

Screening of Thermotolerant Yeast Strain for Ethanol Fermentation

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Ethanol 발효를 위한 내열성 효모 균주의 Screening

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For the purpose of developing new thermotolerant yeast strains for ethanol fermentation, yeasts were isolated from molasses and screened for their fermentation ability at elevated temperatures. Three candidate strains were screened. These strains preferred pH 5.0 and 34°C for their ethanol production. Under such conditions the three strains showed average ethanol productivity of 75g ethanol per liter of fermentation broth in a synthetic medium containing glucose as substrate. These strains were identified as *Saccharomyces cerevisiae* and *Kluveromyces marxianus*.

The conventional yeast alcohol fermentation requires a cooling system to maintain a optimal temperature such as 30°C for a maximum alcohol yield. Yeast strains which tolerate high fermentation temperatures may reduce fermentation cost by eliminating cooling process (1-5). Some substrate prepared by heating needs cooling before yeast is inoculated. If a heat tolerating yeast is used, the fermentation may be started with higher temperature, and thus some part of the cooling cost will be saved (6-8). Other economic gains would be form reduction in mashing time saving in water, and volumetric reduction of effluent. In this respect, it is reasonable to select suitable strains having high temperature tolerance as well as high fermentation power for reducing fermentation costs. Two yeast strains which produce ethanol optimal at temperatures between 35°C and 40°C were screened and identified as *Saccharomyces cerevisiae* and *Kluveromyces marxianus*.

Materials and Methods

Isolation of yeast

Yeast strains were isolated from molasses kindly provided by Il San Co., (Pusan, Korea). The molasses samples were seeded in yeast extract potato dextrose liquid medium and incubated at 30°C to 34°C for 24 hours. The cultures were diluted 10⁴, 10⁶ and 10⁸ fold with sterilized distilled water and then 0.15ml of dilutes were plated on yeast extract potato-dextrose agar plate. After incubation at 30° for 12 days yeast, colonies appeared on the plates were isolated and purified.

Identification of yeast strains

The yeast strains isolated were identified according to Lodder and Kreger-van Rij (9).

Fermentation experiments

In a 1000ml Erlenmyer flask, 400ml of medium

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containing 18% glucose, 1.4% yeast extract, 0.21% NH_4Cl , and 0.018% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, was filled and autoclaved at 120 °C for 20min. After inoculating with the isolated yeast strains, fermentation was carried out with agitation on a shaker.

Growth measurement

Growth of the yeasts was estimated by measuring optical density at 620nm.

Analytical methods

The amounts of alcohol content, total reducing sugar and fusel oil were determined by the methods described in our previous paper (10).

Table 1. Fermentation characteristics of the 12 yeast strains isolated from molasses in a synthetic medium containing total sugar of 17.59%.

Strains	Final pH	Acidity	Alcohol productivity (g/l)	RTS (%)	FR (%)
M231	3.95	3.50	75.04	0.33	82.81
M232	4.12	4.30	21.44	11.13	23.66
M233	4.04	3.85	72.88	0.32	81.08
M234	4.25	3.80	72.40	0.32	80.54
M238	4.00	4.00	75.12	0.25	82.91
M239	4.00	3.65	74.48	0.19	82.20
S341	3.90	4.55	35.36	8.18	39.02
S342	4.33	2.65	34.48	8.31	38.05
S343	4.10	2.45	37.60	7.09	41.50
S344	4.10	2.50	32.64	8.83	36.02
S345	3.90	2.75	39.12	7.28	43.17
W351	4.15	2.70	67.92	0.68	74.96

RTS: Remained Total Sugar

FR: Fermentation Rate(%) = actual alcohol conc./theoretical alcohol conc. \times 100

TS: Total Sugar

Table 2. Morphological properties of isolated yeasts.

	M-231	M-238	IFO-1-84	M-239	IFO-0260
Growth in malt extract (after 3 days at 25 °C)	globose	globose	globose	subglobose	subglobose
Shape and size of cell	(3-5)-(4-10) m	(4-9)-(4-9) m	(3-10)-(4-10) m	(2-6)-(3-10) m	(2-6)-(3-10) m
Vegetative reproduction (after 7 days at 25 °C)	multilateral budding a slight ring and sediment are formed	multilateral budding a sediment is formed	multilateral budding a sediment is formed	budding a sediment is formed and occasionally a slight ring as well	budding a sediment is formed and occasionally a slight ring as well
Growth on Malt agar plate (after 30 days 17 °C, the streak culture)	Abundant growth Brownish smooth the margin is entire	Abundant growth Brownish soft-smooth the margin is entire	Abundant growth Brownish smooth the margin is entire	butyrous Brownish-cream smooth the margin is undulating or lobiform	butyrous Brownish-cream smooth the margin is undulating or lobiform
Formation of ascospore	globose to ellipsoidal	globose to ellipsoidal	globose to ellipsoidal	crescent form to remiform ascospores	crescent form to remiform ascospores

Results and Discussion

Identification of strains

From total of 300 colonies isolated from molasses, 12 colonies which showed higher produc-

Table 3. Fermentation of sugars by isolated yeasts.

sugar	M-231	M-238	IFO-1-84	M-239	IFO-0260
D-glucose	+	+	+	+	+
galactose	v	v	v	+	+
sucrose	w	+	+	+	+
maltose	+	w	+	-	-
lactose	-	-	-	v	v
melibiose	-	-	-		
soluble starch	-	-	-		

+; positive -; negative v; very weak fermentation w; weak fermentation

tivity of alcohol were preselected. Fermentation power of the 12 strains were further tested by fermenting the synthetic medium (pH 5.0). Fermentation parameters of the selected strains are shown in Table 1. The ethanol productivity at 30°C of M-231, M-238, M-239 were 75.04, 75.12 and 74.48 g/l, respectively, which are equivalent to that of *S. cerevisiae* IFO-1-84 was 74.42g/l. The three strains of high fermentation efficiency together with the two reference strains were subjected to taxonomic identification. The two isolated strains, M-231 and M-238, were grown as globose shape at 25°C for 3 days in malt extract medium, but M-239 was grown as subglobose shape (Table 2).

All strains reproduced through multilateral budding or single budding and M-231, M-238 and M-239 formed sediment in the liquid culture. Streak cultures of M-231, M-238 and M-239 were slightly

Table 4. Assimilation of carbon compounds by isolated yeasts.

Carbon compounds	M-231	M-238	IFO-1-84	M-239	IFO-0260
Galactose	v	v	v	+	+
Sucrose	v	v	v	+	+
Maltose	v	v	v	-	-
Lactose	-	-	-	+	+
Cellobiose	-	-	-	+	+
Raffinose	v	v	v	+	+
D-Xylose	-	-	-	+	+
D-Arabinose	-	-	-	+	+
D-Ribose	-	-	-	v	v
L-Rhamnose	-	-	-	-	-
Mannitol	-	-	-	+	+
Inositol	-	-	-	-	-

+; positive -; negative v; very weak

Table 5. Physiological properties by yeasts isolated.

	M-231	M-238	IFO-1-84	M-239	IFO-0260
Assimilation of nitrate	-	-	-	-	-
Assimilation of ethylamine·HCl	-	-	-	-	-
Assimilation of cadaverine·HCl	-	-	-	-	-
Growth in vitamin-free medium	w	w	v	w	w
Growth in the presence of 100ppm of cycloheximide	-	-	-	-	-
Names of species of isolated yeasts	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Kluveromyces marxianus</i>	<i>Kluveromyces marxianus</i>

IFO-1-84; *S. cerevisiae*, IFO-0260; *K. marxianus*, +; positive -; negative w; weak, v; very weak.

brownish, brownish or brownish-cream and were smooth on the malt agar medium after one month at room temperature. All strains fermented glucose vigorously, but could not ferment lactose, melibiose and soluble starch except M-239 which fermented lactose very weakly (Table 3). Strains M-231 and M-238 assimilated carbon compounds other than glucose, nitrates, ethylamine-HCl and cadaverine-HCl very weakly or not. However, M-239 assimilated several carbon compounds vigorously (Table 4 and 5). Their growths were inhibited in the presence of 100ppm cycloheximide. The morphological and physiological characters of the isolated strains were compared with that of the reference strains. From the comparison it was concluded that the strains M-231 and M-238 were identical to *S. cerevisiae* and M-239 be identified as *K. marxianus*.

Growth in a synthetic medium

The growth of the three isolated strains in the synthetic medium was determined and compared with that of the reference strain of *S. cerevisiae* (Fig. 1). The strain M-239 grew better than that of M-231, M-238, and IFO-1-84 at 34°C.

Effect of pH and temperature on fermentation

Using the three isolated yeasts and the *S. cerevisiae* reference strain the synthetic medium was fermented at different temperatures and different pH value and their fermentation characters were

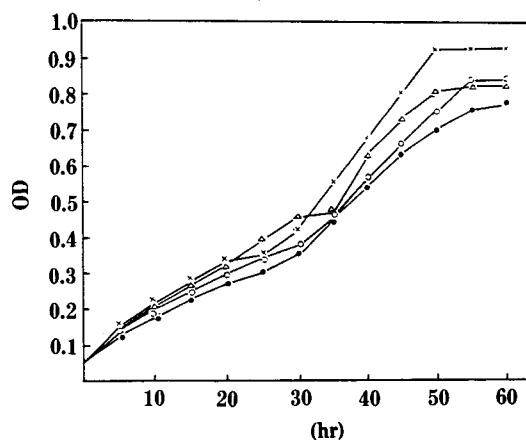


Fig. 1. Growth curves of yeasts isolated from molasses and *S. cerevisiae* IFO 1-84.

Yeasts were incubated at 34°C in the synthetic medium (pH 5.5).

●—●: M-231 strain ×—×: M-238 strain △—△: M-239 strain ○—○: IFO-1-84

Table 6. Alcohol productivity of strains isolated from molasses at temperatures 25°C, 30°C, 34°C and 37°C (pH 5.0).

Strains	Final pH	Alcohol productivity (g/l)	Used sugar (g/l)	FR (%)	Temperature
M231	3.79	68.6	135.0	75.0	25°C
M238	3.86	71.0	139.0	77.2	
M239	3.87	65.3	127.8	71.0	
IFO-1-84	3.89	68.2	133.4	74.1	
M231	3.95	75.0	146.8	76.0	30°C
M238	4.00	75.1	147.0	79.2	
M239	4.00	74.4	145.7	74.3	
IFO-1-84	4.12	74.4	145.6	75.9	
M231	3.97	74.4	145.7	75.8	34°C
M238	3.93	75.2	147.2	78.6	
M239	3.96	74.8	146.4	73.3	
IFO-1-84	3.95	60.3	118.1	65.6	
M231	3.87	36.2	70.9	39.4	37°C
M238	3.81	41.5	81.3	45.1	
M239	3.77	30.8	60.3	33.5	
IFO-1-84	3.83	29.5	57.7	32.1	

FR: Fermentation Rate(%) = actual alcohol conc./theoretical alcohol conc. × 100

observed (Table 6). All strains preferred pH 5.0 for their fermentation than pH 4.0 or 6.0. These strains showed the highest alcohol productivity at 34°C. The alcohol productivity of the three isolated strains at this temperature were averaged as around 75g alcohol per liter of culture broth, which is equivalent to that of the *S. cerevisiae* reference strain. The highest alcohol fermentation rates at 34°C, M-231, M-238 and M-239 strains indicated 75.85%, 78.69% and 73.30%, while IFO-1-84 was 65.63%. The highest alcohol yields and productivities were obtained at 34°C. The strain M-231, M-239 which showed the highest yield at 34°C had the low productivity among the strains selected at 37°C (Table 6).

요 약

고온 알코올 발효에 필요한 새로운 내열성 효모 균주를 개발할 목적으로, 당밀에서 분리하였다. 내열성 효모중 높은 온도에서 알코올 생산능을 검토하여 알코올 생산능이 우수한 3균주를 얻었다.

이들 균주는 pH 5.0, 34°C에서 알코올 발효를 하였을 때 기질로서 포도당을 함유한 합성배지에서 평균 75g/l의 알코올을 얻을 수 있었다. 이들 균주는 *Saccharomyces cerevisiae*와 *Kluveromyces marxianus*로 동정되었다.

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