Bio-Technological Characterization of the Saccharomyces bayanus Yeast Strains in Order to Preserve the Local Specificity

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Abstract
The wine yeasts have multiple and important applications in the industry, aiming to obtain pure cultures and the selection of those strains which, according to the lab investigations, present superior bio-technological properties. In this study we monitored three types of Saccharomyces bayanus yeast strains, isolated from indigenous grapes varieties, Apold Iordana, Italian Blaj Riesling and Royal Feteasca from Jidvei area, which are present in the collection of the Biotechnologies and Microbiology Research Center of SAIAPM University. The yeast strains were subject to alcoholic fermentation in malt must at different temperatures, in the presence of alcohol, sugar and SO2 in various concentrations. The obtained results led to selecting of those strains which had best results regarding the alcoholic tolerance, osmo-tolerance, fermentation speed under stress conditions and resistance to SO2. These results can have practical applications in using the indigenous strains, isolated from grapes which are from inside the country, so that we preserve the local specificity, and reduce imports regarding this area.

Keywords: yeast, Saccharomyces bayanus strains, alcoholic fermentation.

1. Introduction
Since the oldest times, the micro-organisms were used for obtaining products which are useful for the man [1]. These properties which participate to these processes are called biotechnological properties. From the microbiological and biotechnological’s point of view, the yeasts presents different characteristics and act differently depending on the environments in which they are grown and fermented, on the chemicals which are added to these environments [2,3]. In the current study we monitored the alcoholic fermentation of the Saccharomyces bayanus yeasts which were isolated from the wine making centers of Apold, Blaj and Jidvei, which were observed at three different temperatures, in the presence of alcohol, sugar in two different percentages, and in the presence of controlled SO2 [4-6]. The cultures were named as follows: Saccharomyces bayanus yeasts reference sample EC118, Saccharomyces bayanus Iordana named SBIA, Saccharomyces bayanus Italian Riesling named SBRI, Saccharomyces bayanus Royal Feteasca named SBFR.

2. Materials and methods
In the present study we used as growing environment Wort broth malt must, Scharlau with a pH of 4.8, four yeast strains: Saccharomyces bayanus control sample (EC118), Saccharomyces bayanus Iordana (SBIA), Saccharomyces bayanus Italian Riesling (SBRI), Saccharomyces bayanus Royal Feteasca (SBFR), from the collection of the Biotechnological and Microbiological Centre’s...
collection of SAIAPM University, alcohol p.a. of 94%v/v, anhydrous glucose for the analysis of ACS Scharlau Chemie S.A. with \( M = 180,16 \). The fermentation of the yeast strains was monitored by the Startorius fermenter equipped with sensors for: temperature, biomass, \( \text{CO}_2 \), oxygen [4].

In order to monitor the thermal tolerance, the four yeast strains were subject to alcoholic fermentation at three different temperatures: 3ºC, 15ºC and 25ºC, in must growing environment for 9 days.

In the present study, we selected three different values for the alcohol percentage: 8, 10, 12% alcohol, and this alcohol was introduced in the must malt growing environment [5].

In order to improve the fermentative quality, we sampled fermentation processes without alcohol and also with progressive concentrations of alcohol. The tested pure cultures were the ones we selected previously, namely: EC 118, SBIA, SBRI, SBFR. The fermentation lasted 9 days and was at 25ºC [6, 7].

In musts, the carbohydrates are in a much higher quantity compared to other components so that we may assert that the osmotic pressure of the solution depends on the current carbohydrates concentration. In order to metabolize the nutrients, the yeasts must provide an opposing pressure in the growing environment, so that the existing osmotic pressure to be balanced. The yeasts activate usually in growing environments which have a maximum of 300g carbohydrates per litre. If the sugar concentration rises, we may observe a decreasing of the fermentative process. Same yeast strains, EC 118, SBIA, SBRI, SBFR, were monitored in the presence of glucose which was added to the growing environment in two separate percentages: 10% and 40%. The fermentation temperature was 25ºC and the process lasted for 5 days.

In order to inhibit the activity of the unwanted microorganisms during the fermentation process it is a common practice in the wine making industry to use antiseptics such as \( \text{SO}_2 \). These provide an inhibition of the yeasts and also of the bacteria and mold according to the applied dose. For studying the yeasts sensitivity to the \( \text{SO}_2 \) we introduced in the growing environment 200 mg of \( \text{SO}_2 \). We observed the must fermentation at 25ºC for nine days [1-3].

As a result of the experiments, the four yeast strains have been characterized and logged.

3. Results and discussion

The temperature plays an important role in the alcoholic fermentation so that the strains react differently to this element. It is known that, under the lab conditions, the yield and the thermal balance of the fermentation is according to the formulas [2]:

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{C}_2\text{H}_5\text{OH} + 2 \text{CO}_2 + 117 \text{ kj} \ (24 \text{ kcal/mol})
\]

**Figure 1.** The fermentative activity of the EC118, SBIA, SBRI, SBFR yeast strains at 3ºC

**Figure 2.** The fermentative activity of the EC118, SBIA, SBRI, SBFR yeast strains at 15ºC
In the present study we observed that for the 3°C temperature, the EC 118 control sample has the best yield, followed by SBIA strain, and the worst results we obtained for the SBRI strain (figure 1). At 15°C temperature, this strain has the best yield compared to other strains which were subject to this study, followed by SBFR sample, which, according to the figure 2 present close values. If we continue to observe the evolution of the fermentation process at 25°C (figure 3) we notice close values for the EC118 control sample and for SBFR sample, the worst result being for the SBRI strain. From the fermentation of the four yeast strains, we can say that these can be used for maximum yield, so that we can select the strain which presents the best characteristics regarding the behavior at the required temperature.

Saccharomyces bayanus yeasts have a particular feature: they can grow and can participate in fermentation process in the presence of alcohol. The yeasts which are alcohol-resistant are the ones which can be used as re-fermentation in growing environments in which there is alcohol in percentage varying from 6 to 14%. So, from the monitoring of the alcoholic fermentation with various alcohol concentrations, we observed that for 8% the SBFR strain presents the best yield, and the smallest values were obtained for the EC 118 control sample (figure 4). The alcoholic power of the four sample presented slight variations during the nine days of monitoring. After the first day, the released CO₂ was around 0.5g/l, being constant for the four strains. Significant differences of about 40% were observed after the third day of fermentation for the SBRI and SBFR strains. After the fifth day of fermentation we observed significant oscillations for all the four strains, the difference between the control sample and the SBIA strain, which presented the lowest fermentative activity, was about 45%. The next two days show constant and close values, and in the last day, the EC118 and SBRI strains present identical values. Observing this alcoholic fermentation, we may say that the strain which presented the best fermentative properties is SBIA, which presented a constant and balanced evolution until the end of the considered time interval.
In the presence of 10% alcohol, the yeast strains which were considered for this study had an evolution depicted by the figure 5, from where we can conclude that the SBRI strain presented the best results compared to the SBIA strain, which had the minimum values. The EC118 control sample and the SBFR strain showed close values, so that we can say that the differences between minimum and maximum values reached about 50%. At this alcohol concentration, the SBRI strain is considered as being the one presenting the best resistance to alcohol. For 12% alcoholic concentration, the strains show a reduced fermentative activity, so that the values drop, compared to the previous ones, with about 10-20% (figure 6). The best evolution was observed for the SBFR strain which had a fermentation dynamics and yield very high. Following this property, the fermentation activity under the presence of alcohol of the *Saccharomyces bayanus* wine yeasts, we can say that the SBRI strain presents the best fermentative qualities in the presence of 10% alcohol, and the SBFR for 8% and 12% alcohol.
Through osmo-tollerance, we monitored the dynamics of the must fermentation for two separate sugar concentrations for the four yeast strains. The fermentation was monitored during 5 days at 25°C.

Observing the dynamics of the must fermentation, in which we added 10% sugar, we can say that the four strains have a close behaviour. The SBRI show the best yield, and the SBIA the least one. For 40% of added sugar, we can observe that the best yield was for the EC118 control sample. The strains SBRI and SBFR show close values and the SBIA strain has the smallest value. If we compare the two types of fermentation, we can observe a higher dynamics, with about 300% higher for the 10% sugar addition, compared to the must in which we added 40% sugar. It can be observed that 40% of added sugar leads to a decreasing of the fermentation process of about 3 times, the dynamics being presented in figure 7.

![Figure 7. The evolution of the fermentative activity of the EC118, SBIA, SBRI, SBFR yeast strains under the influence of 10% and 40% sugar addition](image)

Watching the evolution of the alcoholic fermentation of the four yeast strains in must malt with an addition of 200 mg SO\(_2\)/l, we can observe that the SBRI strain has a constant and growing evolution up to the fifth day, compared to the SBIA strain whose variation is low due to the sulfur addition. The most important values can be observed for SBRI and SBFR strains, in the 5th day presenting a constant decrease and approximately equal until the last day of fermentation.

![Figure 8. The evolution of the fermentative activity of the EC118, SBIA, SBRI, SBFR strains during the fermentation process with an addition of 200 mg SO\(_2\)/l](image)

In Figure 8 we can observe that during the entire fermentation process of the must in which we added 200 mg SO\(_2\)/l, the significant values are for the SBRI strain.
4. Conclusions

From the present study, we can characterize and rank the four yeast strains, this being helpful in their selection, which can be made depending on their superior biotechnological properties. So, the SBFR adapts the easiest to the temperature variations, having superior alcoholic yield. It also presents the property that it can ferment also in the presence of 8% and 12% alcohol without being impacted too much. At 10% alcohol, this strain is exceeded by the SBRI strain. This yeast presents also the best yield for 10% sugar addition. For 40% sugar addition, we can observe a decreasing of the fermentation speed for all the strains, and it can be concluded that such a high percentage is not economical. Another property is that for this strain, the maximum yield is for addition of 200mg SO₂/l, which is very beneficial for the coming technological processes.

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