	-	H_2S
C3(50)	24	14	-	11,3	42	H_2S
C4(50)	23	12	-	11,0	48	H_2S
C1(100)	21	13	+	13,8	-	-
C2(100)	23	11	+	12,9	15	H_2S
C4(100)	20	13	+	13,5	5	-

From the overall data analysis of the 31 yeast strains, 17 can be considered to be high-performance sources due to the stable features during the fermentation process and the achievement of quality wines.

CONCLUSIONS

1. The waters from the washing of the berries, of the steam, and the must resulted from the processing of the grapes belonging to the varieties of Feteasca alba, Sauvignon blanc and Chardonnay, were the bases from where the 86 yeast strains were isolated. The 14 yeast strains were isolated from the berries and steam washing waters, 24 from the fresh must without composition correction, and 48 yeast from the must treated with variable doses of SO_2 (50 – 200 mg/L).

2. From the analysis of the data collected in the preliminary test, 31 yeast strains were select and 64% of the 86 yeast strains isolated being eliminated.

3. After the verification of the fermentation characteristics (laboratory phase) of the 31 yeast strains isolated from the vineyard with Feteasca alba, Sauvignon blanc and Chardonnay, in the 2nd phase only 17 sources were selected, which can be considered as high-performance ones due to the stable features in the fermentation processes and the achievement of quality wines

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