Enological potential of local yeast isolated from dealurile bujorului vineyard

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Abstract

In our national effort to promote autochthonous wines we have isolated and identified from Dealurile Bujorului vineyard 19 wine yeast strains belonging to Saccharomyces cerevisiae var ellipsoideus, S. bayanus, S. oviformis and S. rosei. Their fermentative properties have been tested on synthetic media. The selected strains have been further tested for their oenological potential on natural grape juice from indigenous grape varieties, respectively Feteasca Regala, Feteasca Alba, Babeasca Gri. The physico-chemical analyses of the obtained wines vary between the following limits: ethanol 8.1 - 14.5%, total acidity 2.74 - 5.68 g/l H₂SO₄, volatile acidity 0.3 - 0.72 g/l acetic acid and 10.1 - 22.31 g/l glycerol. The higher fermentative power has been shown by the strain S. oviformis MI 43 which has lead to a 14.1-14.5%. Three of the strains prove "flor" proprieties which lead to more aromatics wines.

Keywords: local wine yeast, indigenous grapes, film strains, Dealurile Bujorului vineyard

Introduction

It is well known that Romania is situated in top 10 wine producers in the world, with an average production of 5.5 mil hl/year. Almost 1 millions hl goes to the export. Taken into account that the Romanian wines have to compete with high quoted wines coming from other traditional wine-making countries (France, Italy, Spain, etc) or more recent producers as USA, Argentina or South Africa, there is a special need to find solutions to be competitive on the market. In the last years it has been given high importance to promote on the market the Romanian wines with strong indigenous character related mainly to their sensorial profiles (aroma and taste).

The microflora of grapes is highly variable, with a predominance of the low alcohol tolerant strains of Hanseniaspora, Kloeckera and various species of Candida, whereas S. cerevisiae is present only in low numbers (Fleet, 2000). The influence that all of these yeasts will have on the flavour of wine depends on several variables as grape variety, viticultural practices, soil composition, which will influence the sensory descriptive analyses. In addition, oenological practices, including yeast and fermentation conditions, have a prominent effect on...
the primary flavours wines (Lilly et al., 2002; Jose et al., 2005; Callejon et al., 2010; Carrascosa et al., 2012).
The wide use of commercial starter cultures, mainly applied to reduce the risk of spoilage and unpredictable changes of wine flavour, can ensure a balanced wine flavour, but it may also cause a loss of characteristic aroma and flavour determinants (Romano et al., 2003; Molina et al., 2009).

The research in the field has been mainly focused on wine aroma of Sauvignon variety, which is an aromatic variety. Yeast strains that produce the highest levels of volatile thiols were responsible for wines with the highest perceived intensity of fruitiness, and these wines are preferred by tasting panels. While the ‘green’ characters in Sauvignon Blanc wines can be manipulated through vineyard management, the ‘tropical fruity’ characters appear to be largely dependent on the wine yeast strain used during fermentation (Swiegers et al., 2009). Also, the varietal character has been successfully attributed to some polyfunctional mercaptans which are released by the yeast during fermentation (Campo et al., 2005). Therefore, the choice of yeast strain offers great potential to modulate wine aroma profiles to definable styles and predetermined consumer market specifications.

In the case of the grapes made of non-aromatic varieties which do not show intense or clear flavours, the wines obtained after their fermentation often show pleasant aromas which can be related to combination of the varietal origin and the yeast (Delfini et al., 2001).

Our work belongs to a wider national project which proposes the isolation and selection of valuable local wine yeast strains from consecrated vineyards in order to be used for the production of wines made of indigenous grape varieties with specific aromatic profiles (Matei et al., 2011; Visan et al., 2012). In this study the yeast isolation has been performed in Dealurile Bujorului vineyard, from Galati county, recognized for its wines made from indigenous grape varieties as Babeasca Neagra, Feteasca Alba, Feteasca Regala and Sarba. From all the enumerated grape varietis, only Feteasca Regala is considered as a semi-aromatic variety.

**Material and method**

In Dealurile Bujorului vineyard the isolation work has started in autumn 2008 and at the end 19 strains belonging to Saccharomyces specie have been kept for the final selection (Table 1). Some of the isolation and molecular identification results have been already reported (Gageanu et al., 2012). From a molecular point of view all the strains belongs to S. cerevisiae specie, but from technological point of view they belong to different subspecies as S. ellipoisdeus, S. bayanus, S.rosei, S. oviformis. These strains are now preserved in the collection of UASMV Bucharest, Faculty of Biotechnology in subculture and lyophilized.

<table>
<thead>
<tr>
<th>No.</th>
<th>Strain code</th>
<th>Identified specie</th>
<th>No.</th>
<th>Strain code</th>
<th>Identified specie</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MI 10</td>
<td>Saccharomyces oviformis</td>
<td>11</td>
<td>MI 45</td>
<td>Saccharomyces ellipoisdeus</td>
</tr>
<tr>
<td>2</td>
<td>MI 16</td>
<td>Saccharomyces oviformis</td>
<td>12</td>
<td>MI 48</td>
<td>Saccharomyces rosei</td>
</tr>
<tr>
<td>3</td>
<td>MI 19</td>
<td>Saccharomyces rosei</td>
<td>13</td>
<td>MI 56</td>
<td>Saccharomyces bayanus</td>
</tr>
<tr>
<td>4</td>
<td>MI 20</td>
<td>Saccharomyces bayanus</td>
<td>14</td>
<td>MI 72</td>
<td>Saccharomyces ellipoisdeus</td>
</tr>
<tr>
<td>5</td>
<td>MI 24</td>
<td>Saccharomyces oviformis</td>
<td>15</td>
<td>MI 112</td>
<td>Saccharomyces rosei</td>
</tr>
<tr>
<td>6</td>
<td>MI 27</td>
<td>Saccharomyces bayanus</td>
<td>16</td>
<td>MI 125</td>
<td>Saccharomyces ellipoisdeus</td>
</tr>
<tr>
<td>7</td>
<td>MI 33</td>
<td>Saccharomyces bayanus</td>
<td>17</td>
<td>MI 134</td>
<td>Saccharomyces ellipoisdeus</td>
</tr>
<tr>
<td>8</td>
<td>MI 35</td>
<td>Saccharomyces bayanus</td>
<td>18</td>
<td>MI 156</td>
<td>Saccharomyces bayanus</td>
</tr>
<tr>
<td>9</td>
<td>MI 37</td>
<td>Saccharomyces oviformis</td>
<td>19</td>
<td>MI 184</td>
<td>Saccharomyces ellipoisdeus</td>
</tr>
<tr>
<td>10</td>
<td>MI 43</td>
<td>Saccharomyces oviformis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Testing the fermentative power on synthetic medium
The yeast strains have been cultivated on YPG liquid medium (yeast extract 10g/l; peptone 20g/l; glucose 20g/l) in order to obtain biomass for the inoculum. After 24 hours cultivation at 27°C has been prepared for each strains inoculum of 10^6 viable cells/ml (determined by haematological method on Thoma slide; the viability has been checked with blue-methylene). The fermentation trials have been performed in Erlenmeyer glasses containing 200 ml of YPG liquid medium inoculated with 2 ml of preculture, w/o shaking. During three cultivation days every 6 hours have been measured the optical density of the culture at 660 nm.

Testing the oenological potential on natural must
From the first trials have been kept two valuable strains, MI 27 (S.bayanus with pellicular proprieties) and MI 43 (S.oviformis) with high fermentative power. As a control have been used the non-inoculated must containing wild yeast. They have been inoculated in natural must from three indigenous grape varieties, respectively Feteasca Regala, Feteasca Alba and Babeasca Gri. In table 2 are presented the inoculation variants.

Table 2- Inoculation variants in natural must
<table>
<thead>
<tr>
<th>Grape variety/Yeast strain</th>
<th>Wild microflora</th>
<th>S. bayanus MI 27</th>
<th>S. oviformis MI 43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feteasca Regala</td>
<td>V 1.1</td>
<td>V 1.2</td>
<td>V 1.3</td>
</tr>
<tr>
<td>Feteasca Alba</td>
<td>V 2.1</td>
<td>V 2.2</td>
<td>V 2.3</td>
</tr>
<tr>
<td>Babeasca Gri</td>
<td>V 3.1</td>
<td>V 3.2</td>
<td>V 3.3</td>
</tr>
</tbody>
</table>

The inoculum has been obtained in sterile must and its size was 10^6 viable cells/ml. The fermentation has been conducted in 1.5 litres of must, during 14 days and 15°C -17°C. After the fermentation, the wine has been separated from the sediments and sent for analysis.

Wines' analysis
The obtained wines have been analysed for the following characteristics: % volume ethanol content (ebuliometric method), total acidity (titrimetrically method with bromothymol blue), volatile acidity (distillation method with Saunier-Cazenave device), glycerol content (volumetric method based on Malaprade reaction), tartaric acid (rapid volumetric method "Schneyder-Pluher").

Results and discussions
Regarding the preliminary fermentative tests, as it can be seen in figures 1 and 2 all the strains have followed a typical growth curve and for most of the strains the logarithmic growth phase have started in the first 12 hours of fermentation under laboratory conditions on synthetic medium (YPG). It has been noticed only one exception, in the case of S. oviformis MI 16, which has started the fermentation only after 42 hours from the inoculation time.
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Fig. 1. Growth curves on synthetic media (YPG). Left: *S. bayanus* group; Right: *S. oviformis* group

Starting from the same fermentation volume and same inoculum size, the faster fermentation start has been noticed for *S. bayanus* MI 27 and *S. oviformis* MI 43 (within the first 6 hours). In their cases the stationary phase has been reached after 48 fermentation hours. It has to be noticed that the groups *S. ellipsoideus* and *S. rosei* show almost the same growth curves, with a rapid exponential phase, but a less rapid start of the fermentation (fig. 2). During the preliminary trials have been noticed that three strains have formed pellicle/film on the wine surfaces, respectively *S. bayanus* MI 27 and MI 156, as well as *S. oviformis* MI 24. According to the literature the "sherry" type wines are characterized by the presence of *Saccharomyces* species, as *S. rouxi, S. oviformis, S. oxydans, S. hispanica* (Vaughan et al., 2011). In our case, two of the strains belongs to *S. bayanus* which has been rarely reported as a "flor" yeast.

Fig. 2. Growth curves on synthetic media (YPG). Left: *S. ellipsoideus* group; Right: *S. rosei* group

Following the preliminary results, for the further experiments on natural must have been kept the strains with the fastest fermentative start, respectively *S. bayanus* MI 27 and *S. oviformis* MI 43.

The oenological capacities of the two strains have been tested on natural grape must provided by SCDVV Dealurile Bujorului (harvest 2012) from the following indigenous grape varieties: *Fetească Albă, Fetească Regală* and *Babească Gri*; their initial sugar content varied between
284 g/l (*Feteasca Regală*) and 210 g/l (*Babeasca Gri*). The measured pH varied between 3.2 and 3.5, which are in normal limits.

![Growth curves in natural grape must (*Feteasca Regală*, *Feteasca Alba* and *Babeasca Gri*) inoculated with *S. bayanus* MI 27 and *S. oviformis* MI 43. As control has been considered the natural yeast microflora](image)

As a common observation, the fermentative processes has followed typical yeast growth curve for all the variants. The fastest start has been notice in the case of *S. oviformis* MI 43. The non-inoculated musts have a delayed fermentation start with 1-2 days against the isolated indigenous yeast. After 14 fermentation days, the wines have been separated from the sediments and for all the obtained wines have been performed physico-chemical analysis.

Table 3 - Physico-chemical analysis of the wines made of indigenous grape varieties and isolated local yeast strains against natural microflora

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Total acidity (g/l H₂SO₄)</th>
<th>Volatile acidity (g/l acetic acid)</th>
<th>Tartaric acid (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V11</td>
<td>5.10</td>
<td>0.42</td>
<td>1.9</td>
</tr>
<tr>
<td>V12</td>
<td>4.91</td>
<td>0.45</td>
<td>1.6</td>
</tr>
<tr>
<td>V13</td>
<td>5.68</td>
<td>0.72</td>
<td>1.82</td>
</tr>
<tr>
<td>V21</td>
<td>4.16</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>V22</td>
<td>5.34</td>
<td>0.47</td>
<td>1.4</td>
</tr>
<tr>
<td>V23</td>
<td>3.74</td>
<td>0.68</td>
<td>2.3</td>
</tr>
<tr>
<td>V31</td>
<td>3.82</td>
<td>0.38</td>
<td>2.45</td>
</tr>
<tr>
<td>V32</td>
<td>3.47</td>
<td>0.35</td>
<td>2.5</td>
</tr>
<tr>
<td>V33</td>
<td>3.21</td>
<td>0.67</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Legend:
- V11 = *Fetească Regală* + natural microflora;
- V12 = *Fetească Regală* + *S. oviformis* MI 43;
- V13 = *Fetească Regală* + *S. bayanus* MI 27 (facultative film strain);
- V21 = *Fetească Alba* + natural microflora;
- V22 = *Fetească Alba* + *S. oviformis* MI 43;
- V23 = *Fetească Alba* + *S. bayanus* MI 27 (facultative film strain);
- V31 = *Babeasca Gri* + natural microflora;
- V32 = *Babeasca Gri* + *S. oviformis* MI 43;
- V33 = *Babeasca Gri* + *S. bayanus* MI 27 (facultative film strain).

In terms of total acidity and tartaric acid content all the wines registered normal values for the grape varieties and vineyard climatic conditions (table 2). Regarding the ethanol content, the highest alcohol content (13 to 14.5% vol.alc) has been reached, for all the musts, in the case of *S. oviformis* MI 43 (yeast species with high fermentative power), followed by the facultative film strain *S. bayanus* MI 27 (fig.4). The higher alcohol content in the case of *oviformis* specie may be also explained by the oxidative activity of the *bayanus* yeast (facultative film yeast) which can metabolize small parts of the formed ethanol (0.7 to 1.5%). The lowest alcoholic power has been noticed in the case of natural microflora.
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Fig. 4– Alcoholic power of selected local yeast strains on indigenous grape must. *S.oviformis* MI 43 produced the highest content of ethanol.

As expected, in the case of facultative film strain *S. bayanus* MI 27 the volatile acidity has been the highest, as well as the glycerol content (fig. 5). It is well known that glycerol does not contribute to the wine aroma, but is essential for its smoothness. The usual glycerol concentration in wine ranges from 4 to 10 g/liter, while in our wines the content is much higher than the maximum reported values, even double (*S. bayanus* MI 27 on Feteasca Regala grape variety). However, the amount of glycerol produced by yeast in wine is also influenced by other factors as sulfite concentration, pH, fermentation temperature, aeration, inoculation level, grape variety and ripeness, as well as nitrogen composition (Radler et al., 1982). This is why further investigation should be performed on its formation.

Fig. 5 – Glycerol production of selected local yeast strains on indigenous grape must. *S. bayanus* MI 27 produced the highest glycerol content.

Conclusions and perspectives

After a 3 years isolation work in Dealurile Bujorului vineyard, 19 yeast strains belonging to *Saccharomyces* species have been kept to be tested for their fermentative power and oenological potential. From a technological point of view these strains belong to *S. bayanus*, *S. oviformis*, *S. ellipsoideus* and *S. rosei*.

Three of the selected strains have shown “flor” proprieties, forming a film on the wine's surface, leading to wines with improved aromatic profiles. These strains are *S. bayanus* MI 27 and MI 156, as well as *S.oviformis* MI 24 and will be subject of further investigations on olfactometric level.

Two of the strains, *S. bayanus* MI 27 and *S. oviformis* MI 43 have shown rapid start in fermentation, this is why they have been tested for their oenological proprieties on indigenous grape varieties (*Fetească Albă*, *Fetească Regală* and *Babească Gri*). The highest alcohol content has been reached, for all the musts, in the case of *S. oviformis* MI 43, while the highest volatile acidity and glycerol content have been obtained when fermented with *S.*
bayanus MI 27. All the wines obtained with new isolated local yeast have superior qualities against the wines obtained by natural fermentation.

Both new selected strains will be kept in collection as valuable yeast for wine making. Considering the actual market direction, which require low alcoholic wines but with stronger aromatic profile, it is most probable that the strain \textit{S. bayanus} MI 27 will be proposed to be tested on pilot and industrial scale for the fermentation of indigenous grape must, especially for the variety \textit{Feteasca Regala}.

References


