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HEXAPLOID FORMATION THROUGH THE CONVERSION OF THE MATING-TYPE ALLELES BY THE ACTION OF HOMOTHALLIC GENES IN *SACCHAROMYCES* YEAST

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Summary

Haxaploid breeding was attempted through the conversion of mating-type alleles by homothallic genes in triploid cells of ***Saccharomyces*** *yeast homozygous for mating-type. To isolate triploid cells homozygous for mating-type efficiently, a procedure based on ultraviolet light induced reciprocal mitotic recombination monitored by homozygosity for* ***thr4*** *(a recessive marker linked to the mating-type locus) was employed. Fifty-eight threonine dependent clones from Hq triploids and six threonine dependent clones from Hp triploids were isolated. Among those clones, five clones from Hq triploids were considered to be hexaploid because their cellular DNA content was about two times that of their respective parental triploids. However, the cell volume and the segregation of genetic markers did not accord well with the values expected for hexaploid cells. These presumably hexaploid clones produced smaller cells during vegetative growth. This may indicate mitotic reduction or segregation of unequal numbers of chromosomes to the daughter cells during mitotic cell division in the polyploid cells.*

Introduction

For many centuries yeasts have served man in the baking, wine-making and brewing-industries, and nowadays they are even used for biomass-production. Therefore, studies on yeasts are valuable in providing more useful information for their industrial application. When one considers how genetics can be applied to improve industrial microorganisms, at least two approaches come to mind. One is to use mutagenesis to improve their intrinsic abilities and the other is to combine their useful genes or strengthen their metabolic activities by hybridization or polyploidization. Mutagenesis has been successful in the improvement of industrial microor-

ganisms, but hybridization and polyploidzation have not yet been successful as practical techniques. Nevertheless, the yeast strains used in the fermentation industry should be improved by hybridization, because their vegetative cells are generally diploid or polyploid, and recessive mutation induced by mutagenesis cannot be expressed phenotypically. In carrying out this objective, an understanding of the genetic system controlling mating-type interconversion in yeasts could provide new ideas for improving hybridization techniques and developing more logical breeding procedures.

In *Saccharomyces cerevisiae* and its related species, there are two mating-types, designated *a* and *α*, which control the mating response. This mating-type specificity is controlled by a single pair of alleles, *a* and *α*, on chromosome III[1-3]. In a heterothallic diploid strain, cultivation of an ascospore gives rise to a haploid clone having either *a* or *α* mating-type, and diploidization occurs by fusion of two haploid cells of opposite mating-type. In a homothallic strain, the mating-type is unstable[4-5], and conversion from *a* to *α* or from *α* to *a* may occur in every cell division[6]. Diploidization in a homothallic strain occurs by the fusion of the converted and unconverted cells in a single haploid ascospore-culture[7]. Conversion of one mating-type allele to the other is controlled by three kinds of homothallic genes, each consisting of a single pair of alleles, *HO*/*ho*, *HMα*, and *HMa*/*hma*[8]. Depending on the genotype of the three loci and the mating-type alleles in any ascospore, three phenotypically different types of diploid homothallic strain may arise on its cultivation. One type shows a perfectly homothallic life cycle with a homozygous genotype of *a* (or *α*) *HO hmα hma* or *a* (or *α*) *HO HMα HMa* genotype (the Ho type); the other two types are semi-homothallic with homozygous *a HO hmα HMa* (the Hp type) and homozygous *α HO HMα hma* (the Hq type) genotypes. Any spore having a genotype other than these gives rise to a stable heterothallic haploid clone[8]. The relationships between the genotypes and the phenotypes in homothallism has indicated[8] that a combination of the *HMα* and/or *hma* alleles with the *HO* allele is essential for the *α* to *a* conversion, while the *HMa* and/or *hmα* alleles with the *HO* allele is essential for the *a* to *α* conversion. Change of one mating-type allele to the other occurs within a few generations of spore germination and the activity of the homothallic genes is blocked as soon as heterozygosity of the mating-type alleles is established by zygote formation between cells with complementary mating types[7].

Recently, work by Takano *et al*[9] and Klar and Fogel[10] has indicated that the homothallic genes are also active during vegetative growth of diploid cells homozygous for the mating-type alleles. In *a*/*a* and *α*/*α* cells derived from an *a*/*α* cell by mitotic recombination conversion occurred and *a*/*a*/*α*/*α* tetraploid cells were subsequently produced through cell fusion between converted and unconverted cells. It was also shown that single allele-conversion accurred in some *a*/*a* and *α*/*α* diploid cells and produced *α*/*α* cells.

In the present work, a similar regimen was designed to breed hexaploid cells using Hq homothallic strains. The process for breeding comprised two main steps. First, *a*/*α* diploid Hq type cells were cultured with *a* Hq type haploid cells. In the mixed culture, *a*/*a* and *α*/*α* cells were produced in some of the *a*/*α* diploid cells by

mitotic recombination between the mating type locus and the centromere with a frequency of approximately 10^{-5} to 10^{-3}. Then fusion of the diploid a/α cells with haploid a cells occurred and gave rise to a zygote and results in triploid $a/a/\alpha$ cell formation. Second, the triploid cells were irradiated with a low dose of ultraviolet light, and in some of the cells a switch from the $a/a/\alpha$ configuration to $a/\alpha/\alpha$ occured by mitotic recombination between the mating type locus and the centromere. Since the genotype for homothallism in the Hq cell was effective for α to a conversions, the mating type in the $a/\alpha/\alpha$ cell was expected to convert to $a/a/a$ during vegetative multiplication. This was followed by cell fusion between the converted $a/a/a$ cell and the unconverted $a/\alpha/\alpha$ cell. The resultant zygote produced a new hexaploid bud. Results from the determination of cellular DNA content for the supposed triploid and hexapliod cells accorded with the above expectation, while the genetic and cell volume data were ambiguous.

Materials and Methods

Strains. Twelve strains of Hq type (ten diploid strains and two haploid strains) and nine strains of Hq type (four diploid strains and five haploid strains) were obtained from the stock cultures for Yeast Genetics (Department of Fermentation Technology, Osaka University). These strains were purified by streaking on complete medium plates. Their nutritional requirements, sporulation ability and mating response were confirmed. The diploid strains were able to sporulate and did not show mating response. The haploid strains had either a or α mating type and did not show sporulation. Finally, three Hq type diploid strains, C415-305B, C443-2C and C443-2C-5C, and two Hq type haploid strains, C449-1B and C405-22A, from the above 21 strains were used for the hexaploid breeding. Two heterothallic haploid strains, C449-1B (a) and T881-1B (α), were used as the standards for mating type determination. Their genotypes are listed in Table I.

TABLE I: LIST OF STRAINS USED

Strain	Genotype[a]			Remarks
	Mating type	Homothallic gene	Genetic markers	
C415–305B	$\frac{a}{\alpha}$	$\frac{HO\ HM\,a\ hma}{HO\ HM\,\alpha\ hma}$	$\frac{lys7\ met14\ his4\ leu2\ thr4}{lys7\ met14\ his4\ leu2\ thr4}$	Homothallism (Hq)
C443–2C	$\frac{a}{\alpha}$	$\frac{HO\ HM\,a\ hma}{HO\ HM\,\alpha\ hma}$	$\frac{ade1\ lys2\ met2\ his4\ leu2\ thr4}{ade1\ lys2\ met2\ his4\ leu2\ thr4}$	Homothallism (Hq)
•C443–2C-5C	$\frac{a}{\alpha}$	$\frac{HO\ HM\,a\ hma}{HO\ HM\,\alpha\ hma}$	$\frac{ade1\ lys2\ met2\ his4\ leu2\ thr4}{ade1\ lys2\ met2\ his4\ leu2\ thr4}$	Homothallism (Hq)
C449–1B	a	$HO\ HM\,a\ hma$	$arg4\ trp1$	Heterothallism
C405–22A	a	$HO\ HM\,a\ hma$	$his6\ trp1$	Heterothallism
T881–1B	α	$hO\ HM\,a\ hma$	$ade1\ lys2\ ura3$	Heterothallism

[a] The terminology of genetic symbols follows that proposed by Plischke *et al*[11].

Media Composition

Complete medium. The complete medium contained 40 g of glucose, 10 g of polypeptone (Daigo Eiyo Chemicals and Co. Ltd.), 5 g of yeast extract (Daigo Eiyo Chemicals and Co. Ltd.), 5 g of KH_2PO_4 and 2 g of $MgSO_4 \cdot 7H_2O$ per liter.

Minimum medium (Burkholder's medium). The minimum medium contained 20 g of glucose, 2 g of L-asparagine monohydrate (Wako-Pure Chemical Industries, Ltd.), 1.5 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.33 g of $CaCl_2 \cdot 2H_2O$, 0.0001 g of KI, 2 μg of biotin, 200 μg of thiamine, 200 μg of pyridoxine, 200 μg of nicotinic acid, 200 μg of pantothenate, 10,000 μg of inositol per liter and trace metals.

Supplement medium. For testing auxotrophic markers, Burkholder's medium appropriately supplement with amino acids (20 mg of L-histidine, 50 mg of L-leucine, 200 mg of L-threonine, 20 mg of L-tryptophan, 20 mg of L-arginine, 30 mg of L-lysine, and 20 mg of L-methionine per liter of medium), adenine (20 mg per liter) and uracil (20 mg per liter) was used.

All solid media were prepared by the addition of 20 g of agar per litter of medium.

Sporulation medium. The sporulation medium was composed of 5 g of sodium acetate and 20 g of agar per liter.

Genetic techniques

Method for testing sporulation. Sporulation was performed by smearing freshly cultivated cells on sporulation medium. Capable strains sporulated after 2 days incubation at 30°C.

Determination of mating type. Mating type was determined as follows. One loopful of cell suspension was inoculated into each of two test tubes containing 2 ml of liquid complete medium. Then two drops of cell suspension (1×10^8 cells per ml) of the standard strain C449–1B (*a*) was added to one test tube. To the other test tube was added two drops of cell suspension of standard strain T881–1B (*α*). After shaking, the mixed cultures were incubated for 24 h at 30°C, then a loopful of cell suspension was spotted on a minimum medium plate. The plate was incubated at 30°C for 2 days and the mating type was determined by the appearance of prototrophs from the combination with standard strains marked with the complementary auxotrophic markers on minimum medium.

Random spore analysis. The cells were cultivated on a complete medium plate for 24 h at 30°C, then a large amount of cells was collected and smeared on sporulation medium. After incubation for 48 h at 30°C, they were examined for spore formation under the microscope. Then all of the cells were put into a tube containing 2.5 ml Zymolyase (Kirin Beer Co., Ltd. Tokyo; 1 mg per ml). The suspension was incubated at 30°C for 24 h, the whole mixture was sonicated 3 times for 3-min periods, 2 ml of sterile water was added, and the sonicate was centrifuged at 3,000 rpm for 5 min. The pellet was dissolved in 5 ml of sterile water containing 4 mg of streptomycin. After standing for 1 h at room temperature, the suspension was

appropriately diluted and spread on a complete medium plate. The plate was incubated for 3–4 days at 30°C, and colonies showing a red color (adenine auxotrophy) or methionine auxotrophy were selected by the replica method[12].

Assay methods

Determination of cell volume. Cells were cultivated for 3 days at 30°C in complete medium. The cell volume was determined by using a Coulter Counter (Model ZBI, Coulter Electronics, Inc.) at the Central Research Institute of Suntory Ltd.

Determination of deoxyribonucleic acid (DNA) content. Cells were shaken in the complete medium for 48 h at 30°C. DNA was extracted by the method of Schneider[13] with modification. Thirty ml of the culture broth was centrifuged at 3,000 rpm for 5 min and the cells were washed two times with 6 ml of distilled water. Then 5 ml of a 4:1 mixture of 95% ethyl alcohol and distilled water was added and the suspension was centrifuged at 3,000 rpm for 5 min. The cell precipitate was suspended in 5 ml of 95% ethyl alcohol and centrifuged again. The supernatant was discarded and the cell pellet was suspended in 5 ml of a 3:1 mixture of ethyl alcohol and ether, and the whole mixture was again centrifuged. This step was repeated 3 times. After this repeated centrifugation the precipitate was kept at room temperature for about 15 min to evaporate the ethyl alcohol and ether. Then 5 ml of 6% perchloric acid was added and the mixture was heated in a water bath at 90°C for 15 min. The cells were immediately cooled in an ice bath and centrifuged at 3,000 rpm 5 min. The supernatant was used for the determination of DNA. DNA content of the extract was determined by measuring the absorbancy at 600 nm using a diphenylamine reagent[14] and 2′-deoxyadenosine (Sigma Chemical Company, U.S.A.) was employed as standard. For estimation of cellular DNA content, the cell number in each sample was counted by using a hemocytometer.

Results and Discussion

Selection of strains

Form the 21 stock cultures of *Saccharomyces* yeasts, six strains of Hq diploid (a/α), two strains of Hq haploid (a), four strains of Hp diploid (a/α) and two strains of Hp haploid (α) were selected for construction of triploid cells. They were selected according to the criteria that, for diploid cells, they should sporulate and not show mating response, and for haploid cells, that they should not sporulate but be able to mate with either the standard a or α strains. Strains selected by these criteria were again purified by single colony isolation. Four colonies from each strain were tested for their auxotrophic traits, mating and sporulation ability. One clone of these four isolates was used for triploid breeding. In the hexaploid breeding triploid strains constructed from Hq type diploid strains C415–305B, C443–2C and C443–2C–5C, and Hp haploid strains C449–1B and C405–22A, were used.

Construction of triploid cells

Cells of Hq haploid (*a*) or Hp (*α*) strain were mated with cells of Hq diploid (*a*/*α*) or Hp (*a*/*α*) strain, respectively. In this experiment, both haploid and diploid cells were cultured separately in 2 ml of complete medium for 24 h at 30°C and the cells were centrifuged and suspended separately in 1 ml of complete medium. Then 0.1 ml of the cell suspension of each mating pair was put into one tube containing 2 ml of complete medium and incubated at 30°C for 24 h. Cells were centrifuged at 3,000 rpm for 5 min, washed and suspended in 0.2 ml of sterile water. Then 0.1 ml of this suspension was spread on a minimum plate. The prototrophs which appeared on the minimum plate after 3 days of incubation at 30°C were picked up. They were assumed to be triploid cells since they were derived from the zygotes between haploid and diploid cells. In general, under the microscope these isolates consisted of round cells that were larger in size than their respective parental diploid cells.

When Hq diploid cells C415–305B, C415–305B–1A*, C415–305B–1B* (* are the Hq diploid segregants from C415–305B after tetrad dissection), C443–2C, C443–2C–5C, and C443–7C mated with Hq haploid cells C449–1B, they produced Hq triploid clones VQ1, VQ2, VQ3, VQ4, VQ5 and VQ6, respectively. Triploid clones VQ10, VQ11 and VQ12 were produced by mating between Hq diploid cells C443–2C, C443–2C–5C, and C443–7C and Hq haploid cells C405–22A, respectively. When Hq diploid cells C437–14C and C437–10B–1A mated with Hp haploid cells C436–6A, they produced Hp triploid clones VP5 and VP7, respectively.

Three clones isolated from each combination of triploid breeding were cultivated in complete medium. After 2 days of incubation at 30°C they were tested for sporulation, mating ability, auxotrophic traits and cell size. For construction of a hexaploid, one clone was chosen from each of the three isolates that had the biggest cell size and sporulation ability, but no mating ability with either *a* or *α* standard.

Construction of hexaploid cells

In order to isolate hexaploid cells efficiently, *thr4* genetic marker was used as a selective marker. Since the *thr4* locus is located at about 30 stranes distal to the mating type locus, which is situated at 20 stranes from the centromere on chromosome III, 40% of clones having *thr4*/*thr4*/*thr4* configuration would be expected to be a triploid with an *α*/*α*/*α* genotype produced by the crossing over between the matingtype locus and the centromere. Thus it was possible to distinguish *α*/*α*/*α* triploids from *α*/*α*/*a* or *a*/*a*/*α* triploids more efficiently by selecting colonies showing threonine requirement than by random isolation of colonies.

The selected triploid clones were inoculated in complete medium and incubated at 30°C for 1 day. After appropriate dilution of the culture with sterilized water, 0.1 ml of each cell suspension was spread on a complete medium plate. The plates were irradiated with a low dose of ultraviolet light for 11 s (80% to 90% survival.) They were then incubated at 30°C for 2 days, and replica plating was performed on

minimum medium plates supplemented with lysine, methionine, histidine, leucine, arginine, tryptophan and adenine but not with threonine, in order to isolate threonine-dependent clones. The plates were incubated at 30°C for 2 days. Colonies which could grow on complete medium plates but not on the replicated plate were isolated. The triploid clones from which threonine-dependent clones could be obtained were VQ1 (5), VQ2 (3), VQ3 (4), VQ4 (5), VQ5 (1), VQ6 (6), VQ10 (13) VQ11 (11) and VQ12 (11) of the Hq triploids and VP5 (5), VQ7 (1) of the Hp triploids. (The figures in parentheses indicate number of threonine-dependent isolates.)

All of these threonine dependent clones were cultivated on complete medium slants at 30°C for 2 days, then cell size, sporulation, mating potency and auxotrophic traits were tested. It was confirmed that all of them are non-maters, sporogenous and prototrophic except that they required threonine for their growth.

Since cells of higher ploidy are expected to have a larger size[15-17], clones showing larger cell size than the original triploid cells were selected. They were VQ1-5 (5th threonine-dependent isolate from triploid clone VQ5), VQ10-11, VQ10-12, VQ11-10 and VQ11-11. These clones were purified by spreading on complete medium plates.

When suspected hexaploid strain VQ1-5 was purified, 24 subclones were picked up for further study. By testing sporulation and cell size of the subclones, 14 subclones showed bigger cell size and sporulation, 3 subclones showed big cell size but no sporulation, 2 subclones showed medium cell size and sporulation, 2 subclones showed small cell size and sporulation, and the remaining 3 subclones showed small cell size and no sporulation.

Thirty-two subclones were picked up from the purification of suspected hexaploid strain VQ10-11. Of these subclones, 31 subclones showed big cell size and sporulation, while one subclone showed medium cell size and sporulation.

Purification of suspected hexaploid strain VQ10-12, resulted in 32 subclones being picked up. Twenty-two subclones showed big cell size and sporulation ability, and the other 10 subclones showed medium cell size and sporulation ability.

When suspected hexaploid VQ11-10 was purified, 12 subclones were picked up. They were classified as follows: 7 subclones showed big cell size and sporulation, 5 subclones showed medium cell size and sporulation.

Thirty-one subclones were picked up when VQ11-11 was purified. They were classified as follows: 21 subclones showed big cell size and sporulation, and 11 subclones showed medium cell size and sporulation.

All the subclones isolated from VQ1-5, VQ10-11, and VQ10-12 showed white colonial color, and were prototrophic except for threonine requirement. The subclones isolated from VQ11-10 and VQ11-11 showed white colonial color and 1 subclones from VQ11-10 and 13 subclones from VQ11-10 showed methionine and threonine requirement.

A subclone from each of these triploid clones showing the biggest cell size, no mating ability but with ability to sporulate was presumed to be hexaploid and used for further study. Cell volume and DNA content per cell were determined. The results are shown in Tables II, III and IV. The selected subclones, i.e., strain

TABLE II: CELL VOLUME, DNA CONTENT, SPORE FORMATION, MATING TYPE AND RANDOM SPORE ANALYSIS OF TRIPLOID STRAIN VQ1 AND THE SUSPECTED HEXAPLOID STRAIN VQ1–5

Strain (Genotype)	Cell volume (μm^3)	DNA content ($mg/10^{11}$cells)	Spore formation	Mating type	Random spore analysis (+:−)							Possible ploidy
					thr[a]	met[b]	his	leu	trp	arg	lys	
VQ1 (C415–305B × C449–1B)	176.64	6.04	+	non[c]	28:6	0:34	19:15	20:14	25:9	24:10	16:18	triploid
lys7 met14 his4 leu2 thr4 + + *lys7 met14 his4 leu2 thr4* + + + + + + + *arg4 trp1*												
VQ1–5	207.36	12.36	+	non	0:29	0:29	18:11	11:18	15:14	11:18	11:18	suspected[d] hexaploid

[a] The threonine-auxotrophic trait was used as a selective marker in hexaploid breeding.
[b] The methionine-auxotrophic trait was used as a selective marker in random spore analysis of the triploid and the suspected hexaploid.
[c] non-mater.
[d] Ploidy was deduced from DNA data. For further explanation, see text.

TABLE III: CELL VOLUME, DNA CONTENT, SPORE FORMATION, MATING TYPE AND RANDOM SPORE ANALYSIS OF TRIPLOID STRAIN VQ10 AND THE SUSPECTED HEXAPLOID STRAINS VQ10–11 AND VQ10–12

Strain (Genotype)	Cell volume (μm^3)	DNA content ($mg/10^{11}$cells)	Spore formation	Mating type	Random spore analysis (+:−)								Possible ploidy
					thr[a]	red/white[b]	his	leu	trp	arg	lys	met	
VQ10 (C443–2C × C405–22A)	211.2	5.73	+	non[c]	32:5	37:0	20:17	19:18	36:1	34:3	15:22	22:15	triploid
ade1 lys2 + *met2 his4 leu2 thr4* *ade1 lys2* + *met2 his4 leu2 thr4* + + *arg4* + + + + + + + + *his6 trp1*													
VQ10–11	215.04	12.00	+	non	0:30	30:0	23:7	21:9	22:8	29:1	10:20	18:12	suspected[d] hexaploid
VQ10–12	119.68	11.46	+	non	0:28	28:0	12:16	18:10	25:3	24:4	11:17	3:25	suspected hexaploid

[a] The threonine-auxotrophic trait was used as a selective marker in hexaploid breeding.
[b] Red colonies were selected for random spore analysis of the triploid and the suspected hexaploid.
[c] non-mater
[d] Ploidy was deduced from DNA data. For further explanation, see text.

TABLE IV: CELL VOLUME, DNA CONTENT, SPORE FORMATION, MATING TYPE AND RANDOM SPORE ANALYSIS OF TRIPLOID STRAIN VQ11 AND THE SUSPECTED HEXAPLOID STRAINS VQ11–10 and VQ11–11

Strain (Genotype)	Cell volume (μm^3)	DNA content ($mg/10^{11}$cells)	Spore formation	Mating type	Random spore analysis (+:–)							Possible ploidy
					thr[a]	red/white[b]	his	leu	trp	arg	lys	met	
VQ11 (C443–2C–5C × C405–22A) *ade1 lys2* + *met2 his4 leu2 thr4* *ade1 lys2* + *met2 his4 leu2 thr4* + + *arg4* + + + + + + + + *his6 trp1*	176.64	5.62	+	non[c]	27:6	33:0	18:15	20:13	26:7	27:6	17:16	26:7	triploid
VQ11–10	69.12	12.50	+	non	0:30	30:0	21:9	21:9	10:20	13:17	7:23	0:30	suspected[d] hexaploid
VQ11–11	65.28	11.25	+	non	0:30	30:0	12:18	13:17	16:14	29:1	11:19	0:30	suspected hexaploid

[a] The threonine-auxotrophic trait was used as a selective marker in hexaploid breeding.
[b] Red colonies were selected for random spore analysis of the triploid and the suspected hexaploid.
[c] non-mater
[d] Ploidy was deduced from DNA data. For further explanation, see text.

VQ1-5, VQ10-11, VQ10-12, VQ11-10 and VQ11-11 were most probably hexaploids, because their DNA content per cell was almost double that of the original triploid strains from which they were derived.

Since the rate of spore germination of these supposed hexaploid strains was very low, it was very difficult to perform tetrad analysis. Therefore, random spore analysis was carried out. The result of the random spore analysis is also shown in Tables II, III and IV. In this analysis, the methionine-auxotrophic trait was used as a selective marker for the isolation of meiotic segregants from the triploid strain VQ1 and the supposed hexaploid strain VQ1-5 derived from it.

In contrast, the red colonial color was used as a selective marker in random spore analysis of triploid strain VQ10 and supposed hexaploid strains VQ10-11 and VQ10-12 derived from it, and of triploid strain VQ11 and the supposed hexaploid strains VQ11-10 and VQ11-11 derived from it. Since the adenine-auxotrophs showed red colored colonies, meiotic segregants were easily isolated each isolate was picked up on a complete medium plate and its nutritional requirements were tested on omission media. If the clone under consideration were triploid, a 1:1 segregation

of + and – alleles from –/–/+ configuration would be observed. On the other hand, some deviation from a 1:1 segregation of + and – alleles would be expected, if the clone were hexaploid and had the expected –/–/+/–/–/+ configuration. For example, in the case of VQ1-5, segregation of the + and – ratio of *lys7*, *his4* or *leu2* would be 1:1, if VQ1-5 were triploid. If VQ1-5 were hexaploid, some deviation from the 1:1 ratio would be expected in the segregation of these markers.

Determination of cellular DNA content was done one week after threonine-dependent clones were isolated, but the determination of cell volume was done 3 months after threonine-dependent clones were isolated. The results of cellular DNA content and cell volume did not correlate with each other. The data on cellular DNA content strongly suggested that all the suspected hexaploid strains VQ1-5, VQ10-11, VQ10-12, VQ11-10 and VQ11-11 were indeed hexaploid. However, no supporting evidence was obtained from the data for cell volume and segregation of genetic markers.

It is worth mentioning that chromosome loss or mitotic reduction or segregation of unequal numbers of chromosomes might have occurred during mitotic cell division in the polyploid cells. In fact, after several subcultures or after extended storage, all the suspected hexaploid strains VQ1-5, VQ10-11, VQ10-12, VQ11-10 and VQ11-11 consisted of heterogeneous cell populations with respect to cell size. The proportion of small cells is increasing with time. It is not known whether mitotic reduction actually occurred in this study. Further experiments are necessary to decide on this question.

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References

1. Lindegren, C.C., and Lindegren, G. (1943) A new method for hybridizing yeast. *Proc. Natl. Acad. Sci. U.S.A.* **29**, 306-308.
2. Mortimer, R.K., and Hawthorne, D.C. (1966) Genetic mapping, in *Saccharomyces Genetics* **53**, 165-173.
3. Mortimer, R.K., and Hawthorne, D.C. (1969) Yeast genetics, in *The Yeasts* (Rose, A.H., and Harrison, J.S. eds.) vol. 1, pp. 385-460, Academic Press, Inc., New York.
4. Hawthorne, D.C. (1963) Directed mutation of the mating type alleles as an explanation of homothallism in yeast. *Proc. 11th Inter. Congr. Genet.* **1**, 34-35.
5. Takano, I., and Oshima, Y. (1970) Mutational nature of an allele-specific conversion of the mating type by the homothallic gene, *HOα* in *Saccharomyces*. *Genetics* **65**, 421-427.
6. Hicks, J., and Herskowitz, I, (1976) Interconversion of matingt ypes in yeast. I. Direct observation of the action of the homothallism *(HO)* gene. *Genetics* **83**, 245-258.

7. Takano, I., and Oshima, Y. (1967) An allele specific and a complementary determinant controlling homothallism in *Saccharomyces oviformis*. *Genetics* **57**, 875-885.
8. Harashima, S., Nogi, Y. and Oshima, Y. (1974) The genetic system controlling homothallism in *Saccharomyces* yeasts. *Genetics* **77**, 639-650.
9. Takano, I., Oshima, T. Harashima, S. and Oshima, Y. (1977) Tetraploid formation through the conversion of the mating type alleles by the action of the homothallic genes in the diploid cells of *Saccharomyces* yeasts. *J. Ferment. Technol.* **55**, 1-12
10. Klar, A.J.S., and Fogel, S. (1977) The action of homothallism genes in *Saccharomyces* diploids during vegetative growth and the equivalence of hma and *HMα* loci functions. *Genetics* **85**, 407-416.
11. Plischke, M.E., von Borstel, R.C. Mortimer, R.K. and Cohn, W.E. (1976) Genetic markers and associated gene products in *Saccharomyces cerevisiae*, in *Handbook of Biochemistry and Molecular Biology* 3rd ed. (Fasman, G.D. ed.) Nucleic acid Vol. II pp. 767-832, Chemical Rubber Co. Press, Cleveland, Ohio, U.S.A.
12. Lederberg, J., and Lederberg, E.M. (1951) Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63**, 399-406.
13. Schneider, W.C. (1945) Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic aced. *J. Biol. Chem.* **161**, 293-302.
14. Burton, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**, 315-322.
15. Takano, I., Yoshizumi, H. and Terashima, Y. (1966) The ploidy of brewing yeasts. (1) The sporulation and the ploidy. *J. Ferment. Technol.* **44**, 150-157.
16. Takano, I., and Oshima, Y. (1973) The diploid *a* sporogenous strain and a novel method for breeding triploid cells in *Saccharomyces*. *J. Ferment. Technol.* **51**, 71.84.
17. Townsend, G.F., and Lindegren, C.C. (1954) Characteristic growth patterns of the different members of a polyploid series of *Saccharomyces*. *J. Bacteriol.* **67**, 480-483.

บทคัดย่อ

ในยีสต์พวก *Saccharomyces* มี mating-type 2 ชนิด คือ *a* และ *α* ซึ่งควบคุมโดยยีนส์ *a* และ *α* บนโครโมโซมแท่งที่ 3 สายพันธุ์ยีสต์พวก homothallic มี mating-type ไม่คงที่ การที่ mating-type *a* เปลี่ยนเป็น *α* ได้ และ *α* เปลี่ยนเป็น *a* ได้นั้นถูกควบคุมโดย homothallic genes 3 คู่

การศึกษาในที่นี้เป็นการผสมพันธุ์ยีสต์ให้ได้เป็น hexaploid โดยอาศัยการกระทำของ homothallic genes ขบวนการที่ใช้มี 2 ขั้นใหญ่ๆ คือ ขั้นแรกนำ *a*/*α* diploid Hq type เซลล์มาเลี้ยงรวมกับ *a* haploid Hq type เซลล์ ให้ได้เป็น *a*/*a*/*a* triploid เซลล์ ขั้นที่สองนำ triploid เซลล์เหล่านี้ไปฉายแสงอุลตราไวโอเล็ตที่ปริมาณพอเหมาะเพื่อให้ *a*/*a*/*a* triploid เซลล์บางเซลล์กลายเป็น *a*/*a*/*α* triploid เซลล์ โดยอาศัยการกระทำของ homothallic genes ทำให้ *a*/*a*/*α* triploid เซลล์บางเซลล์ถูก *convert* เป็น *a*/*a*/*a* triploid เซลล์ในระหว่างการแบ่งเซลล์ หลังจากนั้น *a*/*a*/*α* triploid เซลล์ซึ่งเลี้ยงอยู่ร่วมกันกับ *a*/*a*/*a* triploid เซลล์ก็เกิด cell fusion เกิดเป็น hexaploid เซลล์ *a*/*a*/*a*/*a*/*a*/*α*

ผลการวิจัย สามารถผสมพันธุ์ยีสต์ให้ได้เป็น hexaploid ทั้งหมด 5 สายพันธุ์ทั้ง 5 สายพันธุ์นี้มีปริมาณ DNA เป็น 2 เท่าของ triploid เซลล์ แต่ขนาดของเซลล์ไม่ได้เป็น 2 เท่าของ triploid เซลล์ เมื่อเลี้ยง hexaploid เซลล์นี้ไว้นานเกิน 3 เดือน ขนาดของเซลล์จะเล็กลง อาจเป็นเพราะเกิด mitotic reduction ในระหว่างที่มี vegetative growth