Note

Characteristics of Wines Made by Saccharomyces Mutants Which Produce a Polygalacturonase under Wine-Making Conditions

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Wines by yeast mutants producing polygalacturonase in high glucose concentration, from Saccharomyces wine-making strains, had higher filterability and more concentrated anthocyanin contents than that of their parent strains. These results suggest that the clarification process was improved at a lower cost by the low viscosity and that high-quality wines result from the increase in the anthocyanin contents.

Key words: Saccharomyces wine yeast; polygalacturonase; filterability; yeast breeding; anthocyanin content

The first impact of a wine is visual, and comes from its brilliancy and its color. The main goal in wine-making technology, whether a white or red wine is going to be made, is to obtain a clear wine after crushing, pressing, and fermenting the grape juice. Soluble pectic polymers from the pulp have a negative effect in the must by restricting juice extraction, delaying must clarification, inhibiting the extraction of color materials in fermentation, and preventing the filtration step after fermentation.1,2 Extraction of color materials and filtration of the stabilized, maturated wine is very important to obtain a clear wine. Filtration is not always an effective method for removing pectin hazes, because filter pads are quickly plugged by pectin and gums, and trying to filter wine with a bad pectin haze often becomes an over-costly step. Usually, a foreign material such as bentonite or a pectic enzyme is added in wine fermentation to solve these problems.1,3 But commercial enzyme preparations have a negative side in the form of impurities. One such impurity, β-glycosidase, can cleave the sugar from the anthocyanins, leaving unstable aglycon that leads spontaneously to color loss.4 Another impurity, pectinmethylsterase, liberates methanol, which is toxic to humans.2,5 Cinnamoyl-esterase, which can liberate hydroxycinnamic acid from corresponding tartaric acid esters, increases the volatile phenol to the detriment of wine aroma.2,3

A few Saccharomyces yeasts used in wine making produce polygalacturonase (PGase),6 but almost no laboratory strains of Saccharomyces cerevisiae can produce PGase.7 Yeast that has PGase activity is expected to decrease the number of steps required for clarification and filtration, but PGase production of wine yeast appears to be repressed in high-glucose conditions.8 It is difficult to induce PGase during the wine-making process, since the glucose and fructose concentration in the grapes is initially about 20% and ultimately about 0.2%, which is too high for PGase production. A very important step in this research was to test the mutants in wine-making conditions. The mutants were tested on must from red grapes, and the wines obtained were analyzed according to their physiochemical characteristics.

The yeast media used were GYP (2% glucose, 0.5% peptone, and 0.5% yeast extract), SD (2% glucose and 0.7% yeast nitrogen base), SD-DYE (an SD plate containing Bordeaux S (0.05 g/l) and aniline blue (0.025 g/l)), and SD-PGA (upper-layering a 1.5% agar plate containing 2% sodium polygalacturonate on an SD plate medium). A natural medium was then prepared using grape must, as follows: The Delaware variety of red grapes from Kawachinagano Fruit Land (Kawachinagano, Japan) was crushed and roughly pressed, and the grape must was collected and sterilized at 121 °C for 5 min. The sugar content of the grapes was determined using a sacharimeter (Hitachi, Tokyo). Commercial wine yeasts, Saccharomyces cerevisiae KW4 and UvaFerm and Saccharomyces bayanus EC1118, were provided by the Iwate Biotechnology Research Center (Kitakami, Japan). The PGase+ mutants 4s1, Fh6, and Es3, which produce PGase in high glucose concentrations, were previously isolated from KW4, UvaFerm, and EC1118 respectively, as follows: The yeast cells were first mutagenized with 5% ethyl-methanesulfonate using Spencer’s method,9 and the mutagenized cells

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Abbreviation: PGase, polygalacturonase
were then haploidized with 0.05% 1-methylbenzimidazole. The haploid cells were selected on a SD-DYE plate. Finally, the colonies that formed a halo on SD-DYE plates were screened as constitutively PGase+ mutants.

Wine fermentation was done in 300 ml of natural medium with 60 ppm Na2SO4 in vessels of 500 ml volume, inoculated with 105–106 cells/ml, for 144 h at 25 °C. Fermentation vessels were rotated every 24 h at 120 rpm for 5 min with a rotary shaker, SCS-R (Sanki-seiki, Osaka, Japan). After fermentation, yeast cells and the other lees were eliminated by centrifugation at 6,000 x g for 5 min. The alcohol content was calculated using an F-kit Alcohol (Rosche-Biopham GmbH, Darmstadt, Germany), according to the manufacturer’s instructions. Total acidity was determined by titration with NaOH 0.1 N in the presence of bromthymol blue, and was expressed in the corresponding H2SO4 content (g/l). Filterability was determined by measuring the time necessary for 100 ml of wine to pass through a 45-μm membrane. The results were expressed in min needed to pass 100 ml of wine.

Total anthocyanins were measured on filtered (0.2-μm, Asahi Techno Glass, Tokyo), according to a principle based on variation in color at various pHs. Anthocyanin was calculated as malvidin-3-glucoside (g/l), using molar absorptivity of 28,000 and a molecular weight of 493.5. Tannin was measured according to a principle based on a property of protoanthocyanidine that it is transformed in anthocyanidine at 100 °C in acid medium. Wine colors were defined by their light absorbance at 420 nm, 520 nm, and 620 nm, respectively. Color intensity (CI), originally defined by the addition of absorbance at 420 nm, 520 nm, and 620 nm. Pigment contents (rates of yellow, red, and violet) were calculated as the rate of absorbance at 420 nm, 520 nm, and 620 nm respectively to CI.

Three PGase+ mutants had fermentative capacities that were resistant to ethanol, an acid pH, and a high level of SO2. The initial sugar contents of the Delaware red grape variety were 196 g/l at pH 3.7. The PGase+ mutants used in this study were constitutively able to produce PGase, even at this high concentration (20%) of glucose. It is well-known that 1% ethanol is theoretically obtained from 17 g/l glucose in the medium obtained after alcoholic fermentation. In our calculations, the maximum ethanol contents were about 12% after wine fermentation. The parents, KW4, UvaFerm, and EC1118, and their PGase+ mutants, 4s1, Fh6, and Es3 respectively, were compared for their fermentative power in a natural medium (grape must). The final content in ethanol was at the same level (11–12%) in all wines (Table 1). The acid concentration of wines obtained by fermentation with commercial strains and their mutants was assayed. The total acidities and final pHs of the wines were 4.6–4.8 g/l and 3.6–3.8 respectively (Table 1). Acidity did not differ among the wines. The organic acid compositions were the same in all wines (data not shown). These results show that there are no differences in alcohol and acid production in wine fermentation using the PGase+ mutants and their parents.

When regard to filterability, as was expected, the mutants showed significantly decreased filtration times (3–6 times) as compared with the parents (Table 1). PGase+ mutants might be effective in wine-making, since the preliminary use of the mutants showed that improved filterability of wine was obtained. Moreover, the filtration efficiency comes not only from the shorter time needed to remove the pectin haze, but also from the subsequently lower costs when expensive commercial pectinase is not used.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Final alcohol content (%)</th>
<th>Final acidity (g/l)</th>
<th>Filterability (min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KW4</td>
<td>11.5</td>
<td>4.6</td>
<td>212</td>
</tr>
<tr>
<td>4s1</td>
<td>11.8</td>
<td>4.6</td>
<td>35</td>
</tr>
<tr>
<td>UvaFerm</td>
<td>11.0</td>
<td>4.8</td>
<td>214</td>
</tr>
<tr>
<td>Fh6</td>
<td>10.9</td>
<td>4.8</td>
<td>43</td>
</tr>
<tr>
<td>EC1118</td>
<td>11.8</td>
<td>4.8</td>
<td>210</td>
</tr>
<tr>
<td>Es3</td>
<td>11.6</td>
<td>4.7</td>
<td>48</td>
</tr>
</tbody>
</table>

*Passing time of 100 ml of wine.

Wines by PGase-Producing Yeast

Table 1. Characteristics of Wines Obtained by Fermentation Using PGase+ Mutants and Their Parents

When anthocyanin and tannin were assayed in the wines produced by the PGase+ mutants (4s1, Fh6, and Es3) and their parent wine yeasts (KW4, UvaFerm, and EC1118 respectively), no significant differences were found in tannin content between the wines of the PGase+ mutants and their parents, but the anthocyanin contents (about 0.36 g/l) in all wines produced by the mutants were about 1.5-fold greater than the anthocyanin contents (about 0.25 g/l) in all wines produced by their parents. These results indicate the possibility that the degradation of the pectic substrate (polygalacturonic acid) in the grapes by the PGase produced by the mutants enhances the release of anthocyanin, much of which is found in the skin of the grapes. To assay the effect of the increase in anthocyanin contents released by the PGase+ mutants on the chromatic characteristics of the wine obtained, the CI and the proportions of the three major pigments in wine —red, yellow, and violet— were measured. The CIs of wines using 4s1, Fh6, and Es3 were 3.9, 3.6, and 3.8 respectively, small increases over the CIs obtained using their parents, KW4, UvaFerm, and EC1118, 3.6, 3.3, and 3.5 respectively. The proportions of the pigments did not differ between the wines produced by the mutants and those produced by their parents. These results suggest that the small increase in the CIs of the wines made by 4s1, Fh6, and Es3 caused the increase in total color materials. The increases in color materials might be attributable to the 1.5-fold increase in anthocyanin content, since the main components of the color in wine...
are anthocyanin and its derivatives. Wines made by red wine fermentation using PGase\textsuperscript{+} mutants can be expected to contain a large amount of color materials (anthocyanin derivatives), and have a deep red color. By convention, a high-quality red wine is defined as a clear wine, having rich color materials. The PGase\textsuperscript{+} mutants, obtained by chemical mutagenesis and isolated for their pectinolytic activity, can replace the fungal commercial enzymes used in the wine industry, which are a source of undesirable methanol. These mutants are expected to be used in wine making out of a desire to improve the final product, the wine, and to please the consumer. In the future, we will carry out taste tests of the wines produced using PGase\textsuperscript{+} mutants.

References


