

YEAST POPULATION DYNAMICS IN SPONTANEOUS FERMENTATIONS

Daniela Ciupeanu-Calugaru

University of Craiova, Faculty of Horticulture, Craiova, Romania, alfdanaciupi@yahoo.com

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Abstract. The research has been made during 2001-2003 and followed the assimilation degree of the carbon and nitrogen sources by isolated yeasts from the winegrowing center of the Didactical Station Banu Maracine, of the University of Craiova. There has been made 61 physiological and biochemical tests. In this study there have been used 17 carbohydrates, 2 polysaccharides, 2 glycosides, 10 alcohols, 5 organic acids and 5 nitrogen sources. In the assimilation tests there has been used 1123 strains of isolated yeasts from the spontaneous flora, belonging to the winegrowing area Banu Maracine, purified and maintained in collection.

INTRODUCTION

Vinification modern technology use the must inoculation with commercial yeasts in order to obtain the desired parameters such as the maximum fermentation ratio, the control of the sanitary practices and wines characteristic, a.s.o. (Fleet and Heard, 1993). Among the controversy aspects to the use of selected yeast in vinification is that there can be reduce and even lost the production of some metabolic compounds offered only by the fermentation with yeast from spontaneous flora (Mateo *et al.*, 1991).

In spontaneous fermentation, the participation level to the fermentation of the non-*Saccharomyces* yeast from the genres *Candida*, *Pichia*, *Rhodotorula*, *Kluyveromyces*, *Kloeckera* and *Hansenula* (Fleet and Heard, 1993) depend on the pedoclimatic conditions of the winegrowing area, on the utilized practice in the vinification process and on the wines characteristics which are to be obtained (Poulard, 1984), the number and the structure of these yeast populations being a characteristic of the winegrowing area. In the fermentation first stage when the ethylic alcohol concentration is low the non-*Saccharomyces* yeast have a maximum developing (105-108 cells/ml) and led the entire fermentative process producing modification in the wine chemical composition. With the increases of the alcoholic concentration, these one give up their places to the yeasts that belong to the *Saccharomyces* yeasts more tolerant to alcohol and more competitive at sugars high concentration (Querol *et al.*, 1990). In the winegrowing center of the Didactical Station Banu Maracine, of the University of Craiova, the fermentation technological processes utilize only yeast from spontaneous flora, being an ideal area in order to study the yeast ecology. The purpose of the activities presented in this paper has been to analyze the presence and the evolution of the yeast conveyor of this area on a period of 3 years (2000-2003) and the dynamic of these yeast during the fermentation.

MATERIAL AND METHOD

The identification of the yeast cells has been made from the must isolated samples, must during the alcoholic fermentation and wines from the winegrowing center Banu Maracine, the purified yeast biocultures being submitted to identification standard tests (Barnett, J. A., R. W. Payne, D. Yarrow, 1990): morphological tests (the cells shape and dimension, colonies characteristic, behaviour in liquid medium forma) and physiological tests (the sugars fermentation and assimilation, the nitrates assimilation, the use of ethylic alcohol as the only nitrogen source, the arbutine scission, growth in the absence of vitamins, growth in the presence ciclohexamide a.s.o. For the experiences there has been used must from white grapes Riesling italian, Sauvignon and varieties of black grapes Merlot, Cabernet Sauvignon. At each experiment there has been followed the alcoholic fermentation duration, determining the sugar quantity, the number of the yeast cells, function the temperature of the must, the endogenous ethylic alcohol and the ethylic alcohol formed during the fermentation and of the fermentation moment.

In order to identify the isolated yeasts and purified take it in to study there has been used the procedures describe by Kreger van Rij, Barnet et al. și Kurtzman. For the physiological investigation there has been prepared liquid medium YNB (yeast-nitrogen-base) containing 1% different carbohydrates as the only source of nitrogen used by yeast through assimilation.

The fermentative features has been determined using the Durham tubes. The results has been recorded at 3, 7, 14, 21 and 28 days. As nitrogen assimilated sources has been investigated diverse substances: nitrates, nitrits, L-lizine, ethylamine, cadaverine and creatine. The reserache that followed the assimilation degree of the carbon and nitrogen sources has been organized for 61 physiological and biochemical tests.

In this study there has been used 17 carbohydrates, 2 polisacharides, 2 glicosides, 10 alcohols, 5 organic acides and 5 nitrogen sources. Simultaneous, there has been organized and followed 4 additional tests: the DBB test, producing of the extracellular amidonates compounds, the urease test for determining the hydrolyse activity and the test for determining the producing of the acides from glucose. In the assimilation tests there has been used 1123 yeast strains isolated from the spontaneous flora belonging to the Banu Maracine winegrowing area, purified and maintained in collection. The purpose of this set of physiological tests has to generate preliminary informations for the systematically determination of the isolated species, correlated with the morphological features previous analyzed. In the 3 years of researche, the initial sugar of the musts has varied between 170-240 gL⁻¹, the samples being collected in each phase of the fermentation: the begining phase (density 1,070-1,100 gL⁻¹), the middle phase (density 1,020-1,040 gL⁻¹) and final phase (density 1,020-1,040 gL⁻¹). The initial concentrations of sulph dioxide has ranged between 50-280 mgL⁻¹, and the fermentative processes doesn't evolve at controled temperatures. The final contain in ethylic alcohol has varied between 12,6- 14,3 % v/v.

RESULTS AND DISCUSSIONS

The soil represent a main compound of the biotope, which through the ecological specific factors (soil temperature, soil humidity etc) and the paedological determinants (humus contain, texture, porosity, soil pH etc) influence, like a host area, the development of the microbians populations.

The brown-redish luvisc soil have the highest weight, being spread on the plane terrain or in the area slightly negative of the S.D. Banu Măracine (Giugea N., Olteanu I., 2001). Due to the accumulation and stagnation of a certain humidity quantity on a long period of time, the alteration processes and eluviation are somehow accentuated. This is the reason for the eliberation of colloidal silica, deposited on the surface of the structural aggregates from the first horizon, inculcating a grey colour.

From the hole soil profile, from the microbiological point of view, present interest, the *Ap* horizon, this one being the main habitat of the yeast after the cropping period.

The dust fraction is of 21,8 % in the surface horizon *Ap*, granting a density of 1,36 g/cm³ and a porosity of 49 %. The hydrophysics index are well correlated with the granulometric composition of the soil and with the contain of organic materia (table 1). Thus the hygroscopicity coefficient has lower value in the surface (5,84 %) where the clay recorde the lowest percentage. The humidity equivalent follow in the same direction the variation of the granulometric fraction percentage being of 24,17 % in the *Ap* horizon, having an average capacity of useful water, which maintain at relative constant values on the entire profile, oscilating between 15 and 16%.

Table 1. The hydrophysics index of the brown-redish luvisc soil of the S.D. Banu Măracine

Horizon	Depth (cm)	CH (%)	CO (%)	EU (%)	CU (%)
Ap	0-19	5,84	8,76	24,17	15,41
Ao	19-38	7,14	10,71	25,71	15,00
AB	38-55	8,56	12,84	29,12	16,28
Bt ₁	55-118	10,11	15,16	30,65	15,49
Bt ₂	Sub 118	8,31	12,46	28,49	16,03

The brown redish luvisc soil is average to low provided with humus (table 2), in the first horizon the percentage of humus being of 2,76 %.

The variation of the nitrogen is the same with the one of the humus, recording a higher value in the surface horizon (0,171 %). It can be notice the very high contain of phosphorus and mobile potassium in the first horizon (80 respectively 320 ppm). The soil reaction is lightly acid on all the thickness of the profile, the pH value growing easily from 6 to 6,7. The hidrolytic acidity has low values (below 4 me/100 g soil), well correlated with the lightly acid reaction. The summ of the changeble base from the soil (SB) and the total capacity of change cationic (T) have medium to low value, and from the base saturation degree point of view, the soil frame at moderate mesobasic.

The temperature and the humidity of the environment, also the rainfall constitute essential features of the climate in a certain viticultural area, with decisive impact on the microorganisms biological, physiological and biochemical processes, with implication on their distribution on the viticultural area.

The granulometric structure of the surface horizon, where there are spreaded the great majority of the winemaking yeasts spores, indicate a weak oxygenation of the soil due to the fine and coarse sand barrier which obstruct the penetration of the air. Also the annual temperature in a continous growing which are accumulated at this levell of first horizon have great implication in the rapide evaporation of the water, offering an drastic habitat for the microorganisms.

For the data base used in the statistically analysis, we have selected only the climatic data with maximum values, the use of the average and temperature degree sums may introduce annual errors in the calculus and results.

In order to obtain correct correlation between the depending factors (the number of yeasts strain isolated) and the independent factors (temperature and air humidity), all the data has been percentage expressed.

For the collecting of the soil sample meant for isolate the yeasts strain from the winemaking area Banu Măracine has been established 25 collecting stations, placed also at the superior quota as well at the slopes base on which is placed the vineyard. Unhomogeneity (the spreading degree) of the experimental data has been verified using the variation analyze and the estimation of the differences between the isolated species has been verified calculating the transgression probabilities, obtaining $p=0,05516\%$ for all the research years; we conclude that the results of the statistical analyze are significant for $t= 3,5984$ and 21 liberty degrees.

Table 2. Chemical structure of the brown-redish luvisc soil of the S.D. Banu Măracine

Horizon	Hum. (%)	N.total (%)	P	K	pH (H ₂ O)	Ah	SB	T	V (%)
			ppm			me/100 g sol			
Ap 0-19	2,76	0,171	80	320	6,05	4,75	14,6	19,42	75
Ao 19-38	1,46	0,093	16	85	6,20	3,62	15,8	19,42	81
AB 38-55	1,20	0,093	10	66	6,30	3,15	17,2	20,35	85
Bt ₁ 55-118	0,84	0,067	4,5	85	6,40	3,10	17,8	20,90	85
Bt ₂ sub 118	0,42	0,041	3,8	59	6,70	2,80	19,1	21,90	87

There has been calculated the coefficients for simple correlation between the distribution frequency of the yeasts strain isolated from the viticultural soil and from the grapes during March-October and the rough values of the monthly temperature and humidity, in all the 3 years of research 2001-2003. Their values are presented in the table 3.

For the mathematical modelling of the entire experiment, has been tested more types of curves: linear, exponential, logarithmic, sigmoid, square and cubic. The curve type that represented most accurate the experiment has been the second degree function, with the interaction of the independent factors, choosing the function has been made through calculus and choosing the regression degree with the highest value (closer to the value 1).

In the table 4 there are presented the regression functions characteristic for each isolated species, where "x" represent absolutely all the values of the air temperature during 2001-2003 and "y" represent absolutely all the values of the air humidity during this period. The fifth term of the equation represent the interaction of the two independent factors and give the cumulated mathematical value of their influence on the distribution of the yeast species in the viticultural ecotope Banu Măracine. We ascertain that all the results of the statistical analysis and mathematical modelling are valid only for the three years research period and can not be extended outside the limits 2001-2003. If the experiment will be continued, in the case that the two components of the researched ecosystem, the vineyard (biocenosis component) and the viticultural soil (viticultural biotope component) will not

suffere major modifications, it can be accumulated an solide data base through their mathematical modelling, the microbiologists and oenologists can find out daily the frequency of any winemaking yeast species, based only on knowing the daily values of the air temperature and humidity (we remind the national value which had the mathematical modelling program gor the potato crop -COF- during 1980).

The regression ecuations has been valued in 23 graphics, where has been represented the evolution curves of the sporogenous speciess monthly evolution frequency and nonsporogenous and the calculated evolution (mathematically modelled) of these frequency during the three years of research.

The frequency of the *Schizosaccharomyces pombe* species had a decrease of 0,02 % in 2002 comparatively with 2001. The influence of the air humidity on the species frequency has been unsignificantly on all the research period, of only 2,40%. The low levell of the rainfall during the winter months can explain these low effects on the species. On the other hand, the continous increase of the temperature has positively influenced the evolution of this species spreading in the viticultural ecotope in a 78,68% proportion. The rest of the influences of aproximatively 19% due to other factors (localized in the ripen phase).

Table 3. The coefficients for simple correlation between the distribution frequency of the yeasts strain isolated and the rough values of the monthly temepature and humidity

Nr. crt	SPOROGENOUS STRAINS	Correlations with the temperature	Correlations with humidity
1	Schyzosacch.pombe	0,887**	-0,155
2	Saccharomycodes	0,719**	0,247
3	Hanseniaspora	0,640**	0,347*
4	S.elegans	0,925**	-0,491**
5	S.oviformis	0,509**	0,464*
6	S.ellipsoideus	0,734**	0,272
7	S.uvarum	0,786**	0,088
8	Debaryomyces	0,873**	-0,743**
9	Dekkera	0,467*	0,591**
10	Hansenula anomala	0,665**	0,117
11	Kluyveromyces	0,793**	0,118
12	P.membranef	0,663**	0,187
13	P.fermentans	0,310	0,682**
14	Zigosaccharomyces	-0,075	0,298
15	Torulaspora	0,675**	-0,310
	ASPOROGENOUS		
16	Metschnikowia	0,712**	0,129
17	Brettanomyces	0,284	0,372*
18	C.valida	-0,035	0,292
19	C.vini	-0,114	0,495**
20	C.stellata	0,520**	0,422*
21	Torulopsis	0,783**	0,189
22	Kloeckera	0,254	0,441*
23	Rhodotorula	-0,071	-0,270

*for p=0,05%; **for p=0,001

After how is described the regression equation, in 2003 the frequency of the *Schizosaccharomyces pombe* species has been increasing, without reaching the frequency level from 2001.

Also in the case of the *Saccharomyces ludwigii* species, the low quantities of rainfall from the winter of 2002 (0,8mm) has negatively influenced the proportion of the species frequency during the all year and further in 2003. The highest frequency of this species has been recorded in 2001 when the influence of the humidity has been approximately 6,10%. During the three years of research the influence of the air temperature on this species has been of 51,69%, thus, after the indication of the regression equation the frequency tend to attain in 2003 the level from 2001.

Hanseniaspora uvarum is the specie which in 2001 had one of the highest frequency among the sporogenous species in 2001. During all the research years, this species had a positive evolution of the frequency, but the low level of the rainfall from August 2003 has not allow to attain the spreading degree from 2001. This was the species which the frequency has been positive and significantly influenced by the modifications of the climatic factors, 40,96% by the air temperature and 12,0% by the air humidity.

Comparative with the species previously described, the *Saccharomyces elegans* had a special evolution, her frequency continuously increase in all the research years, reaching from 0,12% in 2001 to 0,18% in 2003. The zone specificity of this species is that the lower level of the humidity not only hasn't affected the spreading level, but even favorized it. The curve of the regression equation indicate a continuous increase of this species frequency, positively influenced 85,56% by the air temperature variation and significantly negative 24,10% by the humidity variation. The rest of 38,54% percentage of influence are due to other factors present in the viticultural ecotope.

Table 4. The regression functions

	Species	The regression functions
SPOROGENOUS	Schyzosaccharomyces	$3,0933-0,709x-0,9221y+0,0747x^2+0,0536xy+0,0928y^2$
	Saccharomyces	$0,3373+0,0132x-0,2103y+0,0084x^2-0,0074xy+0,0352y^2$
	Hanseniaspora	$-1,9552+0,4618x+0,4073y-0,0556x^2+0,0309xy-0,0529y^2$
	S. elegans	$-1,9618+0,5851x+0,4098y-0,0338x^2-0,0601xy-0,0212y^2$
	S. oviformis	$-0,9114+0,3628x+0,0586y-0,0203x^2-0,0381xy+0,018y^2$
	S. ellipsoideus	$-0,8304+0,3604x-0,04y-0,0163x^2-0,0323xy+0,031y^2$
	S. uvarum	$-1,1563+0,1163x+0,4533y-0,0109x^2+0,0079xy-0,0566y^2$
	Debaryomyces	$1,0465-0,1761x-0,2948y+0,0279x^2+0,0018xy+0,031y^2$
	Dekkera	$0,1028+0,051x-0,1401y-0,0031x^2+0,0046xy+0,022y^2$
	Hansenula	$-4,0323+0,9877x+1,0155y-0,0712x^2-0,0851xy-0,0785y^2$
	Kluyveromyces	$1,2063+0,0312x-0,724y-0,0045x^2+0,0269xy+0,084y^2$
	P. membranefaciens	$-3,2004+0,5234x+1,0698y-0,0349x^2-0,0424xy-0,1058y^2$
	P. fermentans	$-1,0042+0,0642x+0,42y+0,0045x^2-0,0156xy-0,0367y^2$
	Zigosaccharomyces	$-2,7826+1,0565x+0,3599y-0,0797x^2-0,098xy+0,0077y^2$
	Torulaspora	$-0,4615+0,4925x-0,2376y-0,0388x^2-0,0354xy+0,0475y^2$
ASPOROGENOUS	Metschnikowia	$0,5267+0,2865x-0,7055y+0,0041x^2-0,0488xy+0,1234y^2$
	Brettanomyces	$1,3817-0,1807x-0,5878y+0,044x^2-0,032xy+0,0981y^2$
	Candida valida	$-0,426+0,8359x-0,6454y-0,0565x^2-0,0877xy+0,1301y^2$
	Candida vini	$-1,545+0,7809x+0,0042y-0,0576x^2-0,0738xy+0,0414y^2$
	Candida stellata	$-1,032+0,2408x+0,231y+0,0003x^2-0,0475xy+0,0026y^2$
	Torulopsis	$-0,8829-0,0277x+0,3894y+0,022x^2-0,0134xy-0,0327y^2$
	Kloeckera	$6,0797-0,9085x-2,1282y+0,0218x^2+0,1969xy+0,1756y^2$
	Rhodotorula	$3,6399-0,9043x-0,7608y+0,0428x^2+0,1314xy+0,0229y^2$

x= air temperature; y= air humidity.

The species *Saccharomyces oviformis* and *Saccharomyces ellipsoideus* have presented similar evolution of the frequency distribution. The decrease of the humidity from the summer of 2002 has influenced with 21,52% the frequency of *S. oviformis* and with only 7,39% the frequency of the *S. ellipsoideus* species; both species have reached high spreading levels in the viticultural ecotope when the variation of the air temperature has influenced their frequency with 25,90 % respectively 53,70%.

From the results of the statistical analysis we can observe that the evolution of the *S. oviformis* specie has been influenced equal proportionally and significantly by the variation of the two climatic factors, which motivate the spectacular increase from 2003, surpassing the frequency values from 2001. The frequency increase of the species can be considered beneficial for the oenologist from S.D. Banu Măracine, knowing that these are the species which lead and finalize the fermentation from the wine cellar (especially *Saccharomyces ellipsoideus*).

Having in mind that the influences of other factors, not take it into study, has been of 52,57 % for the frequency evolution of *Saccharomyces oviformis* and of 38,73% for the frequency evolution of *Saccharomyces ellipsoideus*, we consider that a factor with important influence in the spreading degree of the two species is represented by the grapes in the rippen pahse, probably the ideal nutrition habitat of these species.

Saccharomyces uvarum has been the species whit a frequency in the viticultural ecosystem viticol Banu Măracine has been aproximatively constant. Maintaining in annual constant limits of this species frequency, due to the significant positive action of the temperature 61,77%, during the summer months and unsignificantly of the air humidity (0,77 %). Although, the rainfall from August 2003 (9,2 mm) have accelerate the decline of the frequency distribution in the viticultural ecotope. Like in all the presented cases, the percentage that quantify the action of an factor (independent) on other factor (depending) it's call in statistic determination coefficient (d) and it's calculated from the correlation coefficient (r), using the formula:

$$d = r^2 \times 100.$$

The *Debaryomyces hansenii* species had a low frequency in 2001 (aproximatively 0,19%) but the temperatures from 2002 have increase the levell of frequency at 0,30%. The frequency decline in 2003 due to the negative action (55,20%) manifested by the air humidity during April-May, with an increase of the distribution in the ecotope at the same time with the decrease of the humidity in August. The conclusion is that the dispersion degree of this species in the viticultural ecotope Banu Măracine is not favorized by the humidity, the species being thermophilic.

Dekkera bruxellensis has been an isolated species from the viticultural ecosystem which has not agree the high temperatures from the summer of 2002 (37,8°C-38,5°C) when the levell of 0,21% from 2001 has decrease to 0,16% during 2002. The tendences to return at the dispersion of over 0,20% in 2003 due to the positive action of the air humidity during this year, the tempearture action being only in proportion of 21,8% but significant.

A particular case in our studies has been the frequency evolution of the *Hansenula anomala* species, which have maintain constantly the dispersion levell at 0,24% with little tendences to surpass in 2003 due to the high significantly action of the temperature on the strains frequency of this species. The air humidity has not an important action on the strains dispersion of the species in this area.

The thermophilic characteristic of the species has made this species to develop and implicit to rapidly spread during summer period of each research year, proving justify the abundant presence in the first two days of alcoholic fermentations of the must.

The strains of the *Kluyveromyces thermotolerans* species prove throughly the denomination in all the research year. The rapide increase of their distribution frequency manifested only in the warmly months from each year when the action of the air temperature has manifested powerfull and significantly in a 62,88% percentage. The temperature variations from 2002 has decrease the strain distribution of the species from 0,31% to 0,24%, in 2003 viticultural ecotope the species increasing the distribution in the viticultural ecotope helped besides the temperatures by other independent factors not taken into the study in a proportion of 35,72%.

Apparently with identical evolution of the frequency, *Pichia membranaefaciens* and *Pichia fermentans* have presented proportion of the distribution aproximatively equal, the presence in the yeast conveyer being pretty consistent (0,22%). The different response of the two species at the action of the two climatic factors has offered to those characteristical evolution curve. Thus, *Pichia membranaefaciens* has been positively influenced significantly strictly by the variation of the temperature in a proportion of 43,95% and the frequency of the *Pichia fermentans* species has been favorized strictly positive by the variation of the air humidity in a 46,51% percentage. Like the regression curves has indicate, the *Pichia membranaefaciens* species strain has the tendence to progress slowly but sure regarding their contribution to the increasing of the oxydative yeasts population. The study of the yeasts frequency of *Pichia fermentans* has shown that the year 2002 was more favourable for obtaining quality wines, the species presenting an important decline in this year. But, the rainfall from during April acted favourable regarding the increase of this species frequency which, to the end of autumn, had the tendence to regain the frequency levell from 2001.

The strains of the *Zygosaccharomyces bailii* species, althoughare very sensitive at temperature that surpass 30°C-32°C, their distribution in the viticultural area Banu Mărăcine hasn't been direct influenced by the two climatic factors take it into study. The high monthly fluctuation of the species frequency presented in all the research years due especially to the rainfall variation. From the statistically analyze result that the most important action on the frequency variation had it other factors not taken into the study, in a proportion of 91,67%. This aspect is presented in the dispersion evolution curves, the most important increase being in every research year during the months of biological and technological maturity of the grapes, which represent the ideal habitat for the development of this species. The high percentage of the frequency in the viticultural ecosystem has been attained by this species at the end of autumn in 2003 when the paedoclimatic factors favorized high yields of grapes.

The strain frequency of the species *Torulaspora delbrueckii* has recorded an important increase during the years 2002-2003 (0,20%) comparatively with the year 2001 when had the lowest levell within the yeasts population, aproximatively 0,16%. The determinant factor which has contribute in the evolution of the species distribution has been the air tempearture, in a 45,56% percentage from the total of the factors action which has contribute to this ascent.

The air humidity hasn't influenced the strain frequency of the sppcies, the fall of the regression curve during the autumn in 2002 due to the high variations of the rainfall. High implication in the increase of the species population density have also other factors not studied from the biocoenosys interior in a proportion of 64,05%.

The very high variations of the climatic factors during 2002 have affected the strain distribution frequency of the speciess *Metschnikowia pulcherrima*. The apparently „disapear” of the strain species during the vintage is justified, knowing that this species is localized especially on the grapes, habitat that favorize the increase of the frequency to a percentage of 47,65%. The humidity hasn't an significant action on the strain distribution

while the air temperature variations has positive contribute with 50,69% to the variation of the yeast species frequency.

The species *Brettanomyces claussenii* has presented an maximum of the strain frequency during the autumn of 2003 (0,25% from the total of isolated yeast population). Although the variation of the air relative humidity had a little action (but significantly 21,89%), the percentage development of the species population from 2003 has been attributed to the high level of rainfall from the spring, being known the rapid development of this species in liquid environment. The variation of the strain frequency hasn't been influenced by the air temperature variations.

Among the species of the genus *Candida* only *Candida stellata* had a lower level of the frequency (approximately 0,21%), the others two species *Candida valida* and *Candida vini* have presented level of the frequency of 0,31% respectively 0,25% from the total of the isolated yeast population. The three species has the frequency curves similar during the research year from average values in 2001, these one suffered a decline in 2002 due to the lack of rainfall during the spring; the rainfall from 2003 acted positively on the development of the strain species frequency. From the climatic factors take it into study, the temperature has a significant positive action only on the frequency of the species *Candida stellata* (27,04%) and the air humidity acted positive significantly only on the frequency of the species *Candida vini*.

The species *Torulopsis bacillaris* had a constant level of the distribution frequency (approximately 0,30%), the evolution tendencies being positive significantly influenced in all the years of research by the temperature variation in a 61,0% percentage.

The high level of the strain distribution of this species in the viticultural area has been also found in the microflora from the fermenting must. The air relative humidity has no influence on the strain frequency of this species

Known as the most spread species in the yeast population which control the fermentation process, *Kloeckera apiculata*, with 0,55 percentage from the total of the yeasts strain in 2002, has presented a severe fall of the frequency level during the summer of 2003 reaching to 0,24% frequency. The factor that act positive significantly to the increase of the species frequency (19,4%) to the end of 2003 has been the air relative humidity also the high rainfall during October.

Among the isolated yeast species from the viticultural ecotope Banu Măracine, *Rhodotorula mucilaginosa* has been the species with the most affected frequency. From the results of the statistical analysis it comes out that no one from the two climatic factors take it into study has no action on the frequency variation of this species. The lack of rainfall from the research period act in the direction of the disappearing of this species which at the end of 2003 represented 0,08% from the total of winemaking isolated yeast.

CONCLUSIONS

The frequency levels of the sporogenous yeast strain, during 2001-2003, comes out that the frequency level of the strain yeast with essential role in the fermentation process has been decreasing to the end of 2003. On the contrary, the frequency level of the oxidative yeast strain has remain constant or has decrease very little, aspect that didn't favorized the oenologists. Globally analyzing the situation of the nonsporogenous yeasts frequency during the three years of research, it comes out that within the viticultural biocoenosis Banu Măracine, the nonsporogenous yeasts population passed through a decline period, the major cause being the climatic changes from the beginning of 2002. In generally, the frequency level

of the nonsporogenous strain species have decrease, the only species that have a recover of the frequency level have been *Metschnikowia pulcherrima* and *Candida valida*. In this case it can be observed that in the case of some normal climatic conditions, the species *Schizosaccharomyces pombe* can realized a distribution of approximately 0,8% from the total of the strain species from the ecotope Banu Măracine.

Based on the monthly monitored frequency in the ecosystem and following the mathematical modelling of the entire biological process, the response surfaces reveal the real view on the frequency evolution of the yeast isolated from the viticultural ecosystem Banu Măracine during the research period 2001-2003. The trusting interval of these response has been of 95%. It can be observed that the strain of the sporogenous species have frequency levels much lower than the nonsporogenous yeast strain. The response way of the yeasts strain to the actions of the climatic factors has been different, emphasizing the different characters of the species.

During 2002 there has been low rainfall, almost drouness, one of the consequence being the alteration of the number of winemaking yeast strain. From the response surfaces it can be observed that there has been even structure modifications within the microbial populations. Thus, in 2003, has increase the number of the oxydative strain species, especially the species *Candida*, *Brettanomyces*, *Dekkera*. Through the positive evolution of the species *Kloeckera* and *Torulopsis*, we can forecast that in the following year these two last species will be majoritary during the first days of the fermentation. We noticed, referring to the yeast conveyer, the decline of the species *Rhodotorula rubra* almost to the disappearance level (0,08 %), followed closely by *Torulaspora* and *Zigosaccharomyces*.

If the aspects of the structural changes of the yeasts populations previously presented, we add the decline of the populations framed in the genera *Saccharomyces*, we notice as an immediate research theme, constituting a collection of yeast specific for the viticultural ecosystem Banu Măracine from the isolated present until now, we have no knowledge of such an homologate collection at the University of Craiova.

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