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Fermentation Characteristics of Wine Yeast Strains

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포도주 양조용 효모의 발효특성

정석태 · 고토나미* · 최종욱**

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Abstract

In fermentation characteristics, R2 was suitable for fast start and fermentation, while UCD530 was not suitable for complete fermentation at 25℃. T73 and AC- strains produced much more total acid compared to other strains and it was concerned with producing large amount of succinic acid and acetic acid. OC-2, UCD530, Beaujolais, and BC strains revealed low fermentation efficiency (below 62.0%), but EC1118, RC212, T73 and BM45 strains showed opposit result (above 70.0%). D254 and Wadenswil 27 seemed to have excellent cohesive ability because these two yeast strains made somewhat hard precipitation at the bottom after complete fermentation. T73 and CEG gave higher amounts of acetic acid (above 630 mg/L), while UCD530, W-3 and Beaujolais recorded low concentration (below 200 mg/L). In sulfur dioxide tolerance, preferable culturing temperature and times were 25℃ and 72 hr respectively. The strains R2, BM45 and L2056 revealed high sulfur dioxide tolerance (above 30mg/L), while 71B, Wadenswil 27 and Beaujolais showed the opposite result (below 5mg/L).

Key words : wine yeast, fermentation characteristic, sulfur dioxide tolerance.

Introduction

Most winery have been using the commercial dried wine yeast for wine making, but they have only limited information about the characteristics of wine strains. Grapes have various flora of microorganism on their surface. To destroy or inhibit the development of undesirable microorganisms such as acetic acid bacteria, wild yeasts and molds, wine maker add sulfur dioxide (sulfite form) to the grape must before the pure culture starter is inoculated (1). Therefore the wine yeast needs tolerance against sulfur dioxide for desirable fermentation. The tolerance of sulfur dioxide is concerned with the internal buffering capacity of organisms and the capacity of production of sulfite binding components (2). To find out the characteristics of commercial wine yeasts, two synthetic medium were applied, one was to examine the fermentation characteristics and the other was to know sulfur dioxide tolerance. In this report, 23 wine yeast

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strains were examined for studying fermentation characteristics by analyzing various components in experimental wine. Also the same strains were examined about sulfur dioxide tolerance using the synthetic medium.

Materials and Methods

In this study, 20 commercial wine yeast strains were used for studying fermentation characteristics and sulfur dioxide tolerance in synthetic media. Also 3 non-commercial wine yeast strains were used for the same study (3).

Table 1. Strains used in this study

Strains	Trade name or other designation	Species	Strains	Trade name or other designation	Species
K1(V1116)	Lalvin[1]	*S. cerevisiae*	RA17	Lalvin	*S. cerevisiae*
BC1118	Lalvin	*S. bayanus*	Wadenswil 27	Lalvin	*S. cerevisiae*
S6U	Lalvin	*S. uvarum*	Montrachet	Red Star[2]	*S. cerevisiae*
RC212	Lalvin	*S. cerevisiae*	Beaujolais	Red Star	*unknown*
R2	Lalvin	*S. bayanus*	CM	Uvaferum[3]	*S. cerevisiae*
T73	Lalvin	*S. cerevisiae*	BC	Uvaferum	*S. bayanus*
AC-	Lalvin	*S. cerevisiae*	CBG	Uvaferum	*S. cerevisiae*
71B	Lalvin	*S. cerevisiae*	CS2	Uvaferum	*S. cerevisiae*
CY3079	Lalvin	*S. cerevisiae*	OC-2	RIFY[4]1022	*S. cerevisiae*
D254	Lalvin	*S. cerevisiae*	UCD530	RIFY 1071	*S. uvarum*
BM45	Lalvin	*S. cerevisiae*	W-3	RIFY 1001	*S. cerevisiae*
L2056	Lalvin	*S. cerevisiae*			

[1] Lallemand Inc. Montreal, Canada
[2] Red Star Yeast & Products, Milwaukee, USA
[3] Danstar Ferment AG, Zug, Switzerland
[4] The Institute of Enology and Victiculture, Yamanashi University, Japan

Fermentation characteristics of wine yeast using a Wickerham medium

Pre-culture

Pre-culture was done at 25℃ for 2 days in YPD medium using L-form tubes. The suspension was centrifuged and the cells were washed with distilled water aseptically. Suspension was adjusted to 2.0 absorbance at 660 nm (A_{660}).

Medium preparation and culture condition

Synthetic medium based on Wickerham medium constitute of yeast carbon base, carbon source (glucose and fructose each 100 g/L), nitrogen source (400 mgN/L of free amino acid; 1043 mg/L of glutamate, 373 mg/L of arginine and 680 mg/L of threonine) and organic acid (6 g/L of tartaric acid and 4 g/L of malic acid). The pH adjusted to 3.5 with 0.1 N NaOH solution and medium was sterilized by filtration using 0.33 ㎛ polyethersulfone membrane. The medium was diluted with autoclaved water aseptically to the 95% of final volume (the rest 5% volume was added pre-cultured yeast suspension). Fermentation was carried out in pre-sterilized 100 ml Erlenmeyer flask containing 60 ml of culture medium which was incubated at 10℃ for 2 days and further incubation went on at 25℃ until the fermentation was completed, without shaking.

Analytical methods

CO_2 production was measured by weight loss of medium during the fermentation. pH was measured by Horiva pH meter F-22, Horiva Ltd, Japan. For the analysis of total acid, 10 ml sample was titrated by 0.1 M NaOH and expressed as tartaric acid. Turbidity was measured at 660 nm using 10 mm cell, 10 times diluted suspension. Extract was calculated from alcohol concentration of distillation and specific gravity using KEM density/specific gravity meter DA-300, Kyoto Electronics Ltd, Japan (4).

Ethanol concentration was determined by fixed enzyme column NJZ1240, Toukaseiki Ltd, Japan, and JRC Enzyme Reactor, Shinnihonmusen Ltd, Japan. Fermentation efficiency was calculated by this way. Fermentable efficiency (%) = {produced alcohol concentration (v/v, %) / initial sugar concentration (g/100ml) × 0.654} × 100. Organic acids in samples were determined by HPLC, LC-workstation CLASS-LC 10, Shimadzu Ltd, Japan. Column, Sim-pack SCR-102H X2 and Guard column SCR-102HG were used under the following conditions; auto injector temperature 20℃, column oven 50℃, mobile phase 5 mM p-Toluensulfonic acid 0.6 ml/min, buffer solution 5 mM of p-Toluensulfonic acid, EDTA and

Bis-Tris 0.6 ml/min, and internal standard 5000 ppm isovaleric acid. The detector condition was gain 1 μS/cm, range 1 and cell temperature 43℃.

Sulfur dioxide tolerance of wine yeast using a Uzuka medium

Pre-culture

1% glucose was added to yeast nitrogen base and incubated at 25℃ (strains indicated to *Saccharomyces bayanus* were incubated both at 15℃ and 25℃) for 3 days, without shaking. The suspensions were centrifuged and the cells were washed with sterile water. The cell suspension was adjusted to 1.0 at 660 nm using sterile water.

Medium preparation

The medium employed for sulfite tolerance test was a modified Uzuka medium (5), which contains yeast nitrogen base (Wickerham medium) w/o amino acid 0.67%, glucose 1% and tartaric acid 0.1 M. The pH was adjusted to 3.0 with saturated NaOH solution.

Sulfur dioxide treatment and assay

After autoclave of the synthetic medium, 1% SO_2 stock solution made from sodium bisulfite was added range from 0 to 60 mg/L as SO_2, an interval of 5 mg/L, respectively. The degrees of sulfite resistance of the wine yeasts were studied by determining the turbidity at 660 nm using 10mm cell, 10 times diluted suspension, cultured on 48 and 72 hours at 25℃ (strains indicated to *S. bayanus* were cultured both at 15℃ and 25℃).

Results

Fermentation characteristics of wine yeast using a Wickerham medium

Fig. 1. shows that R2 recorded the highest CO_2 produce rate, whereas BM45 and UCD530 show lower than those of other yeast strains. Even though the BM45 was slow start somewhat, that strain completed the fermentation similar to other strains. UCD530 started well but on account of increasing the alcohol concentration in the culture medium, the CO_2 production rate was becoming slow and stopped the fermentation at tenth day. This result means that UCD530 is not good fermentation at 25℃ compared with other strains. UCD530 indicated as *S. bayanus* and cryophilic strains is known to adequate for low temperature fermentation (6).

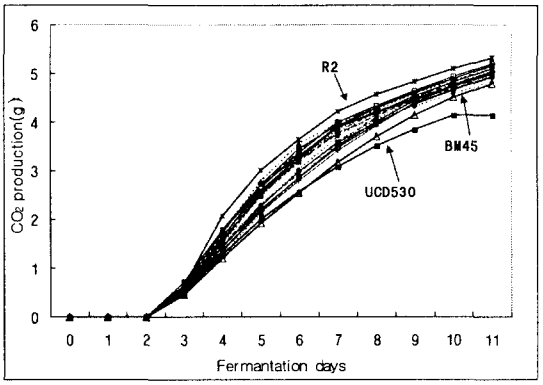

Fig. 1. CO_2 production rate of wine yeast at 25℃ using modified Wickerham medium.

Table 2. Analytical data of experimental wine fermented by different wine yeast strains using modified Wickerham medium

Strains	pH	Total acid (%)	Turbidity (A660nm)	Extract (g/100ml)	Alcohol (v/v, %)	Fermentation efficiency(%)
OC-2	3.66	0.87	0.618	2.29	7.6	62.0
K1	3.67	0.86	0.762	2.24	8.0	65.3
Montrachet	3.66	0.84	0.860	2.24	7.9	64.4
UCD530	3.66	0.83	0.767	4.21	7.6	62.0
EC1118	3.69	0.84	0.698	2.58	8.6	70.1
S6U	3.68	0.86	0.651	2.58	8.3	67.7
RC212	3.63	0.89	0.723	2.42	8.6	70.1
R2	3.66	0.86	0.835	2.34	8.4	68.5
T73	3.64	0.96	0.944	2.58	8.6	70.1
AC-	3.65	0.92	0.894	2.42	8.3	67.7
71B	3.72	0.80	0.664	2.39	8.0	65.3
CY3079	3.66	0.87	0.835	2.26	8.2	66.9
D254	3.70	0.83	0.696	2.24	8.0	65.3
BM45	3.67	0.87	0.651	2.34	8.6	70.1
L2056	3.67	0.86	0.671	2.29	8.1	66.1
RA17	3.67	0.84	0.889	2.19	8.4	65.8
Wadenswil 27	3.67	0.84	0.711	1.98	7.7	62.8
W-3	3.70	0.87	0.644	2.11	7.8	63.6
Beaujolais	3.70	0.84	0.581	2.37	7.5	61.2
CM	3.69	0.78	0.791	2.41	7.8	63.6
BC	3.72	0.77	0.705	2.45	7.6	62.0
CEG	3.69	0.83	0.727	2.45	8.4	68.5
CS2	3.71	0.78	0.605	2.43	8.1	66.1

Table 2 shows the characteristics of experimental wine fermented by wine yeast strains. The pH range of most samples were from 3.66 to 3.70 except samples of RC212, T73 and AC- (below pH 3.66), and 71B, BC and CS2 (above pH 3.70). In total acid, T73 and AC- strains produced more total acid compared with other strains. When comparing with the result of organic acid (Table 3), these two strains produced large amount of succinic acid and acetic acid relatively. The samples fermented by 71B, BC, CEG and CS2 were lower amount of total acid (below 0.8%). The analytical data of turbidity means the amount of yeast strain cultured, the samples of Montrachet, R2, T73, AC-, CY3079 and RA17 were higher, and OC-2, Beaujolais and CS2 were lower turbidity than those of other strains.

The extract of experimental wines were not significant difference except UCD530, recorded 1.8 times as much as average contents of other samples. This data revealed that UCD530 was not suitable for complete fermentation at high temperature (25℃). Most of strains produced alcohol range from 7.6% to 8.4% except EC1118, RC212, T73 and BM45 (8.6%), and Beaujolais, lowest ethanol concentration (7.5%). In fermentation efficiency, OC-2, UCD530, Beaujolais, and BC strains revealed low fermentation efficiency (below 62.0%), but EC1118, RC212, T73 and BM45 strains showed opposit result (above 70.0%)

Additionally (data not shown), Montrachet and R2 produced a lot of bubbles during the fermentation, whereas T73, AC-, BM45 and BC produced lower than other strains. The experimental wine fermented by OC-2, Beaujolais and 71B expressed thin blue color characteristically. After complete fermentation, samples of D254 and Wadenswil 27 made somewhat hard precipitation at the bottom. These two yeast strains seem to have excellent cohesive ability.

Table 3 shows organic acid contents of experimental wine fermented by wine yeast strains. This analytical data shows that the sample of CM contains significantly high amount of Tartaric acid + Pyruvic acid compared with other strains. It is probably due to the much production of pyruvic acid, because tartaric acid is not produced by yeast strains. T73 released more amount of succinic acid than other yeast strains. Acetic acid is the main volatile acid in wine and formed during yeast fermentation as a result of the side reaction of acetaldehyde oxidation. In this experiment, T73 and CEG gave higher amounts of acetic acid (above 630 mg/L), while UCD530, W-3 and Beaujolais recorded low concentration (below 200 mg/L). Acetic acid is a negative effect in wine quality and above 600-700mg/L volatile acid is noticeable and it depreciate wine quality (7).

Table 3. Organic acids contents of experimental wine produced by different yeast strains using modified Wickerham medium

Strains	Organic acids (mg/L)					
	T+P acid[1)]	Malic acid	Succinic acid	Lactic acid	Fumalic acid	Acetic acid
OC-2	7,881	3,194	767	99	18.0	325
K1	8,239	3,385	674	122	13.2	418
Montrachet	7,702	3,023	629	112	17.8	354
UCD530	7,652	2,865	667	191	12.3	160
EC1118	7,917	3,242	557	141	10.7	364
S6U	7,945	3,105	663	77	14.6	363
RC212	7,816	3,175	565	183	14.9	505
R2	7,667	3,009	558	147	17.6	499
T73	8,024	3,244	920	94	12.8	639
AC-	7,752	3,134	730	157	18.3	509
71B	7,777	2,810	616	169	15.4	341
CY3079	7,837	3,248	548	116	16.3	404
D254	7,769	2,929	583	87	14.4	502
BM45	7,725	3,215	498	193	20.2	483
L2056	7,732	3,087	546	185	15.0	436
RA17	7,814	3,158	511	128	18.9	384
Wadenswil 27	7,754	3,094	550	100	11.3	393
W-3	7,587	3,017	486	98	17.1	123
Beaujolais	7,698	3,008	695	197	21.3	188
CM	8,784	3,435	661	62	10.0	336
BC	7,358	2,817	577	69	13.6	345
CEG	7,533	2,955	550	100	13.4	652
CS2	7,519	2,963	488	70	16.6	548

[1)] Tartaric acid + Pyruvic acid

Sulfur dioxide tolerance of wine yeast using a Uzuka medium

The synthetic medium for sulfur tolerance test of yeast strain was developed by the Uzuka et al. (5). Synthetic medium is good for setting the actual sulfur dioxide

concentration, because sulfur dioxide bind various compound in the grape must and lose their toxic effect against yeast cells. The major components which interact with SO2 are carbonyl compound, phenols, pigments and some other specific components (8).

Fig. 2 revealed that 25℃ was more preferable for culture than 15℃, and culturing for 72 hour is more clear tendency of sulfur dioxide resistance than 48 hour.

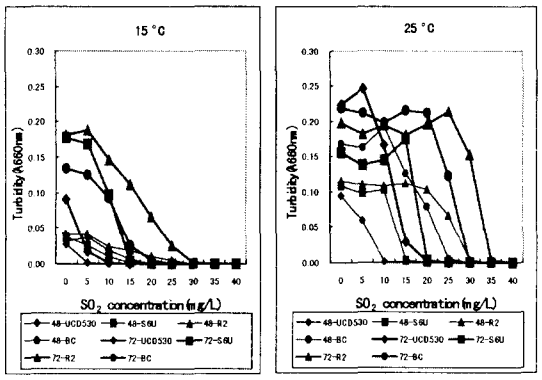

Fig. 2. Growth characteristics of wine yeasts on culture time and temperature in different sulfur dioxide concentration.

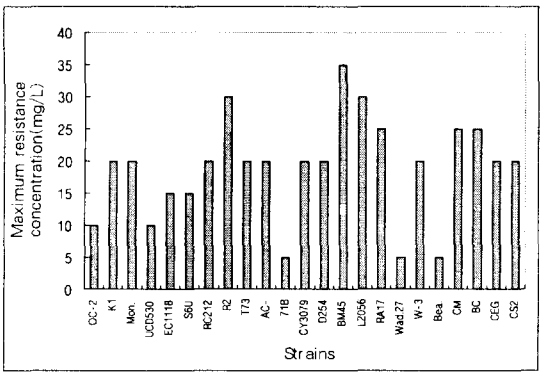

Fig. 3. The degrees of sulfur dioxide tolerance of wine yeast strains using modified Uzuka medium.

Fig. 3 shows the degrees of sulfur dioxide tolerance of 23 wine yeast strains. Sulfur dioxide tolerance of most strains used were included range from 10 mg/L to 25 mg/L except lower and higher tolerance strains. R2, BM45 and L2056 revealed extremely high sulfur dioxide tolerance (above 30 mg/L), whereas 71B, Wadenswil 27 and Beaujolais expressed opposite result (below 5mg/L). OC-2 had tolerance of 10 mg/L sulfur dioxide. This finding had some different result of Uzuka's study (5), OC-2 grew in the presence of 50 ppm of sulfite (about 32 ppm of SO_2). Ozawa's study (9) said that W-3 had extremely high SO_2 tolerance up to 200 mg/L but in this experiment, W-3 had resistance only 20 mg/L. This result revealed that various components of grape must was combined to most of added SO_2 and about 10% of added SO_2 worked toxicity to microorganism.

요 약

포도주 양조용 효모의 발효특성 검정에 있어서 R2 균주가 발효 속도에 있어서 우수하였으며 UCD530 균주는 25℃ 발효에서 완전한 발효가 이루어지지 않아 고온에서의 발효가 적합하지 않았다. T73과 AC- 균주는 총산 생성량이 많았는데 이것은 이 두 균주가 시험에 사용된 다른 균주에 비해 succinic acid 와 acetic aicd을 많이 생성한 결과였다. 발효효율에 있어서 OC-2, UCD530, Beaujolais, BC 균주는 낮은 발효효율(약 62%)을 보인반면 EC1118, RC212, T73, BM45 균주는 비교적 높은 발효효율(약 70%)을 나타내었다. D254와 Wadeswil 27 균주는 발효완료 후 침전물 형성이 우수한 것으로 보아 이 두 효모는 부유물의 응집능이 강한 것으로 생각된다. 발효 중 이취 성분의 하나인 초산생성능에서 UCD530, W-3, Beaujolais 균주는 낮은 초산생성능(약 200 mg/L)을 보인 반면 T73 및 CEG 균주는 높은 초산생성능(약 630 mg/L)을 보여 발효주의 풍미에 나쁜 영향을 줄 것으로 생각된다. 포도주 양조용 효모의 아황산 내성 시험에 있어서 25℃ 72시간 배양한 것이 뚜렷한 아황산 내성 특성을 나타냈으며, 균주별로는 R2, BM45, L2056는 높은 아황산내성을 (약 30 mg/L), 71B, Wadenswill 27, Beaujolais는 낮은 아황산 내성 (약 5 mg/L) 특성을 보였다.

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