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Biotechnological Characteristics of Some *Saccharomyces* species Isolated from Wine Yeast Culture

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Abstract The use of isolated wine yeasts in winemaking processes is preferable to spontaneous fermentation. Selection criteria of wine yeast strains depend also on capacity and rate of fermentation and on alcohologenic capabilities. Our studies have described the dynamics of fermentation of wine musts by some isolated wine yeast strains of *Saccharomyces* genus: strains 6 and 8 of *S. cerevisiae* var. *ellipsoideus* (*S. ellipsoideus*) and strains 5 and 7 of *S. bayanus* var. *oviformis* (*S. oviformis*). All have high technological properties and all are adapted for the specific pedoclimatic conditions of some areas of Sibiu viticultural region. The selected strains were used as inocula to ferment Sauvignon, Muscat Ottonel, Rose Traminer, and Pino Gris musts in controlled laboratory conditions. It was found that higher initial oxygen concentration in must is necessary to accelerate the fermentation of all the wine yeast strains studied. In order to obtain quality wines, strains with considerable fermentative capacity, high alcohologenic capabilities, and a good conversion efficiency are recommended.

Keywords: wine yeast strains, *Saccharomyces* genus, fermentation capacity, dynamics fermentation, alcohologenic capabilities

Introduction

The topic of our work is related to current research on the biotechnological properties of industrial yeasts and on the technological processes that may provide high-quality products. In our country, biotechnological research has not fully clarified certain aspects of this topic. We have decided to continue research done so far to gain a deeper understanding of biological and production aspects of a certain number of yeast strains for industrial use.

The fermentation-based industry requires the use of pure cultures of useful and valuable yeast strains, as well as the identification and elimination of contaminant yeast strains that negatively influence the metabolic activity of the culture yeast strains (1, 8). Considering all these premises, the aim of this research is to accomplish the following major goals:

a) isolation, identification, and selection of wine yeast strains of obvious value for use in the fermentation-vased industry,

b) delineation of the biological and technological peculiarities of isolated wine yeast strains,

c) creation of a collection of wine yeasts with superior productive characteristics that may prove useful to specialists in the field.

This experimental research was performed on isolated pure cultures of certain wine yeast strains selected in the Microbiology Laboratory of the Food Biotechnology Department of Lucian Blaga University of Sibiu (1, 4, 6). We have paid attention to the isolation of local wine yeast strains and to the selection of strains that have superior technologic properties and are adapted to the specific climatic and soil conditions within some important viticultural areas in Sibiu County (9, 10).

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Capacity and rate of fermentation of the musts by wine yeast strains are important selection criteria for these strains. The selected yeast strains must have a good fermentation capacity and high alcohologenic capabilities to ferment sugars from must as completely as possible. Yeasts also need to be able to achieve alcoholic fermentation in the shortest time (7, 11, 12).

This paper describes a study of some isolated wine yeast strains. These strains were used to ferment Sauvignon, Muscat Ottonel, Rose Traminer, and Pino Gris musts under controlled laboratory conditions. The aim was to study the capacity and rate of fermentation of the natural musts in order to determine which strains would be suitable for use as inocula in musts from the Sibiu and Alba regions. The evolution of some biotechnological characteristics was monitored: must fermentation dynamics, fermentation rate of the must sugars, and alcohologenic capabilities of isolated wine yeast strains. The percentage of alcohol produced and the conversion efficiency of sugar to alcohol in the fermentation process were also calculated.

Materials and Methods

Isolation and identification of wine yeast strains We studied four wine yeasts of the genus *Saccharomyces* that were collected, identificated, and selected from fermerting musts and wines supplied by several wineries located in the Sibiu viticultural region (Axente-Sever, Medias, Tarnava, Sebes and Alba Iulia). The experiments were carried out in the Microbiology Laboratory of Food Technology Faculty of Lucian Blaga University in Sibiu. Identification of physiological characteristics was carried out using the physiological tests (2, 3, 13), according to the criterion of Barnett *et al* (1) and Kreger Van Rij (6). For cultivation of wine yeasts, the natural musts of pure grape wines (Sauvignon, Muscat Ottonel, Rose Traminer, Pinot Gris) were used as nutritive media. Two commercial wine yeasts, *Saccharomyces ellipsoideus* M and

Saccharomyces oviformis M, were used as reference standards. These yeasts belong to the collections of the Wine Microbiology Laboratory at the Viticultural Research and Processing Station in Blaj, Romania.

Small-scale fermentations We isolated four local strains of *S. ellipsoideus* and *S. oviformis* and they were used as ferment on musts for laboratory-scale micro-fermentation. The musts were distributed in 200 mL quantities in Erlenmeyer flasks, sterilized, and locked by Mûller valves filled with H_2SO_4. The musts were then inoculated with active leaven made up of a population of $1,2 \times 10^7$ cells/ mL must for each strain. Fermentation temperature was kept at 25°C for 11 days (5, 9, 11).

Yeasts have the capacity to adapt their metabolism to anaerobic conditions of ethanol generation and to facultative anaerobic environments (14, 15). Considering this fact, the experiments were carried out in parallel series of anaerobiosis and facultative anaerobiosis for each yeast strain, using the same variables in both cases.

Chemical monitoring of fermentation The dynamics of the must fermentation was emphasized by gravimetrical determination of the weight of CO_2, which was emitted after 24 hours and was expressed as % weight (g CO_2/100 g must). During fermentation, must sugar levels (g/L, determined by refractometric method) and alcohologenic capabilities of the studied yeasts (by picnometrical method for the distillate, expressed as percent alcohol by volume) were monitored daily. At the end of the fermentation process, we calculated the alcohol fermentation efficiency (as g sugar / alcohol volume percentage) and the conversion ratio (%).

Statistical data processing was performed using the SPSS statistics package for Windows.

Results and Discussion

Biotechnological characteristics of the isolated and selected wine yeast strains are presented in Tables 1-4 and Figures 1-4. As seen in these tables and figures, the isolated wine yeast strains show good fermentative capacity, high alcohologenic capabilities, and they completely ferment the sugars in the must in a relatively short period of time. Under facultative anaerobic conditions these technological characteristics are even more obvious. Among the yeast strains we have isolated, strain 6 of *S. ellipsoideus* and strain 5 of *S. oviformis* show remarkable alcohol yields are. Most of the wine yeast strains we have isolated use about 17 g of sugar or even less to yield one grade of alcohol.

1. The dynamics of natural must fermentation by each of the wine yeast strains that we have isolated and selected are rendered in Figures 1 and 2. This data was used in the characterization of the yeasts' fermentation speed (each point or each column represents the average of 4 repeated measures).

The data from Figures 1 and 2 shows that there is a rapid triggering of fermentation after 24 hours with all the yeast strains we have isolated. We noticed higher values for CO_2 release from facultative anaerobiosis than from anaerobiosis.

With all yeast strains studied, the highest CO_2 volume being released in a 24- hour period was recorded on the third day of fermentation. Upon completion of natural must fermentation, wine yeast strain 6 of *S. ellipsoideus*

Table 1. Capacity of fermentation pasteurised Sauvignon must by *S. oviformis* 5 wine yeast strain and of of the Rose Traminer must by *S. oviformis* 7 wine yeast strain

FT	S. oviformis											
	Anaerobiosis						Facultative anaerobiosis					
	Sugar (g /L)				Ethyl alcohol formed (% vol. alcohol)		Sugar (g /L)				Ethyl alcohol formed (% vol. alcohol)	
	in must	fermented	in must	fermented			in must	fermented	in must	fermented		
	*5	*5	*7	*7	*5	*7	*5	*5	*7	*7	*5	*7
-	207.50	-	198.00	-	-	-	207.50	-	198.00	-	-	-
1	200.10	7.40	187.40	10.60	0.47	0.61	196.90	7.40	185.20	12.80	0.67	0.81
2	171.40	36.10	148.10	49.90	2.24	2.93	152.30	36.10	137.40	60.60	3.36	3.71
3	100.30	107.50	82.30	115.70	6.46	6.84	81.20	107.50	67.40	130.60	7.71	8.03
4	56.80	150.70	48.30	149.70	9.01	8.85	33.40	150.70	32.30	165.70	10.67	10.17
5	33.40	174.10	30.20	167.80	10.42	9.92	13.20	174.10	18.50	179.50	11.94	11.09
6	20.90	189.70	19.50	178.50	11.26	10.51	6.80	189.70	10.00	188.00	12.28	11.59
7	10.90	201.90	12.00	186.00	11.77	11.00	4.30	196.70	4.10	193.90	12.45	11.94
8	5.60	203.2	7.90	190.0	12.11	11.26	1.50	201.90	1.30	196.70	12.62	12.11
9	4.30	205.10	4.20	193.80	12.19	11.43	-	203.2	-	-	12.71	12.19
10	2.40	196.7	1.30	196.70	12.36	11.60	-	205.1	-	-	-	-
11	-	-	-	-	12.45	11.68	-	-	-	-	-	-
Fermented sugar		207.50		198.00				207.50		198.00		
Yield (sugar g / % volume of alcohol)					16.95	16.67					16.24	16.32
Alcohol yield (%)					58.99	59.95					61.56	61.25

*FT = fermentation time (days); *5 = *S. oviformis* 5 ; *7 = *S. oviformis* 7

Table 2. Capacity of fermentation of pasteurizat Pinot gris must, by *S. ellipsoideus* 6 wine yeast strain and of the Muscat Ottonel must, by *S. ellipsoideus* 8 wine yeast strain

FT	*S. ellipsoideus*											
	Anaerobiosis						Facultative anaerobiosis					
	Sugar (g /L)				Ethyl alcohol		Sugar (g /L)				Ethanol formed	
	in must	fermented	in must	fermented	formed (% vol. alcohol)		in must	fermented	in must	fermented	(% vol.alcohol)	
	*6	*6	*8	*8	*6	*8	*6	*6	*8	*8	*6	*8
-	215.00	-	191.60	-	-	-	215.00	-	191.60	-	-	-
1	207.50	8.50	184.20	7.40	0.47	0.54	204.40	9.80	183.10	9.80	0.61	0.74
2	184.20	30.8	152.30	40.80	1.83	2.38	159.70	49.90	141.70	49.90	3.29	3.00
3	125.80	89.2	85.50	106.10	5.92	6.15	87.60	120.00	71.60	120.00	7.63	7.24
4	75.90	139.10	47.20	144.40	8.28	8.36	45.10	160.30	31.30	160.30	10.17	9.68
5	53.60	162.40	27.00	164.60	9.51	9.59	26.00	178.4-	13.20	178.40	11.34	10.76
6	34.50	180.50	15.30	176.30	10.59	10.17	15.30	186.00	5.60	186.00	12.02	11.26
7	21.70	193.30	6.80	184.80	11.43	10.67	7.90	188.70	2.80	188.80	12.45	11.43
8	13.20	201.80	5.40	186.20	11.95	10.84	4.60	190.10	1.50	191.10	12.62	11.51
9	9.00	206.00	3.80	187.80	12.02	10.92	1.60	-	-	-	12.80	11.60
10	6.20	208.80	1.10	190.50	12.28	11.09	-	-	-	-	12.89	-
11	4.10	210.90-	-	-	12.36	11.17	-	-	-	-	-	-
12	-	-	-	-	12.54	-	-	-	-	-	-	-
Fermented sugar		215.00								191.60		
Yield (sugar g/% volume of alcohol)					17.14	17.18					16.67	16.52
Alcohol yield (%)					58.32	58.29					59.92	60.54

*FT = fermentation time (days); *6 = *S. ellipsoideus* 6 ; *8 = *S. ellipsoideus* 8

and strain 5 of *S. oviformis* released the highest quantities of CO_2, proving that these yeast strains have high fermenting speeds.

2. The results obtained in studying the fermentation capacity of each isolated yeast strain in the natural must are presented in Tables 1 and 2 and Figures 3 and 4.

The data in Tables 1 and 2 shows the correlation between time (days) and sugar content fermented daily by each wine yeast strain. This indicates the speed at which the respective yeast strains ferment the sugars in the must. After the first 24 hours of fermentation, there is a considerable decrease in the sugar content of the must. This decrease, due to the action of the wine yeast strains, shows small variations from one strain to another.

The third day of fermentation marks the highest amount of sugar processed in a 24- hour period by the yeast strains we have isolated. The highest quantities of sugar being proccesed, and therefore the highest fermenting speeds of the sugar in the must, have been attributed to strain 6 of *S. ellipsoideus* and strain 5 of *S. oviformis* (in facultative anaerobiosis the values were higher than those from anaerobiosis). At the end of the fermentation of the must, the entire sugar supply was used up by the isolated wine yeast strains in a relatively short span of time.

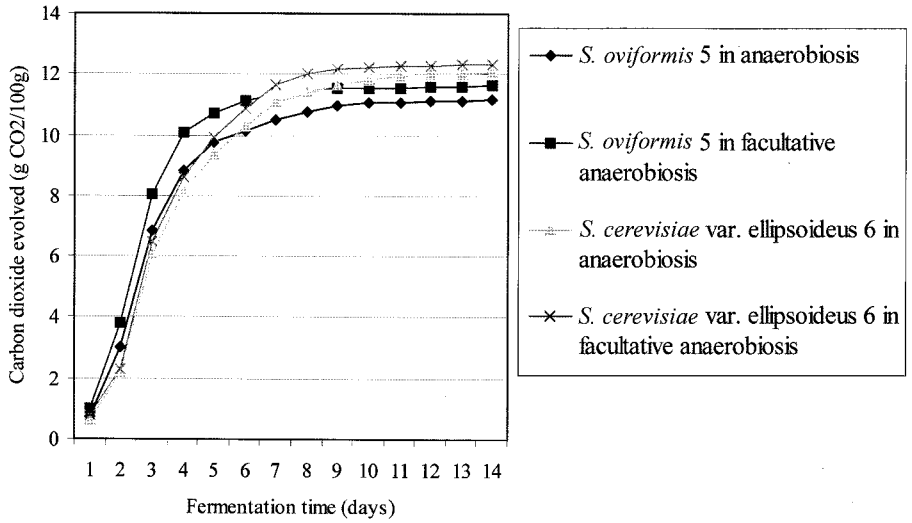

Fig. 1. Dynamics of CO_2 during the wine must fermentation with *S. oviformis* 5 and *S. ellipsoideus* 6 wine yeast strains.

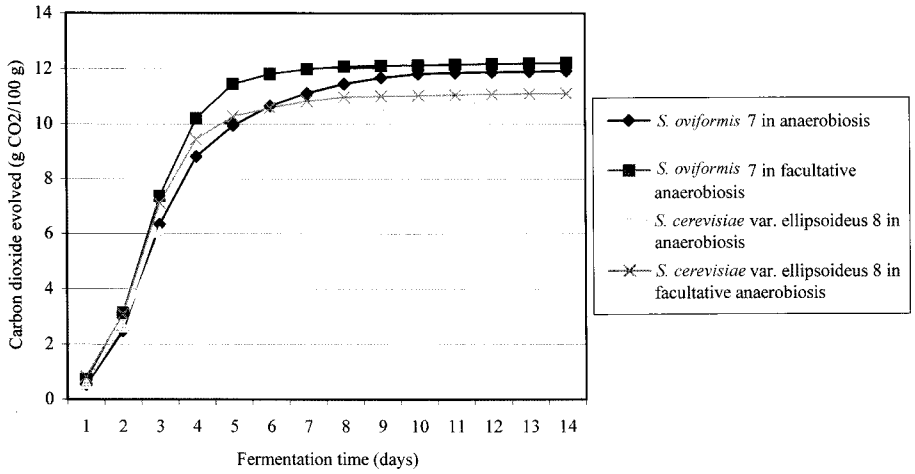

Fig. 2. Dynamics of CO_2 during the wine must fermentation with *S. oviformis* 7 and *S. ellipsoideus* 8 wine yeast strains.

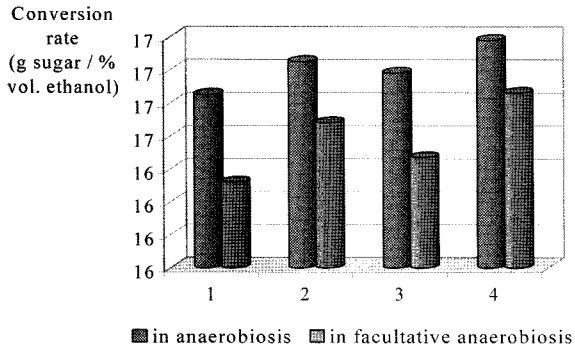

Fig. 3. Quantity of sugar used by the wine yeast strains (1 : *S. oviformis* 5; 2: *S. oviformis* 7; 3 : *S. ellipsoideus* 6; 4 : *S. ellipsoideus* 8) to produce one alcohol grade during musts fermentation.

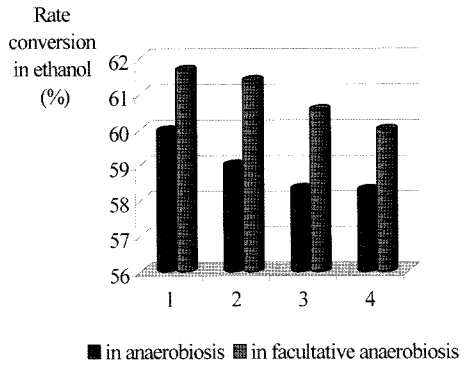

Fig. 4. The alcohologenic capabilities of the wine yeast strains that we have isolated (1: *S. oviformis* 5 ; 2: *S. oviformis* 7 ; 3 : *S. ellipsoideus* 6; 4: *S. ellipsoideus* 8).

The alcohologenic capabilities of the wine yeasts, as shown in Tables 1 and 2 and in Figure 4, are high with most of the strains we have isolated. Ethyl alcohol could be detected after the first 24 hours of fermentation, with all wine yeast strains producing about the same amount of alcohol. Alcohol production levels gradually rose between the second day and the fifth day, with lower daily quantities of ethyl alcohol afterward. The highest alcohologenic capabilities were recorded in the case of the wine yeast strains 6 of *S. ellipsoideus* and 5 of *S. oviformis*, resulting in fermented musts with alcohol concentrations between 11.17% and 12.89% by volume.

The alcohologenic capabilities of the wine yeast strains we have isolated depend on the efficiency of conversion of sugar to alcohol as measured by gram weight of sugar against % alcohol in total volume. We have demonstrated that the efficacy of conversion of grams of sugar/% of alcohol volume represents an important technologic characteristic in distinguishing one yeast strain from another. The distinct sugar content is due to the individualized role of each yeast strain and species in forming larger or smaller quantities of secondary substances under the given fermentative conditions.

The wine yeast strains we have isolated included strains 6 and 8 of *S. ellipsoideus* and strains 5 and 7 of *S. oviformis*. Strain 6 of *S. ellipsoideus* produced a 1% increase in alcohol volume for every 17.14 grams of sugar, while strain 8 of the same species showed a conversion rate of 17.18 g of sugar/percentage of alcohol volume under anaerobic conditions. Strains 5 and 7 of *S. oviformus* showed sugar usage rates of 16.95 and 16.67 g of sugar/ percentage of alcohol volume, respectively, in anaerobiosis. Under conditions of facultative anaerobiosis, strain 6 of *S. ellipsoideus* had a rate of alcohol production of 16.67 g sugar/ % alcohol, while strain 8 of the same species showed a value of 16.52 g of sugar/percentage of alcohol volume. The weight of sugar used by *S. oviformus* strain 5 was 16.34 grams for a 1% volume increase in alcohol under facultative anaerobic conditions. Under the same conditions, strain 7 of *S. oviformus* had a conversion rate of 16.32 g of sugar/percentage of alcohol volume.

Yeast strains 6 of *S. ellipsoideus* and 5 of *S. oviformis* used the lowest amount of sugar to produce one grade of alcohol. These yeasts showed a high level of efficacy in production of alcohol (Tables 1 and 2 , Fig. 3).

The fermentations were more intense in those samples with higher oxygen concentrations (in facultative anaerobiosis conditions) for all yeast strains studied. However, fermentation in these samples was highly diminished after the fifth day of fermentation, demonstrating the necessity of high initial oxygen concentrations in the natural musts to accelerate the fermentation process (4, 5).

Our study has led to the establishment of a rich collection of wine yeast strains that may prove valuable and useful to specialists in this field. This collection is available from the Microbiology Laboratory of the Food Biotechnology Department, Lucian Blaga University of Sibiu.

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