APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1983, p. 1034–1040
0099-2240/83/031034-07$02.00/0

Vol. 45, No. 3

Construction and Characterization of Isogenic Series of *Saccharomyces cerevisiae* Polyploid Strains

ATSUKO TAKAGI, SATOSHI HARASHIMA, AND YASUJI OSHIMA*

Department of Fermentation Technology, Osaka University, 2-1 Yamadaoka, Suita-shi, Osaka 565, Japan

Received 30 August 1982/Accepted 23 November 1982

Tetraploid cells of *Saccharomyces cerevisiae* are generated spontaneously in a homothallic *MAT***a**/*MAT*α diploid population at low frequency (approximately 10^{-6} per cell) through the homozygosity of mating-type alleles by mitotic recombination followed by homothallic switching of the mating-type alleles. To isolate tetraploid clones more effectively, a selection method was developed that used a dye plate containing 40 mg each of eosin Y and amaranth in synthetic nutrient agar per liter. It was possible to isolate tetraploid clones on the dye plate at a frequency of 1 to 3% among the colonies colored dark red in contrast to the light red of the original diploid colonies. Isogenic series of haploid to tetraploid clones with homozygous or heterozygous genomic configurations were easily constructed with the tetraploid strains. No significant differences in specific growth rate or fermentative rate were observed corresponding to differences in ploidy, although the haploid clones showed a higher frequency of spontaneous respiratory-deficient cells than did the others. However, a significant increment in the fermentative rate in glucose nutrient medium was observed in the hybrid strains constructed with two independent homozygous cell lines. These observations strongly suggest that the polyploid strains favored by the brewing and baking industries perform well not because of the physical increment of the cellular volume by polyploidy but because of the genetic complexity or heterosis by heterozygosity of the genome in the hybrid polyploid cells.

Polyploidy has often been observed in saccharomyces yeasts used in the brewing (1, 2, 20), baking (3), and alcoholic fermentation (Y. Oshima, unpublished data) industries. This indicates that polyploid yeasts may have advantages over the regular diploid yeasts under industrial conditions. To examine whether these advantages derive from physical or genetic features of the polyploids, namely, from their larger cellular volume, which enables them to carry more subcellular organelles, their smaller specific cellular surface area in comparison with haploid or diploid cells, or the wider variety of genetic information they may contain if they carry several sets of haploid genomes, we constructed various homozygously or heterozygously isogenic series of yeast strains from haploid or diploid to tetraploid.

A method for the construction of isogenic series of yeast strains of different ploidy, involving switching of the mating-type alleles by the function of the homothallic genes, has been suggested by Takano et al. (19). In homothallic strains, the two mating-type alleles, *MAT***a** and *MAT*α, undergo interchange at high frequency, generally in each cell division cycle, whereas heterothallic cells have stable mating types which switch with very low frequency (approximately 10^{-6}) (6, 7). The mechanism of the mating-type switch in the homothallic cells has been well elucidated (5): transposable genetic elements encoding specific information for either the *MAT***a** or the *MAT*α allele and originating from the *HML* and *HMR* loci (controlling element [4, 13] or cassette [7, 8]) can replace the element installed in the mating-type locus through the function of another homothallic gene, *HO*. Homothallic diploidization occurs by the fusion of two cells of complementary mating types generated in a single-spore culture. Cells having the genotype *HO MAT*α (or *MAT***a**) *HML***a** *HMR*α or *HO MAT*α (or *MAT***a**) *HML*α *HMR***a** show perfect homothallism and are designated Ho (14, 16) type I or type II (4), respectively, whereas those having the *HO MAT***a** *HML*α *HMR*α or *HO MAT*α *HML***a** *HMR***a** genotype show semihomothallism and are designated Hp or Hq, respectively. The mating-type switch does not occur in spore cultures of genotype *HO MAT*α *HML*α *HMR*α (Hp haploid [14, 16]) or *HO MAT***a** *HML***a** *HMR***a** (Hq haploid [14, 16]), because these genotypes have only one kind of mating-type information, α or **a**, respectively, in the genome. Hence, α Hp and **a** Hq

spores give rise to haploid vegetative cells of the same mating type by single-spore culture. The mating-type switch by the function of homothallic genes also occurs in diploid cells if they have mating potential. Once the homozygous *MAT***a**/*MAT***a** or *MAT*α/*MAT*α configuration of mating-type alleles has been generated from a homothallic *MAT*α/*MAT***a** diploid cell through mitotic recombination, the mating-type switch to *MAT***a**/*MAT*α (if only one of the *MAT***a** or *MAT*α alleles in the *MAT***a**/*MAT***a** or *MAT*α/*MAT*α configuration switches to the complementary allele) or the switch from *MAT***a**/*MAT***a** to *MAT*α/*MAT*α or vice versa (if double switching occurs) can be expected during subsequent cell multiplication. In the case of double switching, tetraploid cells isogenic to the original diploid cell might be produced by copulation between the switched and unswitched diploid cells. Since the original diploid is derived by self-diploidization of a haploid homothallic cell, these diploid and tetraploid cells are homozygous for all of the alleles in the genome, except for the mating-type alleles. An isogenic triploid cell can also easily be constructed by spore-to-cell mating between an **a** or α haploid spore and a vegetative diploid cell of *MAT*α/*MAT*α or *MAT***a**/*MAT***a** configuration originating from a spore of the Hp or Hq tetraploid strain of the same genomic series as the haploid spore. Thus, once tetraploids have been constructed, isogenic series of diploid and triploid strains can easily be constructed, as can the haploid strain if an Hp or Hq homothallic strain was adopted as the parental strain.

The greatest difficulty in tetraploid construction by this method is the low frequency of occurrence of the primary tetraploid zygotes: mitotic recombination from which the mating-type homozygosity results is estimated to occur at a frequency of 10^{-6} per cell spontaneously or 10^{-4} by the application of recombinogens, such as a low dose of UV irradiation (18). To overcome this difficulty we investigated the use of a dye plate for effective discrimination of tetraploid colonies from those of the original diploid.

This communication deals with a dye plate for the discrimination of colonies of isogenic diploid and tetraploid clones. Three independent isogenic series of haploid to tetraploid clones were constructed. One of these series was subjected to certain physiological tests, i.e., specific growth rate, fermentation rate, and frequency of spontaneous appearance of respiratory-deficient (RD) cells. No significant differences were found corresponding to the differences in ploidy, except for a high frequency of RD cells in the haploid clones and a lower rate of glucose fermentation in the haploid and diploid clones with mating potential than in the diploid or polyploid nonmaters. However, when diploid and tetraploid clones with heterogeneous genomic configurations were constructed by crossing two different series of pure lines, we observed a significant increment in the fermentation rate of glucose in nutrient medium.

MATERIALS AND METHODS

Yeast strains. The strains of *Saccharomyces cerevisiae* used are listed in Table 1.

Media. The standard nutrient medium (YPAD medium) used for the general cultivation of yeast strains contained 1% yeast extract (Daigo Eiyo Chemicals, Osaka, Japan), 2% polypeptone (Daigo Eiyo Chemicals), 0.2% KH_2PO_4, 0.04% adenine (Wako Pure Chemicals, Osaka, Japan), and 2% glucose. Various modified YPAD media with various concentrations of glucose (10, 20, or 30%) and with or without the addition of 5% ethanol were also prepared to test the specific rate of growth and fermentation of yeast strains. Sporulation was conducted with a medium containing 0.5% potassium acetate and 2% agar. The basal medium for the dye plate consisted of 0.1% yeast extract, 0.1% polypeptone, 2% glucose, 0.15% KH_2PO_4, 0.15% $(NH_4)_2SO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, and 1.5% agar (Wako Pure Chemicals) according to the recipe of Nagai (10). The media were sterilized by autoclaving at 1 kg/cm^2 for 10 min. The dye solutions were sterilized by filtration and added to the basal medium after it was cooled to 60°C. Ethanol (96%) was added to the modified YPAD medium without sterilization.

Genetic methods. Mating types were determined by observing the zygote formation after mixed inoculation of cells with strains KYC53 (**a**) and KYC54 (α) as the standards in a few milliliters of YPAD medium. Tetrad dissection was carried out by digesting the ascus sac with snail gut juice (9). Spore-to-cell mating was carried out under a microscope with the aid of a micromanipulator. For isolation of hybrids of strains SU2′ and TU2′ and of strains SU1 and TU1, single-cell isolation from the mixed culture of cells in YPAD medium was carried out under the microscope with the aid of the micromanipulator.

Biochemical and physiological methods. The frequency of RD cells was scored by spreading cells on nutrient plates. Colonies appearing on the plates after incubation at 30°C for 3 days were overlaid with 2,3,5-triphenyltetrazorium chloride agar (12), and the plates were stood at room temperature for at least 30 min away from direct sunlight. Colonies of the respiratory-competent cells were stained pink to red, whereas those of the RD cells remained white. Cellular DNA contents were determined from the absorbance at 600 nm of the complex of DNA with diphenylamine reagent (17), the DNA being extracted by the method of Ogur and Rosen (11) from approximately 10^9 cells cultured in the standard YPAD medium at 30°C by shaking for 36 h. Salmon sperm DNA (Wako Pure Chemicals) was used as a standard. Cell numbers in each sample were counted three times for each sample (totals were ca. 1,000 to 2,000 cells) with a Thoma hemacytometer for calculation of cellular DNA contents. Cell growth was monitored with a Bioscanner (Ohtake Works, Tokyo, Japan; model OT-BS-12), and the specific growth rate was calculated graphically. Fermentation rates were estimated by measuring the

TABLE 1. Yeast strains used

Strain (genotype)[a]	Mating type	Abbreviation	Source
S line (Hp type; *HO HMLα HMRα his4 leu2 lys2*)			
S-14-9C-1B-15B	α	S1	Spore culture of S2
S-14-9C-U37-2B-4B-5B-18A-6A-1A	α	SU1	Spore culture of SU2
S-14-9C-1B	**a**/α	S2	Spore culture of S-14-9C (19)
S-14-9C-U37-2B-4B-5B-18A-6A	**a**/α	SU2	Spore culture of SU4′
S-14-9C-U37-2B-4B-5B-18A-18C	α/α	SU2′	Spore culture of SU4′
S-100	**a**/α/α	SU3	Cross between SU2′ and a spore of SU2
S-14-9C-U37	**a**/**a**/α/α	SU4	Isolated from S2 by Takano et al. (19)
S-14-9C-U37-2B-4B-5B-18A	**a**/**a**/α/α	SU4′	Spore culture of SU4
C line (Hp type; *HO HMLα HMRα his4 leu2 arg4*)			
C-328-1C-13A-1B	α	C1	Spore culture of C2
C-328-1C-13A-U101-4A-7B-4B-5A-4B-2C-8A	α	CU1	Spore culture of CU2
C-328-1C-13A	**a**/α	C2	Takano et al. (19)
C-328-1C-13A-U101-4A-7B-4B-5A-4B-2C	**a**/α	CU2	Spore culture of CU4′
C-328-1C-13A-U101-4A-7B-4B-5A-4D	α/α	CU2′	Spore culture of CU4′
C-500	**a**/α/α	CU3	Cross between CU2′ and a spore of CU2
C-328-1C-13A-U101	**a**/**a**/α/α	CU4	Isolated from C2 after UV irradiation
C-328-1C-13A-U101-4A-7B-4B-5A	**a**/**a**/α/α	CU4′	Spore culture of CU4
T line (Hq type; *HO HMLa HMRa adel arg4 his4 leu2 lys2 trp1*)			
T-1023-23B-5A	**a**	T1	Spore culture of T2
T-1023-23B-U1-7B-1D-1D-7A-5A-1C	**a**	TU1	Spore culture of TU2
T-1023-23B	**a**/α	T2	Takano et al. (19)
T-1023-23B-U1-7B-1D-1D-7A-5A	**a**/α	TU2	Spore culture of TU4′
T-1023-23B-U1-7B-1D-1D-7D	**a**/**a**	TU2′	Spore culture of TU4′
T-2000	**a**/**a**/α	TU3	Cross between TU2′ and a spore of TU2
T-1023-23B-U1	**a**/**a**/α/α	TU4	Isolated from T2 after UV irradiation
T-1023-23B-U1-7B-1D-1D-7A	**a**/**a**/α/α	TU4′	Spore culture of TU4
ST2	**a**/α		Constructed by SU1 × TU1
ST4	**a**/**a**/α/α		Constructed by SU2′ × TU2′
KYC53 (*ho trp3 ura1 ura2*)	**a**		Our stock culture
KYC54 (*ho trp3 ura1 ura2*)	α		Our stock culture

[a] The S, C, and T series of strains were derived from strains S-14-9C-1B (S2), C-328-1C-1A (C2), and T-1023-23B (T2), respectively, by UV-promoted homozygosity of the mating-type alleles followed by mating-type switch and repeated selection of meiotic products. U in the strain number represents UV irradiation for acceleration of mitotic recombination at the indicated stage of strain construction. Numbers and letters A, B, C, and D in the strain number represent ascus number and spore number, respectively. The nomenclature for genetic symbols recommended by Plischke et al. (15) is used, except for the conventional symbols **a** and α for mating type.

carbon dioxide evolution on inoculation of 25 ml of the overnight culture in the standard YPAD medium into 500 ml of various modified YPAD media in 1-liter Erlenmeyer flasks fitted with Alwood fermentation plugs.

RESULTS

Dye plate for discrimination of tetraploid and diploid colonies. In *Saccharomycodes ludwigii*, it was possible effectively to discriminate diploid colonies generated by direct diploidization in a haploid cell population by using a dye plate containing 15 mg of trypan blue and 10 mg of phloxine B per liter in a synthetic nutrient medium (the basal medium used in the present study) (21). Although the mechanism is not known, the diploid colonies were stained dark violet, whereas the haploid colonies were stained pale violet. In the present study, we investigated a similar method for the discrimination of isogenic diploid and tetraploid colonies of *S. cerevisiae*, using

various dyes, singly or combined in pairs, at two concentrations, 20 and 40 mg per liter, with pure cultures of strains S2 (diploid [Table 1]) and SU4 (tetraploid) and a mixed culture of those two strains. Strains S2 and SU4 were each inoculated into a few milliliters of the standard YPAD medium, and the cultures were stood at 30°C for 1 day and then mixed. Mixed and pure cultures were spread on the dye plates after appropriate dilution with sterilized water. The plates were incubated at 30°C for 2 to 4 days. Dyes examined were trypan blue, phloxine B, eosin Y, alizalin red S, rose bengal, bluish schalet WS, amaranth, fuchsine S (acid), neutral red, methylene blue, Bordeaux R, Nile blue, aniline blue, Niagara sky blue, Evans blue, and Ponceau 3R. The combination of 40 mg each of eosin Y (Wako Pure Chemicals; color index no. 45380) and amaranth (Daiwa Chemicals, Osaka, Japan; color index no. 16185) per liter of basal medium gave the highest color contrast between the isogenic diploid and tetraploid colonies so far examined; the tetraploid colonies were stained dark red, and the diploid colonies were stained light red.

We applied the same dye plate for the detection of tetraploid colonies arising in the diploid cell population of strain S2. Strain S2 was cultivated in the standard YPAD medium by standing at 30°C overnight, and the cells were spread on the eosin Y-amaranth plates after appropriate dilution. The plates were then irradiated with UV light (Matsushita Electric Ind. Co., Ltd., Kadoma, Japan) (18 W) for 15 s at a distance of 30 cm to promote mitotic recombination (these conditions killed approximately 50% of the cells). After 2 to 4 days of incubation at 30°C, dark red colonies appeared on the plates with a frequency of about 10^{-2} among the light red colonies. We examined 117 dark red colonies for cell size under a microscope and found that 11 consisted of large cells and 6 were a mixture of large and small cells, whereas the remaining 100 colonies consisted of small cells.

Since the original strain, S2, is an Hp diploid of homozygous genotype for the *HO HML*α *HMR*α alleles, it showed only 2 nonmater:2 α segregation when the cells were self-sporulated and four-spored asci were dissected. On the other hand, a tetraploid clone generated in the S2 cell population, having the same genotype for the homothallic genes and the *MAT***a**/*MAT***a**/*MAT*α/*MAT*α genotype for the mating-type locus, should produce self-sporulated asci showing 4 nonmater:0 α, 3 nonmater:1 α, and 2 nonmater:2 α segregations. When 5 of the 11 clones derived from the colonies staining dark red on the dye plate and consisting of large cells were subjected to tetrad analysis after self-sporulation, one isolate showed 4 nonmater:0 α (12 asci), 3 nonmater:1 α (8 asci), and 2 nonmater:2 α (2 asci) segregations, and another isolate showed 4 nonmater:0 α (6 asci), 3 nonmater:1 α (10 asci), and 2 nonmater:2 α (4 asci) segregations. The remaining three isolates, however, showed solely the 2 nonmater: 2 α segregation.

To confirm the effectiveness of the dye plate for other strains, cells of strain C2 (Hp-type diploid [Table 1]) were spread on the dye plates, and the plates were incubated at 30°C for 2 to 4 days. We examined 229 dark red colonies among approximately 10^4 colonies on the dye plates and found six clones of larger cell size, whose polyploidy was confirmed by the segregation of mating type after self-sporulation.

The trypan blue-phloxine B plate was extremely effective for the discrimination of the diploid colonies among the haploid colonies of *Saccharomycodes ludwigii* (21), as almost all of the isolated dark violet colonies were identified as diploids. In comparison with this result, the dye plate for discrimination of isogenic diploid and tetraploid colonies of *S. cerevisiae* is ineffective. It is, nevertheless, at least 100 times more effective than random selection of the colonies. We also constructed another series (T series) of isogenic strains of haploid to tetraploid strains by the same procedure with independent original diploid strains (Table 1). An example of the variation in cell morphology with ploidy is that of the C series strains shown in Fig. 1.

Effects of polyploidy on cellular activities. Since test strains of the same series are isogenic, their qualitative characteristics (for example, ability to ferment or assimilate various carbohydrates, and vitamin requirement) were obviously the same (data not shown). To investigate the effect of polyploidy, we compared the specific growth rate and fermentation rate of eight strains belonging to the S series, S1, SU1, S2, SU2, SU2′, SU3, SU4, and SU4′ (Table 1). Their specific growth rates were examined in the standard and modified YPAD media adjusted to various concentrations of glucose (10, 20, or 30%) and with or without ethanol (5%). Although significant differences were observed corresponding to differences in cultural conditions in each strain, all of the strains showed nearly equal specific growth rates under the same conditions, regardless of differences in ploidy (Table 2). This result indicates that differences in ploidy do not affect the specific growth rate. In the fermentation of glucose, all of the strains showed almost the same final weight of carbon dioxide evolution (data not shown). Hence, only the fermentation rates (weight of carbon dioxide evolved per day) in days 1 and 2 were compared (Table 2). Haploid strains showed lower rates than strains of higher ploidies. The reason is not clear, but it may be

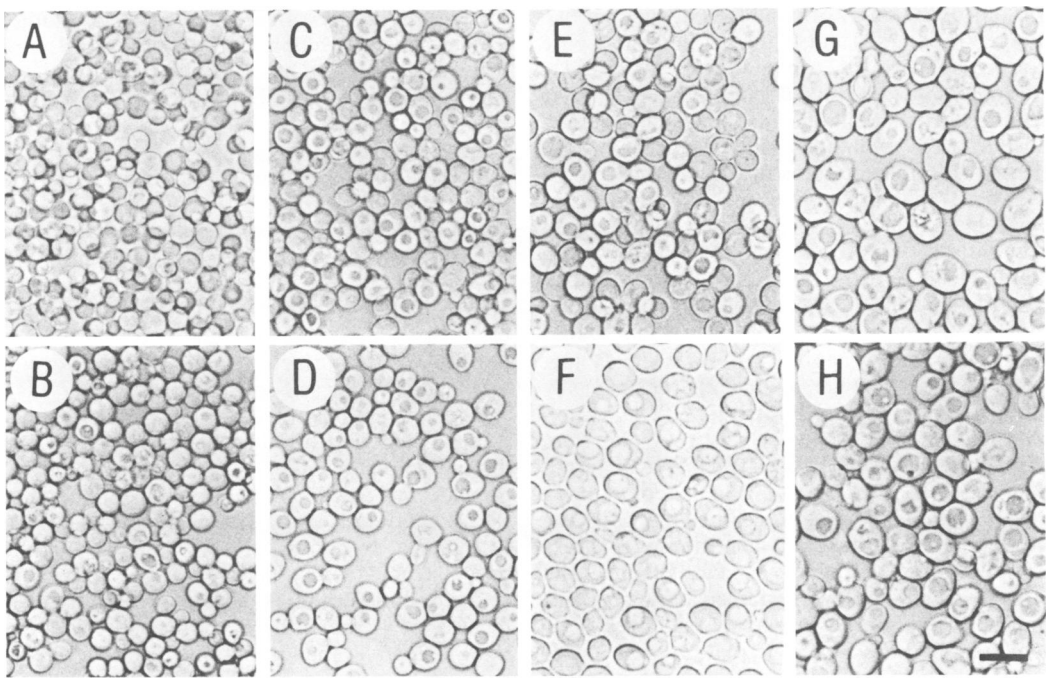

FIG. 1. Photomicrographs of yeast cells of isogenic C series strains (Table 1). (A) α haploid strain C1. (B) α haploid strain CU1. (C) **a**/α diploid strain C2. (D) **a**/α diploid strain CU2. (E) α/α diploid strain CU2′. (F) **a**/α/α triploid strain CU3. (G) **a**/**a**/α/α tetraploid strain CU4. (H) **a**/**a**/α/α triploid strain CU4′. All photomicrographs were taken at the same magnification. Bar, 10 μm.

related to the mating ability of the haploid cells because strain SU2′, a diploid strain with mating ability, also showed a slightly lower rate than the others. (The same explanation was also suggested by the data in Table 3 [see below].) No essential differences were observed among the fermentation rates of diploid, triploid, and tetraploid nonmater strains.

TABLE 2. Comparison of DNA content, specific growth rate, fermentation rate, and frequency of appearance of spontaneous RD cells among isogenic strains of different ploidies

Strain	Mating type	Ploidy	DNA content[a] (μg/cell [$\times 10^{-8}$])	Specific growth rate under the following conditions[b]:						Fermentation rate[c] (g of CO_2 evolved at 30°C) during:		RD frequency[d] (%)
				20, 2, 0	30, 2, 0	30, 10, 0	30, 20, 0	30, 30, 0	30, 2, 5	0 to 24 h	24 to 48 h	
S1	α	*n*	1.9	0.17	0.52	0.43	0.33	0.31	0.30	3.5	6.1	1.22
SU1	α	*n*	2.4	0.15	0.46	0.47	0.29	0.29	0.22	3.3	5.4	0.55
S2	**a**/α	2*n*	4.1	0.17	0.53	0.52	0.30	0.30	0.25	7.2	8.0	0.10
SU2	**a**/α	2*n*	3.7	0.17	0.53	0.53	0.32	0.32	0.28	6.8	8.9	0.13
SU2′	α/α	2*n*	3.1	0.16	0.48	0.45	0.29	0.29	0.24	5.7	7.3	0.02
SU3	**a**/α/α	3*n*	5.6	0.18	0.46	0.48	0.34	0.30	0.24	9.0	8.4	0.13
SU4	**a**/**a**/α/α	4*n*	6.5	0.18	0.53	0.48	0.30	0.30	0.25	8.8	8.2	0.11
SU4′	**a**/**a**/α/α	4*n*	6.9	0.18	0.52	0.45	0.33	0.33	0.25	6.7	9.0	0.06

[a] DNA was extracted from cells shaken at 30°C for 36 h in 30 ml of the standard YPAD medium.

[b] Specific growth rate (hour $^{-1}$) in the standard and modified YPAD media under the indicated conditions. The first number represents the incubation temperature (°C); the second and third numbers represent the percent glucose and ethanol concentrations, respectively, in the medium.

[c] Carbon dioxide evolved from culture on 500 ml of the modified YPAD medium containing 10% glucose.

[d] Yeast cells shaken in 100 ml of the standard YPAD medium in a 500-ml Sakaguchi flask for 24 h at 30°C were plated after appropriate dilution, and colonies appearing after 2 to 3 days of incubation were tested for RD mutation by the 2,3,5-triphenyltetrazorium chloride agar overlay method (12). RD frequency was estimated by counting 2,165 to 7,014 (average, 4,990) colonies.

Effect of polyploidy on the appearance of RD cells. The specific growth rate and fermentation rate depend on the comprehensive activities of cellular metabolism, whereas the appearance of most RD cells is due to variation of the mitochondrial genome. To investigate the effect of polyploidy on the stability of the mitochondrial genome, the frequencies of spontaneous appearance of RD cells were examined. The results showed that the haploid strains produced RD cells with higher frequency than the diploid, triploid, and tetraploid strains (Table 2). This may be explained by assuming that the number of mitochondria in haploid cells is smaller than that in diploid or polyploid cells, and cells with fewer mitochondria would more easily lose those bearing the wild-type genome, either by mitochondrial mutation or by the unequal partition of the mutant and wild-type mitochondria at the sprouting of a new bud. No significant differences in RD frequency were observed corresponding to the difference in ploidy between the diploid, triploid, and tetraploid strains. This implies that diploid and polyploid cells contain sufficient mitochondria for the mechanism described above.

The observations described above strongly suggest that the advantages of the polyploid strains under industrial conditions are due neither to the simple multiplication of the same genome nor to the physical capacity of the polyploid cells, but to the greater complexity of genetic information contained in them than in haploid or diploid cells.

Effect of heterozygosity on cellular activity. Heterosis is known to occur in animal and plant breeding; a number of hybrids show greater vigor than pure lines. To test this possibility, we constructed a hybrid tetraploid strain, ST4, by crossing two diploid strains, SU2′ (*MAT*α/*MAT*α; Hp diploid) and TU2′ (*MAT***a**/*MAT***a**; Hq diploid), and a hybrid diploid strain, ST2, by crossing the haploid strains SU1 (*MAT*α; Hp haploid) and TU1 (*MAT***a**; Hq haploid) (Table 1). The two resultant strains are isogenic with each other, but strain ST2 is of half the genomic size of strain ST4. In all of these strains the final weights of carbon dioxide evolved with the modified YPAD medium were almost equal (data not shown), whereas the kinetics of the fermentation differed from strain to strain. The fermentation rate (weight of carbon dioxide evolved per day) in days 1 and 2 was higher in strains ST4 and ST2 than in the pure lines, whereas each hybrid showed almost the same value as the other (Table 3). These results indicate the occurrence of hybrid vigor in the fermentation of glucose; the heterozygosity of the genome gives rise to a significant alteration of cellular activity compared with that of the parent. Since no significant differences were observed between the fermentation rates of strains ST4 (tetraploid) and ST2 (diploid), it is clear that the hybrid vigor is not due to the polyploidy per se of hybrids.

TABLE 3. Fermentation rate of strains of different ploidies in YPAD containing 10% glucose at 30°C

Strain	Mating type	Ploidy	Genotype[a]	CO_2 evolved[b] (g) during:	
				0 to 24 h	24 to 48 h
SU1	α	*n*	S	1.2	5.6
TU1	**a**	*n*	T	2.5	4.2
ST2	**a**/α	2*n*	S/T	12.6	7.1
SU2	**a**/α	2*n*	S/S	7.9	6.3
TU2	**a**/α	2*n*	T/T	5.3	7.3
SU2′	α/α	2*n*	S/S	3.6	4.7
TU2′	**a**/**a**	2*n*	T/T	3.9	5.1
ST4	**a**/**a**/α/α	4*n*	S/S/T/T	12.3	6.9
SU4′	**a**/**a**/α/α	4*n*	S/S/S/S	7.4	6.4
TU4′	**a**/**a**/α/α	4*n*	T/T/T/T	3.5	4.9

[a] S and T, whole genome of strains SU1 and TU1, respectively, except for the mating-type alleles.

[b] Carbon dioxide evolved from culture on 500 ml of the modified YPAD medium containing 10% glucose.

DISCUSSION

Polyploidy has often been observed in saccharomyces yeasts used in brewing, baking, and alcoholic fermentation. To investigate the reason for this, isogenic series of yeast strains with different ploidal levels were constructed by making use of the action of the homothallism genes. No significant differences were found in the specific growth rate among strains from haploid to tetraploid or in the fermentation rate and the spontaneous appearance of RD cells among strains from diploid to tetraploid. However, hybrids of the S series and T series strains showed hybrid vigor in their fermentation rate. These observations suggest that industrial polyploid yeasts are selected for the hybrid vigor resulting from their greater genetic heterogeneity compared with that of diploid or haploid strains, rather than for an increased capacity due to the greater physical volume of the cells. This means that the strains favored industrially should be hybrids, an inference which is borne out by the numerous examples of industrial strains segregating many varieties of various phenotypes by both the mitotic and the meiotic processes that have been found since the beginning of yeast genetics (22).

At the same time, this argument requires the hybridization of yeasts under brewing and baking conditions. In practice, yeasts were probably transferred successively from batch to batch of beer mash or dough. Throughout the history of these processes, in family kitchens and local

factories, there must have been ample opportunity for hybridization between different yeast strains through their coexistence, sporulation, and mating. In our experience, homothallic strains are widely distributed in nature, and they are likely to have been involved in such hybridization processes. These processes probably predate the introduction of modern microbiological techniques into the brewing and baking industries, as most of the favored industrial strains were selected or derived from traditional fermenting materials.

ACKNOWLEDGMENT

This study was supported by Grant-in-Aid for Scientific Research no. 248053 (to Y.O.) from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Emeis, C. C.** 1961. Polyploide Kulturhefer, p. 205–215. *In* C. Christian and C. C. Emeis (ed.), Proceedings of the European Brewers Convention, 8th Congress, Vienna. Elsevier/North Holland Publishing Co., Amsterdam.
2. **Emeis, C. C.** 1965. Stabilität diploider und polyploider Kulturhefestämme, p. 156–163. *In* C. Christian and C. C. Emeis (ed.), Proceedings of the European Brewers Convention, 10th Congress, Stockholm. Elsevier/North Holland Publishing Co., Amsterdam.
3. **Gunge, N.** 1966. Breeding of bakers' yeast—determination of the ploidy and attempt to improve practical properties. Jpn. J. Genet. **4**:203–214.
4. **Harashima, S., Y. Nogi, and Y. Oshima.** 1974. The genetic system controlling homothallism in saccharomyces yeasts. Genetics **77**:639–650.
5. **Herskowitz, I., and Y. Oshima.** 1981. Control of cell type in *Saccharomyces cerevisiae*: mating type and mating-type interconversion, p. 181–209. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Hicks, J. B., and I. Herskowitz.** 1976. Interconversion of mating types in yeast. I. Direct observations of the action of the homothallism (*HO*) genes. Genetics **83**:245–258.
7. **Hicks, J. B., and I. Herskowitz.** 1977. Interconversion of yeast mating types. II. Restoration of mating ability to sterile mutants in homothallic and heterothallic strains. Genetics **85**:373–393.
8. **Hicks, J. B., J. N. Strathern, and I. Herskowitz.** 1977. The cassette model of mating-type interconversion, p. 457–462. *In* A. J. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Johnston, J. R., and R. K. Mortimer.** 1959. Use of snail digestive juice in isolation of yeast spore tetrads. J. Bacteriol. **78**:292.
10. **Nagai, S.** 1963. Diagnostic color differentiation plates for hereditary respiration deficiency in yeast. J. Bacteriol. **86**:299–302.
11. **Ogur, M., and G. Rosen.** 1950. The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. Arch. Biochem. **25**:262–276.
12. **Ogur, M., R. St. John, and S. Nagai.** 1957. Tetrazolium overlay technique for population studies of respiration deficiency in yeast. Science **125**:928–929.
13. **Oshima, Y., and I. Takano.** 1971. Mating types in saccharomyces: their convertibility and homothallism. Genetics **67**:327–335.
14. **Oshima, Y., and I. Takano.** 1972. Genetic controlling system for homothallism and a novel method for breeding triploid cells in *Saccharomyces*, p. 847–852. *In* G. Terui, (ed.), Fermentation technology today. The Society of Fermentation Technology, Osaka, Japan.
15. **Plischke, M. E., R. C. von Borstel, R. K. Mortimer, and W. E. Cohn.** 1976. Genetic markers and associated gene products in *Saccharomyces cerevisiae*, p. 765–832. *In* G. D. Fasman (ed.), Handbook of biochemistry and molecular biology, vol. 2, 3rd ed. Chemical Rubber Co. Press, Cleveland, Ohio.
16. **Santa Maria, J., and D. Vidal.** 1970. Segregación anormal del "mating type" en Saccharomyces. Inst. Nac. Invest. Agron. (Madrid) **30**:1–21.
17. **Schneider, W. C.** 1945. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. J. Biol. Chem. **161**:293–303.
18. **Takahashi, T.** 1964. Gene conversion of the mating-type locus in *Saccharomyces*. Bull. Brew. Soc. **10**:11–22.
19. **Takano, I., T. Oshima, S. Harashima, and Y. Oshima.** 1977. Tetraploid formation through the conversion of the mating-type alleles by the action of homothallic genes in the diploid cells of *Saccharomyces* yeasts. J. Ferment. Technol. **55**:1–12.
20. **Takano, I., H. Yoshizumi, and Y. Terashima.** 1966. The ploidy of brewing yeasts. I. The sporulation and the ploidy. J. Ferment. Technol. **44**:150–157.
21. **Yamazaki, T., and Y. Oshima.** 1979. Direct diploidization and occurrence of polyploidy in *Saccharomycodes ludwigii*. J. Gen. Microbiol. **111**:271–281.
22. **Winge, Ö., and O. Laustsen.** 1937. On two types of spore germination, and on genetic segregation in saccharomyces, demonstrated through single-spore cultures. C. R. Trav. Lab. Carlsberg Ser. Physiol. **22**:99–117.