Studies on Osmophilic Yeasts

Part XII. Characteristic Aspects on the Assimilation and Fermentation of Sugars by Osmophilic Yeasts in the Environment of a High Concentration of Sodium Chloride

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Assimilation of galactose and maltose by *Saccharomyces rouxii*, which is a typical salt-tolerant yeast playing an important role in soy-brewing, was negligible or extremely poor in the medium containing 18% NaCl, although the assimilation in the ordinary medium was vigorous. The yeasts which were able to assimilate and ferment galactose, maltose and/or saccharose in the high-saline medium were limited to a few strains. From the studies on balances of fermentation products, it was revealed that ethanol yield based on sugar consumed in the 18% NaCl-medium was lower than that in the ordinary medium, suggesting that other fermentation products than ethanol should be accumulated. Analytical results of the fermented broths showed much polyalcohol production in the high-saline medium.

INTRODUCTION

Comparative studies on the metabolism of osmophilic yeasts in the ordinary medium and in the high-saline environment have been reported by the author¹⁻³⁾.

Takahashi and Yukawa⁴⁾ and Ishimaru⁵⁾ presented the analytical data of the broths fermented by soy-yeasts, although they did not refer to the special behavior of yeasts in the high-saline medium.

In this paper will be described some characteristic assimilation and fermentation of sugars by various salt-tolerant yeasts in the saline condition with reference to the shift of metabolism as compared with the ordinary culture.

The studies on these characteristics of the salt-tolerant yeasts might be not only of great use in the manufacture of soy-sauce and miso paste, but also full of interest in terms of the taxonomy of osmophilic and halophilic micro-organisms.

EXPERIMENTAL AND RESULTS

[I] Influence of a High Concentration of Sodium Chloride on Sugar Assimilation by Salt-tolerant Yeasts.

Sixty-nine salt-tolerant yeast strains^{6,7)} of various genera and species were examined as for their sugar assimilation in the ordinary media and those containing 18% NaCl. The media employed were as follows: (1): KH_2PO_4 0.1%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05%, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.01%, NaCl 0.01%, Bacto vitamin-free casamino acid 0.4%, Bacto yeast ext. 0.1%, either glucose, galactose, maltose or saccharose 3%. (2): (1) + NaCl 18%.

One loopful of the yeast taken from the fresh culture on koji extract agar was suspended in sterile saline. Attention was paid so as not to touch the agar

1) H. Ōnishi, This Journal, **21**, 143 (1957).

2) H. Ōnishi, *ibid.*, **23**, 332 (1959).

3) H. Ōnishi, *ibid.*, **23**, 351 (1959).

4) T. Takahashi and M. Yukawa, *Memoirs of the College of Agr., Tokyo Imp. Univ.*, **5**, 227 (1915).

5) Y. Ishimaru, *J. Fermentation Technol.*, **13**, 295 (1935).

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7) H. Ōnishi, *ibid.*, **24**, 386 (1960).

TABLE I. INFLUENCE OF A HIGH CONCENTRATION OF SODIUM CHLORIDE ON THE SUGAR ASSIMILATION BY SALT-TOLERANT YEASTS

Strains		Glucose		Galactose		Maltose		Saccharose	
		NaCl 0%	NaCl 18%	NaCl 0%	NaCl 18%	NaCl 0%	NaCl 18%	NaCl 0%	NaCl 18%
<i>Sacch. rouxii</i>	m 1	++	++	++	—	++	—	++	±
"	m 2	++	++	+	—	++	—	+	±
"	m 3	++	++	++	—	++	—	++	±
"	m 4	++	++	++	—	++	—	++	+
"	m 5	++	++	++	—	++	—	++	±
"	m 6	++	++	++	—	++	—	++	±
"	m 8	++	++	++	—	++	—	++	±
"	m 9	++	++	++	—	++	—	++	±
"	m 10	++	++	++	—	++	—	++	±
"	m 11	++	++	++	—	++	—	+	±
"	m 14	++	++	++	—	++	+	++	+
"	s 2	++	++	++	—	++	—	++	+
"	s 4	++	++	++	—	++	—	++	±
"	s 5	++	++	++	—	++	—	++	++
"	s 8	++	++	+	—	++	—	+	±
"	s 11	++	++	+	—	++	—	+	±
"	s 12	++	++	++	—	++	—	+	±
"	sal 1	++	++	++	—	++	—	+	±
"	sal 2	++	++	++	—	++	—	+	±
"	sal 3	++	++	++	—	++	—	+	±
"	sal 5	++	++	++	—	++	—	+	±
"	j 4	++	++	±	—	++	—	±	—
"	j 5	++	++	±	—	++	—	±	—
"	R 4	++	++	+	—	++	—	+	±
"	R 5	++	++	+	—	++	++	+	+
"	E 7	++	++	++	—	++	—	+	±
"	N 3	++	++	++	—	++	—	++	—
"	N 4	++	++	+	—	++	—	+	±
"	N 5	++	++	±	—	++	—	±	±
"	N 6	++	++	++	—	++	—	+	±
"	N 8	++	++	++	—	++	—	+	±
"	N 9	++	++	+	—	++	—	±	±
"	N 10	++	++	++	—			+	±
"	N 11	++	++	±	—			±	±
"	N 11a	++	++	++	—			+	±
"	N 15	++	++	±	—			±	±
"	N 13a	++	++	++	—			±	±
"	N 18	++	++	++	—			++	+
"	N 28	++	++	+	—			+	±
"	A 34	++	++	+	—	++	—	+	±
<i>Sacch. rouxii</i> var. <i>halomembranis</i>	sal 4	++	++	++	—	++	—	++	++
"	sal 6	++	++	++	—	++	—	++	±
"	j 3	++	++	++	++	++	++	++	++
"	j 6	++	++	++	+	++	++	++	++
"	N 16	++	++	++	++	++	—	+	+

TABLE I. (Continued)

Strains		Glucose		Galactose		Maltose		Saccharose	
		NaCl 0%	NaCl 18%	NaCl 0%	NaCl 18%	NaCl 0%	NaCl 18%	NaCl 0%	NaCl 18%
<i>Sacch. rouxii</i> var. <i>halomembranis</i>	N 22	+++	+++	+++		++		+	++
"	N 26	+++	++	+++		++		++	±
"	A 31	+++	+++	+++	—	+++	++	++	++
<i>Sacch. acidifaciens</i>	s 9	+++	+++	+	—	++	—	+	±
<i>Sacch. acidifaciens</i> var. <i>halomembranis</i>	R 6	+++	+++	+++	—	+	—	++	++
<i>Torulopsis halophilus</i>	N 24	+++	+++	+++	+	+	++	++	+
<i>Torulopsis nodaensis</i>	N 21	+++	+++	+++	+++	—	—	+	+
<i>Torulopsis famata</i>	E 29a	+++	++	++	++	++	±	++	++
<i>Torulopsis versatilis</i>	F 5	+++	+++	++	+	++	++	++	++
"	F 7	+++	+++	++	++	++	+++	++	+++
<i>Torulopsis sphaerica</i>	F 8	+++	+++	++	±	±	—	++	++
"	F 28	+++	+++	++	+++	+	±	++	+++
<i>Torulopsis sake</i>	F 11	+++	+++	++	+	++	++	++	++
<i>Torulopsis etchellsii</i>	A 23	+++	+++	+++	—	++	++	+++	+++
<i>Pichia farinosa</i>	A 6	+++	+++	+++	+++	++	++	+++	++
<i>Candida polymorpha</i>	Ek	+++	+++	+++	+++	+++	+++	+++	+++
<i>Trichosporon behrendii</i>	E 3a	+++	+++	+++	+++	+++	±	+++	++
<i>Hansenula subpelliculosa</i>	H 79	+++	+++	+++	±	+++	—	+++	++
<i>Debaryomyces hansenii</i>	H 70	+++	+++	+++	+++	+++	++	+++	+++

layer. One drop of this suspension was inoculated to each medium. After 15 and 30 days' incubation at 30°C, the extent of assimilation was judged from the growth of cells. The results are shown in Table I.

As expected, all the strains tested were able to assimilate glucose well in both media, ordinary and 18% NaCl. When galactose or maltose was used as a sole carbon source, *Sacch. rouxii* and *Sacch. rouxii* var. *halomembranis* showed good growth in the ordinary medium, whereas negligible or extremely poor growth was observed in the 18% NaCl-medium even after incubation for 30 days. Most of *Pichia* and *Torulopsis* could utilize galactose and maltose even in the 18% NaCl medium.

[II] Influence of a High Concentration of Sodium Chloride on Sugar Fermentabilities by the Salt-tolerant Yeasts.

From the experiment [I], it was found that assimilations of sugars other than glucose by the salt-tolerant yeasts were suppressed by the presence of 18% NaCl. Sugar fermentabilities were then examined on the selected strains which showed relatively good assimilation of the miscellaneous sugars even in the 18% NaCl medium.

First, semiquantitative fermentation test employing

Durham tube was carried out. As shown in Table II, the fermentation of sugars other than glucose in the 18% NaCl medium was poor.

Second, the time course of fermentation was examined quantitatively. Eighty ml of each of the media (the same as those in [I] except 5% sugars) were placed in a 150 ml Erlenmeyer flask, sterilized, inoculated with an organism and incubated at 30°C setting alwood tube. Evolution of CO₂ was gravimetrically estimated from the decrease in the total weight (Fig. 1). The salt-tolerant yeasts which were more or less able to ferment galactose, maltose or saccharose in the high-saline medium, were limited to few strains as follows:

(1) Yeasts which ferment galactose....*Torulopsis nodaensis* and *Torulopsis sake*.

(2) Yeasts which ferment maltose....*Torulopsis versatilis*, *Torulopsis etchellsii* and *Torulopsis sake*.

(3) Yeasts which ferment saccharose.....*Hansenula subpelliculosa* and *Torulopsis sphaerica*.

Sacch. rouxii, which plays an important role in soy-sauce brewing, could ferment glucose and maltose in the ordinary medium. In the presence of 18% NaCl, however, the yeast fermented only glucose, but not maltose. The time courses of fermentation by *Sacch.*

(1) Glucose Media

(2) Galactose Media

(3) Maltose Media

(4) Saccharose Media

FIG. 1. Influence of a High Concentration of Sodium Chloride on the Fermentation of Sugars by Salt-tolerant Yeasts.

Solid line: NaCl 0% Broken line: NaCl 18%

rouxii in the four types of media containing mixtures of glucose and maltose at various ratios are illustrated in Fig. 2. Similar curves showing the absence of effects of the mixing ratios of glucose and maltose were obtained in the media free of NaCl. On the contrary, four curves in the 18% NaCl-media were quite different; the fermentation proceeded in proportion to the amounts of glucose added.

[III] Influence of a High Concentration of Sodium

Chloride on Balances of the Fermentation Products by the Salt-tolerant Yeasts.

Highly salt-tolerant 18 strains of various genera and species, which were found in soy-sauce mashes and miso-paste, were employed.

The media used are as follows. (1) Glucose 10%, KH_2PO_4 0.1%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05%, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.01%, NaCl 0.01%, Bacto vitamin-free casamino acid 0.4%, and Bacto yeast ext. 0.1%. (2): (1) + NaCl 18%.

TABLE II. INFLUENCE OF A HIGH CONCENTRATION OF SODIUM CHLORIDE ON THE SUGAR FERMENTABILITY BY SALT-TOLERANT YEASTS

Strain		NaCl	
		0%	18%
(1) Maltose			
<i>Sacch. rouxii</i>	m 14	##	+
"	R 5	##	+
<i>Sacch. rouxii</i> var. <i>halomembranis</i>	sal 4	##	—
"	j 3	##	—
"	j 6	##	—
"	N 16	##	—
"	N 22	##	±
"	A 31	##	—
<i>Torulopsis sake</i>	F 11	##	##
<i>Torulopsis versatilis</i>	F 5	##	+
"	F 7	##	##
<i>Torulopsis etchellsii</i>	A 23	##	##
(2) Galactose			
<i>Torulopsis nodaensis</i>	N 21	##	+
<i>Torulopsis versatilis</i>	F 5	##	—
"	F 7	##	—
<i>Torulopsis sake</i>	F 11	##	+
<i>Torulopsis sphaerica</i>	F 8	##	—
"	F 28	##	—
<i>Trichosporon behrendii</i>	E 3a	##	—
<i>Debaryomyces hansenii</i>	H 70	##	—
<i>Pichia farinosa</i>	A 6	##	—
(3) Saccharose			
<i>Torulopsis versatilis</i>	F 5	##	—
"	F 7	##	—
<i>Torulopsis sake</i>	F 11	##	—
<i>Torulopsis sphaerica</i>	F 8	##	+
"	F 28	##	+
<i>Hansenula subpelliculosa</i>	H 79	##	##

For stationary culture, 100 ml each of one of these media were placed in a 150 ml Erlenmyer flask, sterilized, inoculated and incubated at 30°C. Shaking culture was carried out with 80 ml of these media placed in 500 ml shaking flasks. The flasks were shaken on a reciprocal shaker operating at 120 r.p.m. with a stroke of 75 mm at 30°C.

The fermentation broths after removal of yeast cells by filtration, were analysed according to the following methods.

Glucose: The Fehling-Lehmann Schoorl method.

Ethanol: After distillation in neutral, the oxidation method by dichromate was applied.

FIG. 2. Influence of a High Concentration of Sodium Chloride on the Fermentation of *Sacch. rouxii* in the Media Containing Glucose and Maltose at Various Ratio.

○ : glucose : maltose=8 : 0 × : glucose : maltose=4 : 4
 △ : glucose : maltose=2 : 6 ● : glucose : maltose=0 : 8
 solid line : NaCl 0% broken line : NaCl 18%

pH: pH test paper (Toyo Roshi Kaisha).

Total acid: Ether extracts from the broth acidified with H_2SO_4 were titrated with $N/20$ NaOH.

Polyalcohol: The method of Neish^{8,9)} was used, i.e. the polyalcohols were oxidized with periodic acid and formaldehyde formed was determined colorimetrically with a chromotropic reagent. The values obtained were corrected for glucose by running a standard glucose sample in parallel with the fermentation solutions.

2,3 Butanediol: A method was Desnuelle and Naudet's¹⁰⁾ as described by Neish⁸⁾, i.e. the oxidation with periodic acid followed by colorimetric determination of acetaldehyde formed with piperazine and nitroprusside solution. A correction was made for acetoin present.

Acetoin: The method of Westerfeld¹¹⁾ as described by Neish⁸⁾, i.e. the colorimetric determination with creatine and α -naphthol.

Sodium chloride did not interfere in the determination of polyalcohol and acetoin, but interfered in the determination of 2,3-butanediol. In the latter case, a correction curve was prepared for the sample containing NaCl.

Analytical results of the fermented broths are given

8) A.C. Neish, Analytical methods for bacterial fermentations 2nd rev. Natl. Research Council. Can., Prairie Regional Lab., Sask. N.R.C. No. 2952 (1952).

9) M. Lambert and A.C. Neish, *Can. J. Research*, **B 28**, 83 (1950).

10) P. Desnuelle and M. Naudet, *Bull. Soc. Chim. France*, **12**, 871 (1945).

11) W.W. Westerfeld, *J. Biol. Chem.*, **101**, 495 (1945).

TABLE III. INFLUENCE OF A HIGH CONCENTRATION OF SODIUM CHLORIDE ON FERMENTATION PRODUCTS BY SALT-TOLERANT YEASTS. (1) STATIONARY CULTURE

Strains	Glucose consumed %	Ethanol formed %	Ethanol yield on glucose consumed*	pH	Total acid % (as succinate)	Polyalcohol formed % (as glycerol)	Polyalcohol yield on glucose consumed (as glycerol)	2, 3-Butanediol formed %	Acetoin formed %
(a) NaCl-free medium (15 days' cultivation)									
<i>Sacch. rouxii</i> N 28	5.25	1.82	67.8%	3.8	0.069	0.409	7.8%	0.033	0.009
" E 7	6.20	2.24	70.6	3.4	0.099	0.504	8.1	0.027	0.003
<i>Sacch. rouxii</i> var. <i>halomembranis</i> A 31	7.62	3.01	77.3	3.4	0.111	0.345	4.5	0.025	0.002
<i>Sacch. rouxii</i> var. <i>halomembranis</i> sal 4	6.41	2.49	75.9	3.4	0.111	0.290	4.5	0.023	0.003
<i>Sacch. acidifaciens</i>	6.88	2.25	64.0	3.6	0.078	1.128	16.4	0.030	0.010
<i>Sacch. acidifaciens</i> var. <i>halomembranis</i>	8.55	3.19	73.0	3.4	0.114	0.762	8.9	0.025	0.002
<i>Torulopsis nodaensis</i>	4.81	1.68	68.3	3.2	0.141	0.544	11.3	0.011	0.001
<i>Torulopsis halophilus</i>	3.52	1.18	65.3	3.4	0.075	0.363	10.2	0.016	0.001
<i>Torulopsis versatilis</i>	2.52	0.71	55.1	3.6	0.099	0.387	15.3	0.007	0.001
<i>Torulopsis sphaerica</i>	4.53	1.34	57.9	2.4	0.159	1.014	22.3	0.014	0.002
<i>Torulopsis sake</i>	3.91	1.34	67.1	3.4	0.105	0.433	11.0	0.015	0.001
<i>Torulopsis etchellsii</i>	3.06	1.07	68.3	3.4	0.069	0.243	7.9	0.007	0
<i>Torulopsis famata</i>	1.29	0.06	39.3	4.2	0.027	0.096	7.4	0.003	0.001
<i>Pichia farinosa</i>	4.99	1.09	42.7	3.8	0.087	0.777	15.5	0.008	0
<i>Candida polymorpha</i>	1.15	0.19	32.3	4.8	0	0.172	14.9	0.003	0.001
<i>Trichosporon behrendii</i>	3.15	1.09	67.2	3.6	0.069	0.229	7.2	0.004	0
<i>Hansenula subpelliculosa</i>	5.21	2.00	75.1	3.8	0.069	0.325	6.2	0.007	0
<i>Debaryomyces hansenii</i>	3.08	0.62	39.3	4.0	0.045	0.611	19.8	0.004	0
(b) NaCl 18% medium (18 days' cultivation)									
<i>Sacch. rouxii</i> N 28	5.51	1.63	57.3%	3.4	0.075	0.573	10.3%	0.039	0.003
" E 7	5.10	1.34	51.4	3.4	0.078	0.759	14.8	0.036	0.003
<i>Sacch. rouxii</i> var. <i>halomembranis</i> A 31	6.03	1.92	62.2	3.2	0.075	0.509	8.4	0.029	0.001
<i>Sacch. rouxii</i> var. <i>halomembranis</i> sal 4	4.82	1.38	56.0	3.2	0.099	0.702	14.5	0.024	0.001
<i>Sacch. acidifaciens</i>	5.36	1.53	55.8	3.4	0.090	0.854	16.0	0.025	0.003
<i>Sacch. acidifaciens</i> var. <i>halomembranis</i>	3.92	1.15	57.4	3.4	0.096	0.683	17.4	0.023	0.001
<i>Torulopsis nodaensis</i>	3.64	1.11	59.6	3.2	0.093	0.366	10.0	0.010	0.001
<i>Torulopsis halophilus</i>	4.27	1.39	63.6	3.2	0.078	0.300	7.0	0.010	0.001
<i>Torulopsis versatilis</i>	2.60	0.69	51.9	3.4	0.069	0.419	16.1	0.017	0.002
<i>Torulopsis sphaerica</i>	4.58	1.56	66.6	3.4	0.069	0.308	6.7	0.013	0.001
<i>Torulopsis sake</i>	4.16	1.34	63.0	3.4	0.063	0.372	8.9	0.021	0.001
<i>Torulopsis etchellsii</i>	4.03	1.29	62.6	3.2	0.075	0.256	6.3	0.001	0
<i>Torulopsis famata</i>	0.88	0.04	8.9	4.0	0.006	0.130	14.7	0	0
<i>Pichia farinosa</i>	4.44	0.07	3.1	3.2	0.063	1.162	26.1	0.001	0
<i>Candida polymorpha</i>	1.14	0.13	22.3	4.0	0.003	0.142	12.4	0.002	0.001
<i>Trichosporon behrendii</i>	1.92	0.19	19.3	4.0	0.003	0.238	12.3	0.002	0
<i>Hansenula subpelliculosa</i>	5.10	1.59	61.0	3.4	0.069	0.571	11.2	0.031	0.004
<i>Debaryomyces hansenii</i>	3.75	0.06	3.1	3.6	0.039	0.991	26.4	0.001	0

* This value was conventionally calculated from Gay-Lussac formula of ethanol fermentation.

TABLE IV. INFLUENCE OF A HIGH CONCENTRATION OF SODIUM CHLORIDE ON FERMENTATION PRODUCTS BY SALT-TOLERANT YEASTS. (2) SHAKING CULTURE

Strains	Glucose consumed %	Ethanol formed %	Ethanol yield on glucose consumed*	pH	Total acid % (as succinate)	Polyalcohol formed % (as glycerol)	Polyalcohol yield on glucose consumed (as glycerol)	2, 3-Butanediol formed %	Acetoin formed %
(a) NaCl-free medium (6 days' cultivation)									
<i>Sacch. rouxii</i> N 28	9.02	1.12	24.3%	3.8	0.009	1.282	14.2%	0.167	0.064
" E 7	9.18	0.07	1.5	3.4	0.024	1.995	21.7	0.031	0.003
<i>Sacch. rouxii</i> var. <i>halomembranis</i> A 31	8.50	0.28	6.4	2.6	0.054	1.664	19.5	0.028	0.006
<i>Sacch. rouxii</i> var. <i>halomembranis</i> sal 4	7.06	1.60	44.3	3.4	0.033	0.899	12.7	0.158	0.080
<i>Sacch. acidifaciens</i>	6.42	1.23	27.4	3.4	0.078	1.108	17.2	0.072	0.038
<i>Sacch. acidifaciens</i> var. <i>halomembranis</i>	9.26	0.04	0.7	3.4	0.051	2.587	25.2	0.016	0.003
<i>Torulopsis nodaensis</i>	1.65	0.05	5.9	2.2	0.210	0.055	3.3	0.008	0.001
<i>Torulopsis halophilus</i>	1.76	0.05	5.6	2.6	0.048	0.056	3.1	0.003	0
<i>Torulopsis versatilis</i>	2.41	0.03	2.4	2.2	0.099	0.849	35.2	0.005	0
<i>Torulopsis sphaerica</i>	2.07	0.01	1.0	2.0	0.189	0.544	26.2	0.007	0
<i>Torulopsis sake</i>	2.19	0.07	6.3	2.2	0.159	0.400	18.2	0.008	0
<i>Torulopsis etchellsii</i>	5.02	0.02	0.8	2.8	0.117	0.067	1.3	0.007	0
<i>Torulopsis famata</i>	7.24	0.02	0.5	4.0	0	1.657	22.9	0.005	0
<i>Pichia farinosa</i>	9.18	0.03	0.6	2.6	0.009	1.695	18.4	0.001	0
<i>Candida polymorpha</i>	8.23	0.02	0.5	4.0	0	1.952	23.7	0.002	0
<i>Trichosporon behrendii</i>	9.21	0.05	1.0	4.0	0	0.246	2.6	0.008	0.001
<i>Hansenula subpelliculosa</i>	9.11	0.18	3.8	2.4	0.129	0.541	6.0	0.043	0.028
<i>Debaryomyces hansenii</i>	4.15	0.67	21.5	3.4	0.054	0.569	13.7	0.011	0.001
(b) NaCl 18% medium (10 day's cultivation)									
<i>Sacch. rouxii</i> N 28	7.66	0.07	1.8%	3.2	0.057	2.843	37.1%	0.025	0.006
" E 7	7.83	0.08	2.0	3.0	0.030	2.248	28.7	0.028	0.002
<i>Sacch. rouxii</i> var. <i>halomembranis</i> A 31	6.43	0.03	0.9	3.0	0.057	1.567	23.5	0.025	0.001
<i>Sacch. rouxii</i> var. <i>halomembranis</i> sal 4	1.25	0.22	34.4	3.8	0.009	0.167	13.3	0.010	0.008
<i>Sacch. acidifaciens</i>	3.89	0.37	18.6	3.4	0.039	1.344	34.5	0.024	0.008
<i>Sacch. acidifaciens</i> var. <i>halomembranis</i>	1.79	0.21	22.4	3.8	0.027	0.227	12.7	0.007	0.002
<i>Torulopsis nodaensis</i>	1.53	0	0	2.0	0.093	0.102	6.6	0	0
<i>Torulopsis halophilus</i>	2.30	0.01	0	3.6	0.003	0.312	13.5	0	0
<i>Torulopsis versatilis</i>	1.37	0.23	32.8	2.6	0.069	0.171	12.4	0.005	0.002
<i>Torulopsis sphaerica</i>	1.05	0.15	27.9	2.6	0.066	0.146	13.9	0.005	0.001
<i>Torulopsis sake</i>	1.05	0.11	20.5	2.6	0.166	0.086	8.2	0	0
<i>Torulopsis etchellsii</i>	2.05	0.03	3.0	3.6	0	0.037	1.8	0.002	0
<i>Torulopsis famata</i>	6.93	0	0	4.2	0	0.058	0.8	0	0
<i>Pichia farinosa</i>	7.96	0.01	0	3.6	0.012	1.228	15.4	0.001	0
<i>Candida polymorpha</i>	7.28	0	0	4.2	0	0	0	0	0
<i>Trichosporon behrendii</i>	3.76	0	0	4.2	0	0.237	6.3	0.001	0
<i>Hansenula subpelliculosa</i>	5.72	0	0	2.6	0.033	1.667	29.1	0	0
<i>Debaryomyces hansenii</i>	3.39	0.02	1.0	3.6	0.009	1.131	33.3	0.007	0.001

* This value was conventionally calculated from Gay-Lussac formula of ethanol fermentation.

in Table III and IV. It was revealed that the amount of ethanol produced in the 18% NaCl-medium was much less than that expected from the amount of glucose consumed. It might be that some alternative products were formed in the high-saline medium. From the analyses of acids, polyalcohols, 2,3-butanediol and acetoin, it was found that much polyalcohol was produced in the high-saline medium. Such polyalcohol production had been previously reported by the author in detail¹²⁻¹⁴⁾.

DISCUSSION

Sugar assimilability and fermentability are the most important criteria in the taxonomy of yeasts. Present observation that these properties of the salt-tolerant yeasts can be dras-

tically altered by changing the concentration of NaCl in the medium, should be noteworthy not only in the taxonomy of the osmophilic and halophilic microorganisms, but also in the industrial utilization of these microorganisms.

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12) H. Ōnishi, This Journal, **23**, 359 (1959).

13) H. Ōnishi, *ibid.*, **24**, 126 (1960).

14) H. Ōnishi, *ibid.*, **24**, 131 (1960).