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Ecological Distribution and Phenotypic Diversity of *Saccharomyces cerevisiae* Strains from the Wine－Producing Area in Yamanashi, Japan

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The analysis of yeast ecology in the enological environment is important for knowing the distribution and diversity of yeasts and for evaluating their potential for winemaking. The *Saccharomyces cerevisiae* strains were isolated from vineyard soils, grapes, grape juices and grape pomaces in the Yamanashi area and were classified into 35 karyotypes. Seven of the 35 karyotypes appeared more frequently in the environment. The isolates were classified into 249 strains according to their karyotype. The population of *S. cerevisiae* in the vineyard soils differed from location and season, and yeasts in vineyard soil are thought to be the main sources of their occurrence in the environment. The indigenous yeasts in wineries also contributed to yeast flora of grape musts. The presence of the same karyotypes of wine strains which have been used for starter culture in the wineries of the Yamanashi area was observed. There were killer, resistant（or neutral）and sensitive strains among the *S. cerevisiae* strains; most of the strains were copper resistant and H_2S productive; and minor numbers of the strains showed active forming and film-forming properties. Many strains had good fermentability and were evaluated as useful potential strains for wine fermentation.

Key words: yeast ecology, *Saccharomyces cerevisiae*, wine yeast, enological property, fermentability of grape must

INTRODUCTION

Yeast flora of grapes, fermenting－musts and wines have been widely investigated in wine－producing countries to analyze the distribution and the evolution of yeast composition during the winemaking process, and also to differentiate useful yeast flora or yeast strains for wine fermentation（3, 8, 12）. The yeasts distributed in ripened grapes and in the early stages of spontaneous fermentation are commonly dominated by non－*Saccharomyces* yeasts belonging to the genera *Kloeckera*（its telemorphic genus *Hanseniaspora*）, *Rhodotorula*, *Cryptococcus* and others, and the occurrence of *Saccharomyces* yeasts, especially *S. cerevisiae* strains, is normally very rare or in small proportion（2, 3, 10, 13）. However, *S. cerevisiae* strains grow and conduct alcoholic fermentation during the middle and last stages of fermentation, and ethanol－tolerant yeasts survive in the fermented wines. In Japanese vineyards and wineries, almost the same distributions of wild yeasts have been demonstrated（4, 5, 15, 16, 24）.

The distribution of *Saccharomyces* and non－*Saccharomyces* yeasts in musts and wines varies

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according to vineyard, grape variety, harvesting practice and winemaking technology. The diversity of yeast flora is considered to affect alcoholic fermentation and wine quality. Wine yeast strains (*S. cerevisiae*) with useful fermentation properties are obtained by selective breeding from the isolates from grapes, fermenting-musts and wines and the use of starter cultures of wine yeast strains is common in winemaking at the present time. Recent progress in the analysis of DNA polymorphism has been used to monitor yeast populations of *Saccharomyces* strains during wine fermentation. Pulsed field gel electrophoresis (PFGE) karyotypes and analyses of restriction fragment length polymorphism (RFLP) of mitochondrial DNA and of amplified specific DNA sequences are employed for the purpose. A survey of spontaneous fermentation in European wine-producing regions has shown some particularities of the indigenous *Saccharomyces* yeasts and the dominance of several strains of *S. cerevisiae* during fermentation (14, 19, 21, 22). It indicated that DNA analysis of *Saccharomyces* yeasts is practical to analyze yeast ecology and to characterize proper strains in a wine-producing region. In the area of Yamanashi, yeast flora of vineyards and wineries have been studied (5, 24); however, the distribution and the diversity of indigenous *Saccharomyces* yeasts in the enological environment have not been analyzed well.

In this study, we performed an ecological and seasonal survey of *S. cerevisiae* strains isolated from vineyards and wineries of the Yamanashi area, which is a principal wine-producing area in Japan. This survey covers two successive years and the isolated strains were examined for PFGE karyotype and biological and enological properties. The diversity of *S. cerevisiae* strains in the environment was demonstrated by this study.

MATERIALS AND METHODS

Samples for the isolation of yeasts

Soils, grapes, grape juices and grape pomaces were collected from vineyards and wineries of three viticultural districts, Katsunuma, Ichimiya and Kofu in Yamanashi, which are popular areas for grape-growing and wine-producing in Japan. About 50 to 300 grams of samples were taken aseptically into plastic bags or bottles during the vinification period in 1994 and 1995; the vineyard's soils were collected at the same locations every time for four seasons.

Isolation and identification of *Saccharomyces* yeasts

Grapes were crushed in sterile bags. The juices from crushed grapes and the grape juice samples from wineries were allowed to ferment spontaneously at 25 ℃ for 3 to 5 days in our laboratory. A few milliliters of pre-fermented grape juice and a few grams of soil or pomace were incubated in an isolation broth (0.3% yeast extract, 0.3% polypeptone, 3% glucose, 3% ethyl alcohol, 0.015% chloramphenicol, pH 4.5) at 25 ℃ for 15 days. Aliquots of 0.2 mL of diluted broth culture (*ca* $1-10\times10^2$ cfu/mL) were plated onto plates of an isolation agar medium (3% agar was added to the isolation broth) and incubated anaerobically in an anaerobic jar (BBL GasPas, USA) with oxgen absorbing agent at 25 ℃ for 7 days. Three to ten yeast colonies were taken according to the yeast populations and the yeast cells of *S. cerevisiae* shape were isolated with a micromanipulator and cultured on YM agar medium (0.3% yeast extract, 0.5% polypeptone, 0.3% malt extract, 1% glucose, 1.5% agar) at 25 ℃ for 3 days. The cultures were preserved at 5 ℃.

Identification of the purified cultures was carried out according to the morphological and cultural characteristics published by Kreger-Van Rij (7).

Electrophoretic condition of PFGE karyotyping

PFGE karyotyping was performed to differentiate the *S. cerevisiae* strains by the procedure of Yanagida et al. (25).

Test of biological and enological properties

1) Killer activity and resistance to killer: This was determined by using 3 reference killer strains (K_1, K_2, K_3; see Table 3) (27) and a sensitive strain (*Candida glabrata* IFO 0622) according to the method described previously (5). Resistance (or neutral) to killer was determined by using the 3 killer strains according to the same manner.

2) Resistance to copper: This was examined using a YM agar medium at 100 to 1000 mg/L of $CuSO_4$, which was supplemented into the medium after autoclaving. The yeast strain was precultured in the YM agar medium

Table 1. Isolation of *S. cerevisiae* from the enological environment in the Yamanashi area

Sampling sites	Sources				Sum of isolates
	Soil	Grape	Grape juice	Pomace	
Vineyard−1 (Kofu)[d]	26[a]/57[b]	16/29	nt[c]	nt	42
Vineyard−2 (Katsunuma)	119/46	42/14	nt	49/16	210
Vineyard−3 (Ichimiya)	40/58	20/20	nt	nt	60
Winery−1 (Kofu)	nt	nt	64/24	nt	64
Winery−2 (Katsunuma)	nt	nt	20/6	nt	20
Total	185/161	78/63	84/30	49/16	396

a) Isolates b) Samples c) Not tested d) Location

and the culture suspension in sterile water was inoculated onto medium plates, which were cultured for 3 days at 25 ℃. Two wine yeast strains, RIFY 1001 and RIFY 1022, served as references (18).

3) Production of hydrogen sulfide (H_2S): This was assessed by the blackening of yeast streaks on BiGGY agar (Difco Laboratories, Detroit, USA) medium after 3 days incubation at 25 ℃ (18).

4) Fermentation tests: These were performed in 25 ml test tubes containing 10 ml of Koshu grape juices of 18% and 35% sugar content (ameliorated with glucose). The juices were inoculated (*ca* 10^6 cells/mL) with pre−cultured yeast in grape juice and fermented at 25 ℃ (or 15 ℃). The fermentation times were as follows: (a) 10 days for the evaluation of fermentation rate and 25 days for the observation of foaming and film−forming at 25 ℃ in grape juice of 18% sugar content, (b) 25 days for the evaluation of fermentation rate at 25 ℃ in grape juice of 35% sugar content, and (c) 20 days for the evaluation of fermentation rate at 15 ℃ in grape juice of 18% sugar content. The tests were done in pairs. The alcoholic fermentations were terminated by cooling at 3 ℃, then the test lots were filtered with filter paper (Advantec, No.5C, Toyo Roshi Co., Tokyo) and analyzed for ethanol content by gas chromatography (16). Wine yeast strain RIFY 1001 (*S. cerevisiae*) served as a reference.

5) Foaming and film formation: Foaming and film−forming activities were observed at 25 ℃ in fermenting grape juice of 18% sugar content for 25 days.

RESULTS AND DISCUSSION

S. cerevisiae strains isolated from the enological environment

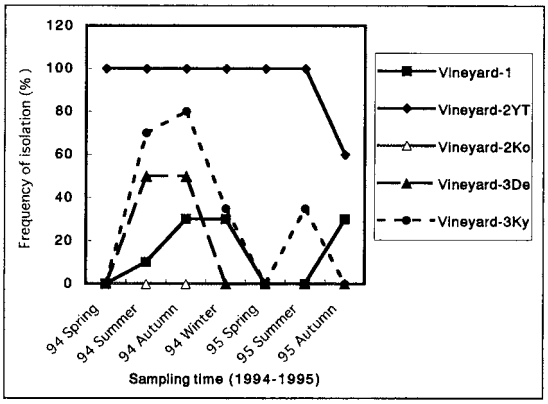

Fig. 1. Seasonal changes of *S. cerevisiae* strains isolated from vineyard soils during 1994 and 1995

Frequency of isolation indicates the percentage of occurrence of *S. cerevisiae* strains in the samples (4 to 8 samples). The sampling sites are shown in Table 1. Vineyard−2YT, Vineyard−3Ko, Vineyard−3De and Vineyard−3Ky: the vineyard numbers and the initial letters indicate the sampling sites.

A total of 396 strains, which were isolated from soils, grapes, grape juices and pomaces of the vineyards or the wineries in the Yamanashi area, were identified as *S. cerevisiae* (Table 1). As the yeasts were classified according to the classification of genus *Saccharomyces* (*Saccharomyces sensu strict*, Yarrow, 1984) (26), the yeast culture in this study included several species (*S. cerevisiae*, *S. bayanus*, *S. pastorianus*, *S. paradoxus*) that have recently appeared in the grouping of *Saccharomyces* species (Kurtzman and Fell, 1998) (9). *S. cerevisiae* strains were always isolated from grape juices and pomaces, but the yeast was not isolated from some samples of soils and grapes. The seasonal

Table 2. Electrophoretic karyotypes of *S. cerevisiae* strains and their distribution in the enological environment in the Yamanashi area

Analysis	Sources				Sum
	Soil	Grape	Grape juice	Pomace	
Origin of the karyotype	17	5	8	5	35 types [a)]
Appearance of the karyotype (A)	23	20	15	14	
Distribution of the strains (B)	116	42	56	35	249 strains [b)]
Karyotypic diversity (B/A)	5.0	2.1	3.7	2.5	

a) 35 karyotypes. No.1 to 17: SI−1, SI−2, SI−3, SI−4, SK−1, SK−2, SK−3, SK−4, SK−5, SK−6, SK−7, SK−8, SK−9, SK−10, SK−11, SK−12, SIM−1 (originated from soils); No.18 to 25 and 29: GI−1, GI−2, GI−3, GI−4, GI−5, GI−6, GI−7, GI−8, GK−4 (originated from grape juices); No.26 to 28 and 30: GK−1, GK−2, GK−3, GIM−1 (originated from grapes); No.31−35: PK−1, PK−2, PK−3, PK−4, PK−5 (originated from pomaces)

b) The 396 isolates were reduced to 249 strains by sorting the isolates according to karyotype.

changes of *S. cerevisiae* strains isolated from vineyard soils are shown in Fig.1. In the vineyard (Vineyard−2YT; YT: initials of the vineyard's name), they were almost always isolated from soils at sampling time. In three vineyards (Vineyard−1, Vineyard−3De, Vineyard−3Ky; De, Ky: indication of sampling sites) their isolation rate changed with time, increasing from summer to autumn and decreasing in winter and spring. There was no isolation of the *S. cerevisiae* strain in Vineyard−3Ko (Ko: indication of sampling site). More *S. cerevisiae* populations were observed in organic soils, where much manure was applied, than in clean and sandy soil. The results indicated that vineyard soils are habitats of *S. cerevisiae* yeasts and the yeast population differed according to sites and seasons. This seems to be related to ecological conditions such as fertility of soils, vegetation of vines and ripening of grapes. The yeasts in vineyard soils may have been transferred to grapes by insects and small animals, as has been reported for the yeasts associated with grapes (11, 19) and living plants (1).

Electrophoretic karyotype and ecological distribution of *S. cerevisiae* strains

The PFGE karyotype of *S. cerevisiae* strains was examined and classified into 35 karyotypes. Then the 396 strains were reduced to 249 strains by sorting the isolates in a sample by the karyotypes (Table 2), as it was supposed that the isolated strains having the same karyotype from a sample were the segregants of a wild yeast. PFGE band patterns of some isolates from vineyard soil, grape, juice and pomace are shown in Fig.2. Model patterns for the 35 karyotypes are shown in Fig.3. The origin (the karyotypes by the original source) and the appearance (the karyotypes of the isolates by sampling site) of the 35 karyotypes are demonstrated in Table 2. The most diverse karyotypes (17 types) originated from vineyard soils, and less diversity was shown in grape juices (8 types), pomaces (5 types) and grapes (5 types). Among the 35 karyotypes, 7 types, SK−1 (34 strains / bar−No.5 in Fig.4), SI−2 (22 strains / bar−No.2), SK−2 (17 strains / bar−No.6), SK−3 (15 strains / bar−No.7), SK−5 (14 strains / bar−No.9), GI−5 (13 strains / bar−No.22) and PK−3 (12 strains / bar−No.33), appeared more frequently in the environment. As for the number of karyotypes in the collections of *S. cerevisiae* strains isolated from grapes and grape musts, it was reported that there were 49 karyotypes for Champagne vineyards and 12 types of mitochondrial DNA restriction profiles for Loire valley (22), 70 karyotypes for Charentes (21), 46 karyotypes for Western Cape (19), and 4 karyotypes for sherry wine in barrels in Jerez (6). Various karyotypes appeared in every source and this indicated a diverse distribution of *S. cerevisiae* in the enological environment.

The distribution of *S. cerevisiae* strains by sampling site is shown in Fig.4. V1, V2, V3, W1 and W2 correspond to Vinyard−1, 2, 3 and Winery−1 and 2 (Table 1); S, G, J and P indicate the samling sources (soil, grape, grape juice, pomace). The yeast population is expressed as a bar chart for the the 35 karyotypes (Bar−No.1 to 35 in

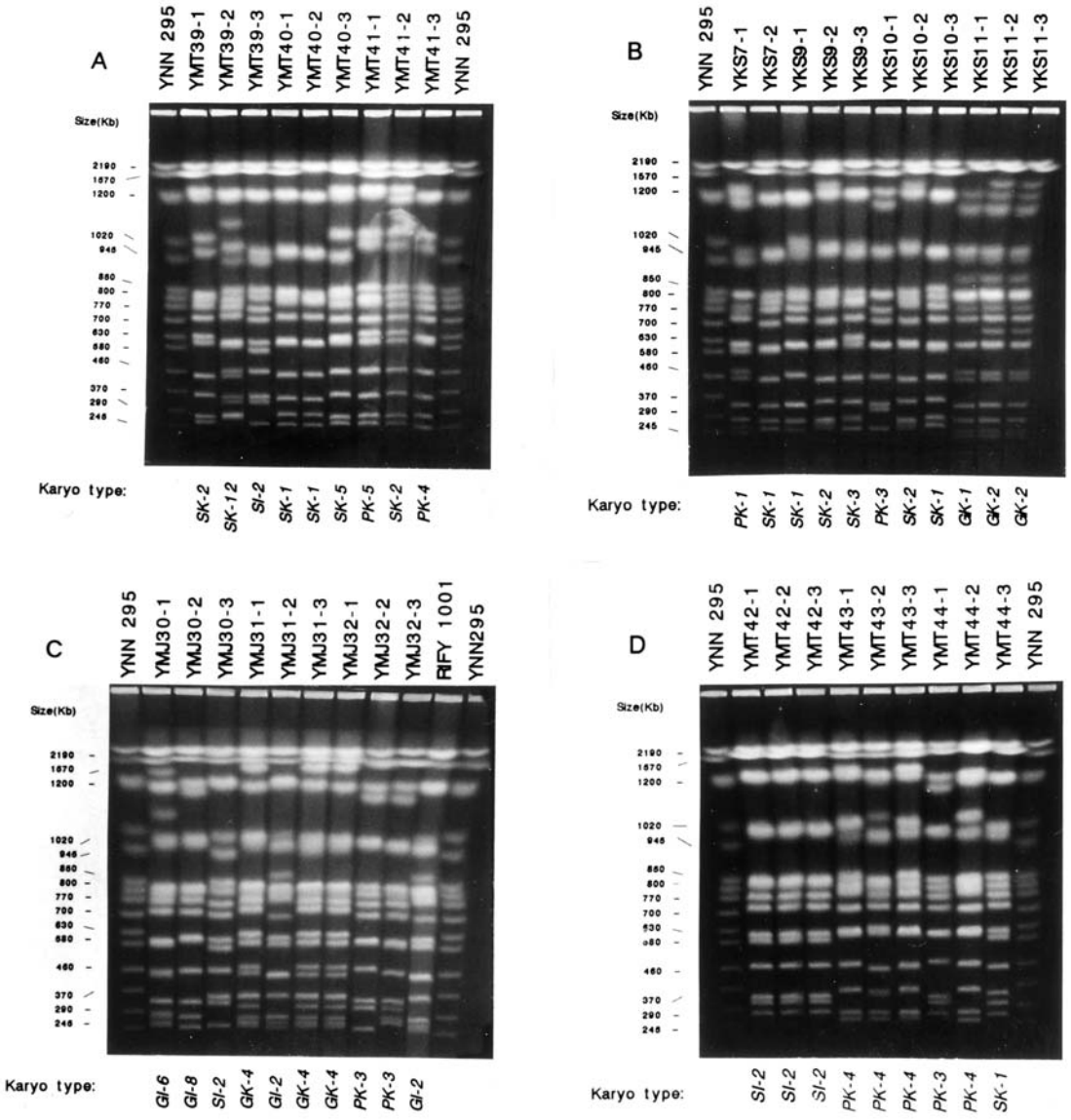

Fig. 2. Chromosomal DNA patterns of *S. cerevisiae* strains isolated from Vineyard－2 and Winery－2

Samples: A, soils; B, grapes; C, grape juices; D, pomaces. The upper side indicates strain number and the lower side indicates karyotype. YNN 295: DNA size marker; RIFY 1001 (wine yeast strain): Reference of karyotype, GI－2 (in sample C)

Fig.4; bar－No.36: scale marker, 10 strains). The distributions of *S. cerevisiae* strains between the soils and the grapes were almost the same in Vineyard－1 and Vineyard－3, but a different distribution between grapes and grape juices was demonstrated in Winery－1. In the soils of Vineyard－2, various karyotypes of *S. cerevisiae* appeared; however, fewer karyotypes were distributed in the grapes (Vineyard－2), the grape juices (Winery－2) and the pomaces (Vineyard－2). A specific distribution of karyotype No.18 was demonstrated in the grape juices, and this seemed to be one of the indigenous yeasts (Winery－1). The results indicate that the yeast population in vineyard soils is a main source of yeast occurrence in the enological environment, and the yeast flora of grape musts and pomaces is also influenced by the indigenous yeasts living in the winery and the environ-

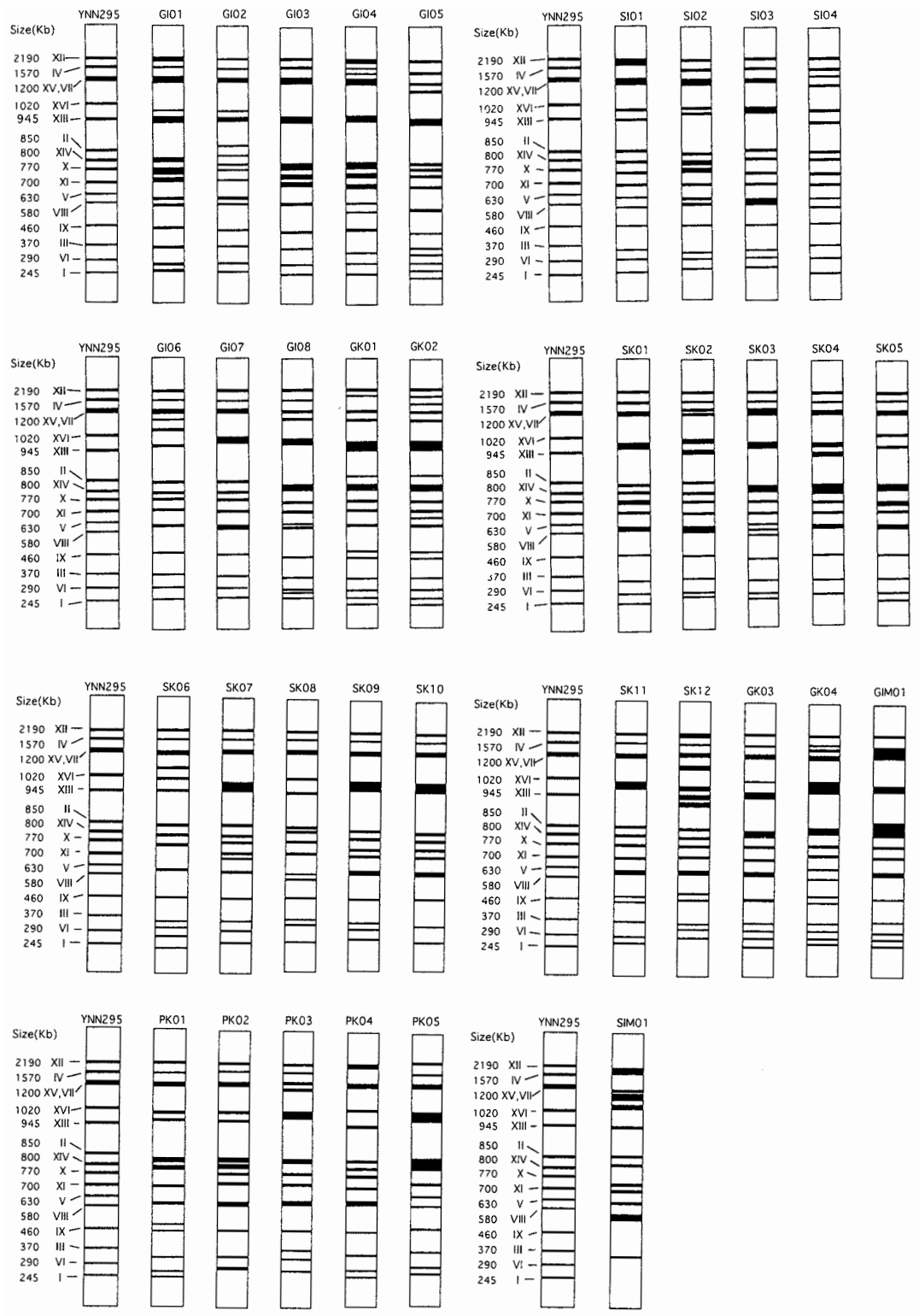

Fig. 3. Model patterns of the chromosomal DNA bands for 35 karyotypes

YNN 295: DNA size and chromosome marker. The origin and karyotyping are shown in Table 2.

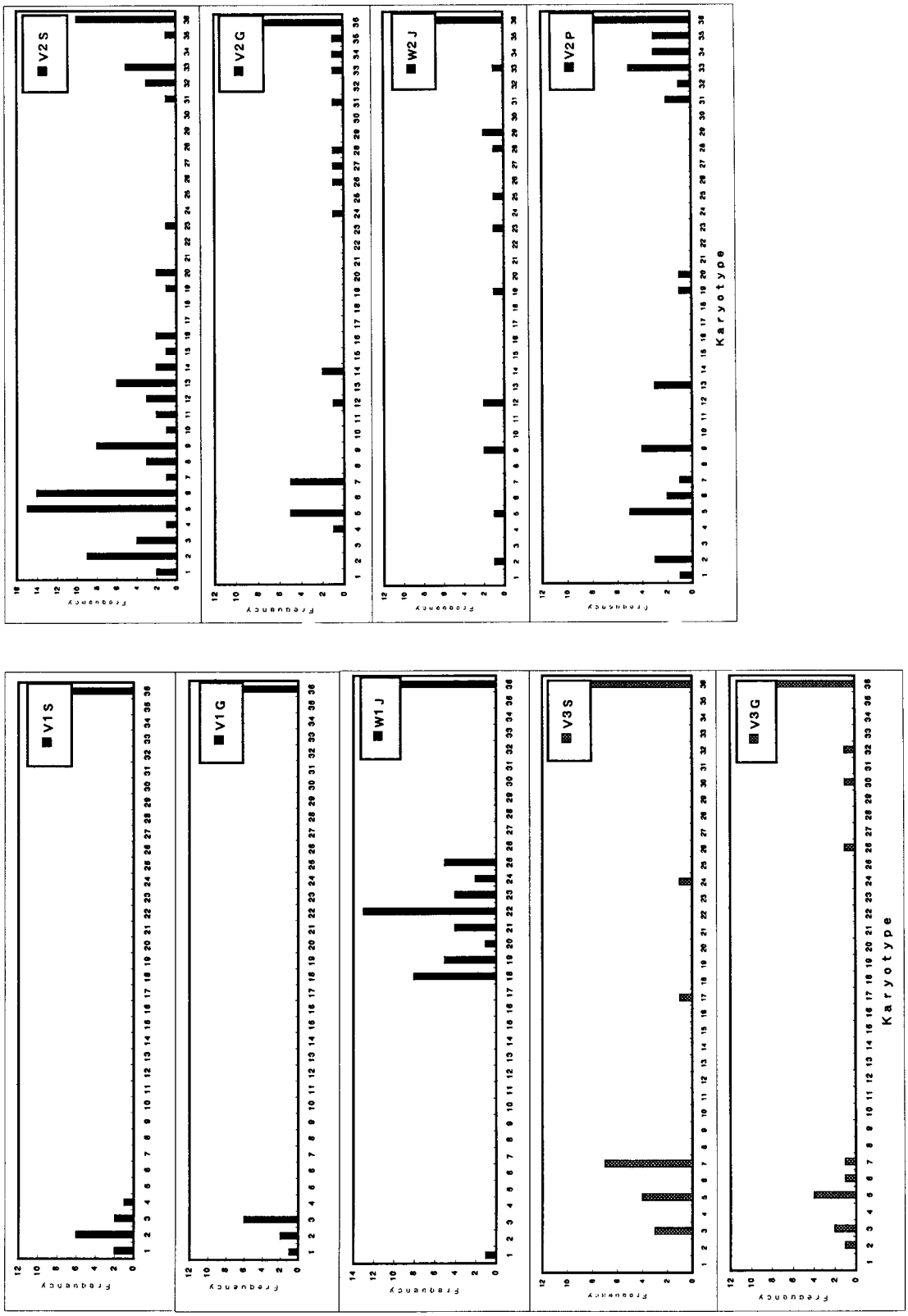

Fig. 4. Karyotypic distribution of *S. cerevisiae* strains in the enological environment

V1S: Vineyard−1/soils, V1G: Vineyard−1/grapes, W1J: Winery−1/juices, V3S: Vineyard−3/soils, V3G: Vineyard−3/grapes, V2S: Vineyard−2/soils, V2G: Vineyard−2/grapes, W2J: Winery−2/juices, V2P: Vineyard−2/pomaces. Frequency: the number of *S. cerevisiae* strains; bar−No.36 is scale marker (10 strains). Sampling sites are shown in Table 1.

Table3. Killer *S.cerevisiae* strains isolated from the enological environment in the Yamanashi area

No. [a]	Killer phenotypes [b]				Sources				Sum	Supposed
	K_1	K_2	K_3	IFO 0622	Soil 116	Grape 42	Juice 56	Pomace 35	249 [c]	killer type (K_1~K_{11}) [d]
KL−1	+	+	+	+	1	0	0	0	1	K_{10}
KL−2	+	+	+	−	4	1	0	0	5	K_{10}
KL−3	+	+	−	+	2	0	4	0	6	K_{11}
KL−4	+	−	+	+	6	0	0	1	7	K_5/K_7
KL−5	+	−	−	+	2	1	10	0	13	K_2/K_3
KL−6	−	−	+	+	1	0	0	0	1	
KL−7	−	+	−	−	0	0	2	0	2	
KL−8	−	−	+	−	0	1	0	0	1	
KL−9	−	−	−	+	32	13	25	8	78	KHS/KHR [b]
Active killer (A = KL − 1~8):					16	3	16	1	36	
Frequency (A/D [c] × 100, %):					13.8	7.1	28.6	2.9	14.5	
Weak killer (B=KL − 9):					32	13	25	8	78	
Frequency (B/D × 100, %):					27.6	31.0	44.6	22.8	31.3	
Total killer strains (A + B = C):					48	16	41	9	114	
Frequency (C/D × 100, %):					41.4	38.1	73.2	25.7	45.8	

a) This study

b) Killer activity to tester strains; +: active, −: no activity. Tester strains: K_1, *S. cerevisiae* A8209B; K_2, *S. cerevisiae* NCYC 1001; K_3, *S. cerevisiae* NCYC 761; *C. glabrata* IFO 0622, tester of KHS/KHR killer

c) Strains tested (D)

d) K_1~K_{11}: In literature (27)

ment.

In the Yamanashi area, two wine strains (RIFY 1001 / W−3, RIFY 1022 / OC−2) are usually used for the starter culture of wine fermentation and the same karyotypes of the two strains, GI−2 (8 strains / bar−No.19) and GI−8 (6 strains / bar−No.25), were also observed in juice samples (see Fig.2−C). It is quite normal that wine strains used in wineries were distributed in the environment, as the domestification of wine yeasts used for starter culture has been reported (2).

Killer activity and resistance (or neutral) to killer of *S. cerevisiae* strains

Thirty−six strains had active killer and their activity was classified as KL−1 to KL−8 (Table 3). Six strains (KL−1, 2) were very active and 14 strains (KL−1, 2, 3, 7) were active to K_2 killer strain, so these strains are very causal for wine yeast strains, as most wine yeast strains are non−killer and some wine yeast strains have K_2 killer (19, 20). There were 13 strains having K_2 killer (or K_3 killer) in this examination. Seventy−eight strains had weak killer (KL−9:KHS, KHR) (5). The results showed about half of *S. cerevisiae* strains tested were killer strains (114 strains), and this revealed a high frequency of killer yeasts in the environment of the Yamanashi area. This indicates the need for caution against contamination by killer yeasts in must fermentation when using starter cultures of sensitive wine yeast strains.

Almost half of *S. cerevisiae* strains tested were resistant (or neutral) strains (119 strains) and these strains were classified into 5 phenotypes (Table 4). Twenty−seven strains were resistant (or neutral) to the three killers (No.1: R_1+, R_2+, R_3+) and 30 strains (No.1, No.2: R_1+, R_2+, R_3−, No.4: R_1−, R_2+, R_3+) were resistant (or neutral) to K_2 killer. The other strains (89

Table 4. Killer resistance (or neutral) of *S. cerevisiae* strains isolated from the enological environment in the Yamanashi area

No.	Phenotype [a]	Sources				
		Soil	Grape	Juice	Pomace	Sum
		116	42	56	35	249 (100%) [b]
1	R_1+, R_2+, R_3+	13	6	6	2	27 (10.8%)
2	R_1+, R_2+, R_3-	0	0	0	1	1 (0.4 %)
3	R_1+, R_2-, R_3+	23	9	2	6	40 (16.0%)
4	R_1-, R_2+, R_3+	0	2	0	0	2 (0.8 %)
5	R_1+, R_2-, R_3-	23	13	6	7	49 (19.7%)
6	R_1-, R_2-, R_3-	57	12	42	19	130 (52.2%)
Resistant strains (No.1 ～ 5):		59	30	14	16	119
Frequency (%) [c]:		50.9	71.4	25.0	45.7	47.8

a) Resistant/neutral (R+) and sensitive (R−) to K_1, K_2 and / or K_3 killers; see Table 3.
b) Strains tested
c) Resistant strains / Strains tested × 100

Table 5. Copper resistance and H_2S production of *S. cerevisiae* strains

Source	Copper resistance (mg/L) [c]						H_2S production [d]		
	<100	100	200	300	500	700	(++)	(+)	(−)
Wine strains (2) [a]	0	0	2	0	0	0	2	0	0
Soil (116) [b]	33	6	9	42	25	1	46	55	15
Grape (42)	13	0	3	23	3	0	13	28	1
Grape juice (56)	1	0	8	31	16	0	11	31	14
Pomace (35)	6	1	5	6	17	0	8	23	4
Total (A):	53	7	25	102	61	1	78	137	34
Frequency (%) [e]	21.2	2.8	10.0	40.9	24.5	0.4	31.3	55.0	13.6

a) RIFY 1001, RIFY 1022
b) Number of strains tested
c) Concentration of $CuSO_4$
d) H_2S production: ++, very productive; +, productive; −, no production
e) Total strains (A)/249 strains × 100

strains) were resistant (or neutral) to K_1 and K_3 killers (No.3: R_1+, R_2-, R_3+, No.5: R_1+, R_2-, R_3-), but were sensitive to K_2 killer. In the previous data, there were 36 killer strains having the three killers (Table 3); however, 119 strains were resistant (or neutral) to these killers in this experiment. The results demonstrated that a higher number of the resistant (or neutral) *S. cerevisiae* strains were present than the killer strains in the sources. This seems to be natural for their survival in the yeast ecology and it may influence the yeast flora in the enological environment.

Resistance to copper

One hundred and ninety-six strains were resistant to the concentration of 100 to 700 mg/L·$CuSO_4$ (Table 5). Among these strains, 25 strains were resistant to 200 mg/L, which is the same resistance level for the two wine strains, and 164 strains (65.9% of the strains tested) were resistant to more than 300 mg/L. The result showed the frequent presence of copper resistance in the enological environment. Almost the same copper resistance has been reported for wild *Saccharomyces* strains from grape musts and this property was used to detect wild yeasts in grape musts and wines (18, 23).

Table 6. Fermentation properties of *S. cerevisiae* strains

Property	Sources				
	Soil	Grape	Juice	Pomace	Sum
	116	42	56	35	249 (100%)[a]
1. Alcoholic fermentation [Strains of good fermentabiliy][b]					
1) Fermentability at 25 ℃	85	32	33	25	175 (70.3%)[c]
2) Fermentability at 15 ℃	41	10	40	14	105 (42.2%)
3) Fermentability at 15 ℃ & 25 ℃	31	22	12	9	74 (29.7%)
4) Fermentability at 35% sugar content	34	4	23	11	72 (28.9%)
2. Foaming					
1) Active foaming	22	8	6	5	41 (16.5%)
2) Weak / no foaming	94	34	50	30	208 (83.5%)
3. Film－formation					
1) Film－forming	26	4	7	7	44 (17.7 %)
2) No film－forming	90	38	49	28	205 (82.3%)

a) Strains tested
b) Good (or better) fermentability as wine yeast strain RIFY 1001
c) Frequency (%): Sum of the strains / 249 strains × 100

Production of H_2S

Two hundred and fifteen strains (86.3% of the strains tested) produced H_2S: 78 strains were very productive as the two wine yeast strains and 137 strains were moderately productive (Table 5). This shows that most of the *S. cerevisiae* strains tested were H_2S productive. However, high H_2S productivity of wine yeast damages the flavor, so a fermentation test is always conducted to evaluate fermentability and aroma productivity (17).

Fermentation properties

1) Fermentability of grape juices: The fermentability of 249 strains was compared to the wine strain RIFY 1001 at 25 ℃ and 15 ℃, and in grape juice of 35% sugar content at 25 ℃. The number of *S. cerevisiae* strains having almost the same levels (or superior levels) of fermentability is noted in Table 6. Good fermentability was shown for 70.3% of the strains tested at 25 ℃, for 42.2% at 15 ℃, for 29.7% at both 25 and 15 ℃, and for 28.9% in the fermentation of 35% sugar content. The results indicate the presence of useful strains for wine fermentation in the *S. cerevisiae* strains tested.

2) Foaming: Active foaming was observed for 41 strains (16.5% of the strains tested) and the rest were weak or non－foaming strains (Table 6). This shows a minor presence of foaming *S. cerevisiae* strains in the enological environment. The foaming of yeasts is undesirable in fermentation.

3) Film formation: Film－forming was observed for 44 strains (17.7% of the strains tested, Table 6). This shows the normal presence of film－forming strains in the environment and the importance of controlling wild yeast, as the film－forming yeast is a spoilage microbe.

This study has demonstrated the distribution and the biological diversity of *S. cerevisiae* yeasts in the enological environment of the Yamanashi area based on karyotype and phenotype. It is suggested that some *S. cerevisiae* strains are useful as potential yeasts for wine-making.

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山梨県のワイン生産地環境における *Saccharomyces cerevisiae* 野生株の分布と多様性

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1994 年から 1995 年にかけて山梨県内のブドウ園やワイナリーの土壌，ブドウ果実，果汁および圧搾粕から野生酵母（*S.cerevisiae*）396 株を分離して核型を調べた．分離株は 35 核型を示し，このうち 7 核型が主たるものであった．これらの核型により分離株は 249 株に整理された．ブドウ園土壌からの分離株が多く，その分離頻度は採取地と季節により変化した．分離株の分離源を核型の比較から，土壌およびワイナリーに生息する酵母株がブドウ果実と発酵もろみの酵母相に反映することが示唆された．また，ワイナリーで使用される酒母用ワイン酵母 2 株と同様な核型株が分離された．分離株の性質は，多様なキラー性とキラー耐性（あるいは中性）を示し，多数株が銅耐性および硫化水素生産性であり，少数株が泡立性および皮膜形成能を有し，また，有用な発酵性を有する酵母株があった．