Fermentation analysis of top-fermenting yeast

Research Laboratories for Alcohol Beverage Technologies
KIRIN Company, Limited

Hironori Inadome, Saori Takahashi, Taku Ota, Shigehiro Yoshizaki, Hiroyuki Yoshimoto

Quality with Surprise
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2. Evaluation of the fermentation properties in the top-fermenting yeast
3. Single nucleotide polymorphisms (SNPs) related to the production of 4-VG.
4. Regulation of 4-VG production
5. Conclusions
1. Introduction
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Craft beer has become a trend all over the world. There are also a lot of craft beer breweries in Australia. And now craft beer trend has come to Japan.

**U.S. BEER SALES VOLUME GROWTH 2014**

- **Overall Beer** 0.5% growth, 197,124,407 bbls
- **Craft Beer** 17.6% growth, 21,775,905 bbls
- **Import Beer** 6.9% growth, 29,430,185 bbls
- **Export Craft Beer** 36% growth, 383,422 bbls

**Craft Beer Market**

- $101.5 BILLION overall market
- $19.6 BILLION craft beer market
- 22% DOLLAR SALES GROWTH

**Shipping quantity of craft beer in Japan**

- 2007: 13,747 KL (+1.7%)
- 2008: 13,979 KL (+1.9%)
- 2009: 14,241 KL (+4.5%)
- 2010: 14,888 KL (+9.3%)
- 2011: 16,277 KL (+14.1%)
- 2012: 18,568 KL
- 2013: 21,802 KL (+17.4%)

Source: National Tax Agency (国税庁)

**Source:** Brewers Association, Boulder, CO
Craft beer in Kirin

Kirin has been committed to Craft Beer Market, establishing its own Spring Valley Brewery, a subsidiary that operates independently as a craft brewery, and also made an early move by taking a stake in Yo-Ho Brewing Company, producing the most popular craft beers in Japan.
Alcoholic beverages in Japan

A large variety of alcoholic beverages can be found in Japan. Some of the most popular ones are Beer, Wine, Sake, Whisky, and Sho-chu.

**Beer**
- Pilsner style beer is most popular in Japan, although low-malt beer and no-malt beer have been developed.

**Wine**
- Recent year, domestic wine of Japan is scoring high evaluation in international competition.

**Sake**
- Sake is a Japanese traditional alcoholic beverage that is made from rice koji.

**Whisky**
- Japanese whiskey that is achieved Japan own evolution is scoring high evaluation in the world.

**Sho-chu**
- Sho-chu is a Japanese traditional distilled spirit that is made from rice, sweet potatoes, wheat and/or sugar cane.
Yeast plays one of the most important role

Yeast can produce ethanol and flavor compounds such as esters, organic acids, diacetyl, 4-VG etc. from the liquids of wort, grape, rice etc. These compounds have a considerable effect on the taste, aroma and other characteristic properties.
Use of the yeast type depends on the category of the alcoholic beverage. In this study, we focused on the Top-fermenting yeast.

<table>
<thead>
<tr>
<th>Category of alcoholic beverage</th>
<th>Type of yeast</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer</td>
<td>Pilsner style</td>
<td>Bottom-fermenting yeast</td>
</tr>
<tr>
<td></td>
<td>Ale style etc.</td>
<td>Top-fermenting yeast</td>
</tr>
<tr>
<td>Whisky</td>
<td></td>
<td>Distiller yeast</td>
</tr>
<tr>
<td>Wine</td>
<td></td>
<td>Wine yeast</td>
</tr>
<tr>
<td>Sho-chu</td>
<td></td>
<td>Sho-chu yeast</td>
</tr>
<tr>
<td>Sake</td>
<td></td>
<td>Sake Yeast</td>
</tr>
</tbody>
</table>

Kirin possess approximately 1,100 strains of brewer’s yeast
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Strategy for evaluation of yeast

Evaluation of the fermentation properties in the top-fermenting yeast in our Yeast Bank were done using lab scale fermentation and chemical analysis and sensory evaluation were performed.

More than 200 strains of Top-fermenting yeast were examined

Lab scale fermentation with all malt wort was performed.

Chemical analysis and Sensory evaluation were done.

Fermentation tube: 500 ml scale tube
Wort: All malt wort, OE 12.5 °P
Yeast pitching rate: 0.4 %(w/w)
Fermentation temperature: 20 °C
Fermentation time: 5 days

Analysis of Sugar, Organic acids, Amino acids, Esters, 4-VG, sulfur dioxide(SO₂), and Sensory evaluation
Chemical analysis of Top-fermenting yeast

More than 200 strains of top-fermenting yeast were examined for chemical analysis to evaluate the fermentation properties.

*Chemical analysis List

<table>
<thead>
<tr>
<th>Fermentable sugars (total)*</th>
<th>Organic acids (total)*</th>
<th>Amino acids (total)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Oxalic acid</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>Malic acid</td>
<td></td>
</tr>
<tr>
<td>Maltotriose</td>
<td>Citric acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Succinic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyruvic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactic acid</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethyl acetate</th>
<th>Acetaldehyde</th>
<th>Diacetyl</th>
<th>4-VG</th>
<th>SO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twenty kinds of amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

More than 200 strains of top-fermenting yeast were examined for chemical analysis to evaluate the fermentation properties.
Cluster analysis of top-fermenting yeast

Top-fermenting yeast strains were divided into 6 groups by cluster analysis using data of utilization of sugar and amino acid, and production of ester, organic acid, 4-VG, diacetyl, acetaldehyde, and SO$_2$.

Cluster 1: Extremely low ability of fermentable sugar utilization
Cluster 2: Low ability of fermentable sugar utilization
Cluster 3: High ability of SO$_2$ production
Cluster 4: High ability of 4-VG production
Cluster 5: Low ability of organic acid production
Cluster 6: High ability of organic acid production
Sugar utilization ability of cluster 1 and 2

Main characteristics of strains in cluster 1 and 2 was low utilization ability of fermentable sugars.
Strains in cluster 1 had low utilization ability of maltose and maltotriose. Strains in cluster 2 had low utilization ability of maltotriose.
Main characteristics of yeast in cluster 3 was high production of SO$_2$, which accounted for only 1.1% of the total.
SO₂ production of each cluster

SO₂ serves as antioxidant, protecting beer from oxidation. Generally, bottom-fermenting yeast produces SO₂, but top-fermenting yeast doesn’t. In the extremely rare case, a few of top fermenting yeast strains produced SO₂.
Main characteristics of strains in cluster 4 was 4-VG production.
4-VG production ability of top-fermenting yeast was divided into two peaks. There is only a few yeast strains which produce low concentration of 4-VG.
Organic acids production in cluster 5 and 6

Cluster 5 and 6 was major clusters (Cluster 5: 37.4%, Cluster 6: 18.3%) and consisted of ale type strains. Cluster 5 and 6 were characterized by production of organic acid.
The organic acid production of cluster 6 was higher than that of cluster 5.
The pH of fermented wort in cluster 6 was lower than that in cluster 5. The pH was correlated with amino acids and negatively correlated with pyruvate.

- **Relationship of pH, amino acid and pyruvate**

  - **Cluster 5**
  - **Cluster 6**
  - **Other clusters**

  - **Amino acids (total)**
  - **Pyruvic acid**

  - $R^2 = 0.449$
  - $R^2 = 0.279$
Kirin possess approximately 1,100 strains of brewing yeasts, and basic data is collected for beer brewing.
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What is 4-VG?

Characteristic flavor of clove-like or phenolic bitter.
Threshold in beer is 0.3 ppm.
4-VG is synthesized from ferulic acid by decarboxylation of yeast.

Ferulic acid

\[ \text{CH}=\text{CHCOOH} \]

Decarboxylation

\[ \text{CH} = \text{CH}_2 \]

4-VG

\[ \text{CH} = \text{CH}_2 + \text{CO}_2 \]

\[ \text{CH} = \text{CHCOOH} \rightarrow \text{CH} = \text{CH}_2 + \text{CO}_2 \]
There is only a few yeast strains which produce low concentration of 4-VG. It was desired to regulate low concentration of 4-VG production by fermentation control.
Evaluation of 4-VG production by SNP

The genotype of *PAD1* gene can be used as a marker of 4-VG production

**PAD1** gene  
(Ferulic acid decarboxylase)

Functional  **305G type**

Dysfunctional  **305A type**

Function of *PAD1* gene of 305A type was lost by nonsense mutation*.

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Effect of ferulic acid in wort on 4-VG production

4-VG production can be controlled by the contents of ferulic acid in wort.

**Conditions**

- **Strain**: TFY024 (Top-fermenting yeast)
- **Pitching rate**: 0.4 %(w/w)
- **Wort**: all-malt wort
- **OE**: 12.5 °P
- **Fermentation temperature**: 20 °C
- **Fermentation time**: 5 days

1 : wort
2 : fermented wort (no addition)
3 : fermented wort (+2 ppm ferulic acid)
4 : fermented wort (+4 ppm ferulic acid)
Effect of pre-culture conditions on 4-VG production

Pre-culture conditions of aeration and temperature didn’t affect 4-VG production.
Effect of pitching rate and fermentation temp.

Yeast pitching rate and fermentation temperature affected 4-VG production.

**Pitching rate**

<table>
<thead>
<tr>
<th>Pitching Rate</th>
<th>4-VG Relative Value (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wort</td>
<td>2</td>
</tr>
<tr>
<td>0.2%</td>
<td>4</td>
</tr>
<tr>
<td>0.4%</td>
<td>6</td>
</tr>
<tr>
<td>0.8%</td>
<td>8</td>
</tr>
</tbody>
</table>

**Temperature**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>4-VG Relative Value (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wort</td>
<td>2</td>
</tr>
<tr>
<td>15°C</td>
<td>4</td>
</tr>
<tr>
<td>20°C</td>
<td>6</td>
</tr>
<tr>
<td>23°C</td>
<td>8</td>
</tr>
</tbody>
</table>
Regulation of 4-VG production in pilot plant

4-VG production was regulated in pilot plant by control of fermentation temperature.

**Fermentation profile**

- **Apparent Extract (°P)**
- **Yeast in suspension (mio Cells/ml)**

**4-VG**

- **Relative value (fold)**

**Ethyl acetate**

- **(ppm)**

**Isoamyl alcohol**

- **(ppm)**

**T-DA**

- **(ppm)**
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Conclusions

1. Top-fermenting yeast strains were evaluated and divided into 6 clusters. Feature of these clusters were defined by sugar utilization, SO₂, 4-VG, and organic acid production.

2. We developed the yeast database. Using this database, we can quickly select the yeast strain candidate containing proper character for development new products.

3. The 4-VG production ability was able to be predicted by analysis of PAD1 gene.

4. The 4-VG production level was able to control by changing the ferulic acid in wort, yeast pithing rate, and fermentation temperature.
In the future

1. To expand our Yeast database, an interesting new yeast strain continues to be collected and evaluated for the fermentation properties.

2. By selecting the proper yeast strain and utilizing genomic information and regulation of fermentation properties, Yeast database will be applied to develop a characteristic craft beers.

3. We want to contribute to create the Japanese own beer culture!
Thank you for your attention!