BISEXUAL MATING REACTION IN *SACCHAROMYCES CHEVALIERI*

TOSHIAKI TAKAHASHI¹ AND YONOSUKE IKEDA

Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan

Received July 7, 1958

IN spite of the accepted belief that homothallic strains of *Saccharomyces* yeasts have no mating type, it was found by TAKAHASHI, SAITO, and IKEDA (1958) that the homothallic strain isolated by them (*Saccharomyces cerevisiae* S-341) does possess a mating type, but in a concealed form, and that its homothallism controlled by three pairs of alleles, e.g., strains possessing $HM_1 HM_2 hm_3$ and $hm_1 HM_2 HM_3$ are diploids, while those carrying $HM_1 hm_2 hm_3$, $hm_1 HM_2 hm_3$, and $hm_1 hm_2 hm_3$ are haploids (TAKAHASHI 1958).

This paper is concerned with studies on the mating reaction of *S. chevalieri*, and an attempt was also made to determine whether the diploidization gene *D* of this strain (WINGE and ROBERTS 1949) is equivalent to one of the *HM* genes. The material, *S. chevalieri* strain No. 9, was supplied from the Carlsberg Laboratory through the courtesy of Dr. C. ROBERTS.

MATERIALS AND METHODS

Six strains were employed.

S-2141: A single spore culture of *S. chevalieri* strain No. 9; homothallic diploid carrying the gene *D*; adenine and arginine independent; galactose² and sucrose fermenter; maltose and melibiose nonfermenter.

714-41: A homothallic diploid carrying the genes HM_1 and HM_2 ; concealed mating type α ; adenine independent and arginine dependent; galactose, sucrose, and melibiose fermenter; maltose nonfermenter.

753-44: A homothallic diploid carrying the genes HM_1 and HM_2 ; concealed mating type α ; adenine and arginine dependent; galactose, sucrose, and melibiose fermenter; maltose nonfermenter.

733-51: A heterothallic haploid carrying the genes HM_1 and hm_2 ; mating type α ; adenine and arginine dependent; galactose, maltose, and melibiose fermenter; sucrose nonfermenter.

733-53: A heterothallic haploid carrying the genes HM_1 and hm_2 ; mating type α ; adenine and arginine dependent; sucrose, maltose, and melibiose fermenter; galactose nonfermenter.

H-965: A heterothallic diploid strain; heterozygous for mating type and maltose fermentation; homozygous for HM_1 and hm_2 , adenine and arginine dependency, galactose and melibiose fermentation, and sucrose nonfermentation.

Crosses between diploid strains were performed by the spore-to-spore copula-

¹ Present address: Brewing Scientific Research Institute, Asahi Breweries Co. Ltd., Suita-shi.

² According to WINGE and ROBERTS (1948), *S. chevalieri* is a slow-fermenter.

tion technique (WINGE and LAUSTSEN 1938). The mating type of the haploid segregants was determined by the mass mating method involving the presence or absence of a mating reaction (LINDEGREN and LINDEGREN 1943); these types will be described hereafter as mass mating types in contrast to the concealed mating types, which were detectable only by the minimal plate mating method (TAKAHASHI, SAITO, and IKEDA 1958).

Nutritional requirements were determined by the presence or absence of positive growth on minimal medium supplemented with adenine (20 mg/1000 ml) and/or arginine (100 mg/1000 ml). Sugar fermentation was tested by the use of B. C. P. indicator medium (HAWTHORNE 1955).

Modified Lindegren's yeast broth (see SAITO and TAKAHASHI 1957), Burkholder's medium (see LINDEGREN 1949), and FOWELL's medium (FOWELL 1952) were used as complete, minimal, and sporulation media, respectively.

Genotypes are to be expressed in the following way: homothallism (diploidization) controlling gene in *S. chevalieri*, *D*; homothallism controlling complementary genes in strains 714-41 and 753-44, *HM*₁ *HM*₂; mating type, *a/a*; independence/dependence of adenine and arginine, *AD/ad* and *AR/ar*; ability/inability to ferment galactose, sucrose, maltose, and melibiose, *GA/ga*, *SU/su*, *MA/ma*, and *ME/me*.

RESULTS

Hybrids H-891 and H-899 were produced by the spore-to-spore copulation technique.

	714-41	<i>HM</i> ₁ <i>HM</i> ₂	<i>a</i>	<i>AD</i>	<i>ar</i>	<i>GA</i>	<i>SU</i>	<i>ma</i>	<i>ME</i>
H-891:	S-2141		<i>D</i>	<i>AD</i>	<i>AR</i>	<i>GA</i>	<i>SU</i>	<i>ma</i>	<i>me</i>
	753-44	<i>HM</i> ₁ <i>HM</i> ₂	<i>α</i>	<i>ad</i>	<i>ar</i>	<i>GA</i>	<i>SU</i>	<i>ma</i>	<i>ME</i>
H-899:	S-2141		<i>D</i>	<i>AD</i>	<i>AR</i>	<i>GA</i>	<i>SU</i>	<i>ma</i>	<i>me</i>

In the tetrads arising from these two hybrids, every character except sexuality showed a 2:2 segregation as seen in Table 1 and Table 2. With regard to the sexual behavior, the single spore cultures could be classified into three types, as follows:

Type I: The strains showed positive mass mating reaction and negative sporulation. They are heterothallic haploids carrying either *HM*₁ or *HM*₂.

Type II: The strains showed negative mass mating reaction, positive sporulation, and positive concealed mating reaction. They mated with either *a* or *α* only when the minimal plate mating method was employed. They are homothallic diploids carrying *aa* or *αα* and *HM*₁*HM*₁, *HM*₂*HM*₂.

Type III: The strains showed negative mass mating reaction, positive sporulation, and positive concealed mating reaction. They mated with both *a* and *α* only when the minimal plate mating method was employed. They are homothallic diploids probably possessing *DD*, and they are bisexual.

Through tetrad analysis, the hybrids were found to segregate out as either two Type III and two Type II, or as two Type III, one Type II, and one Type I.

TABLE 1
Segregation data of H-891

Segregant	Segregation	Sexual behavior			Type
		Mass mating type	Concealed mating type	Sporulation	
891-11	<i>ar me</i>	—	<i>b</i> *	+	III
12	<i>AR me</i>	—	α	+	II
13	<i>AR ME</i>	—	<i>b</i>	+	III
14	<i>ar ME</i>	<i>a</i>	— †	—	I
891-21	<i>ar ME</i>	—	α	+	II
22	<i>AR me</i>	—	<i>b</i>	+	III
23	<i>AR me</i>	—	<i>b</i>	+	III
24	<i>ar ME</i>	—	α	+	II
891-31	<i>AR me</i>	—	<i>b</i>	+	III
32	<i>ar me</i>	—	<i>b</i>	+	III
33	<i>ar ME</i>	—	α	+	II
34	<i>AR ME</i>	—	<i>a</i>	+	II
891-41	<i>AR ME</i>	—	<i>b</i>	+	III
42	<i>AR me</i>	—	α	+	II
43	<i>ar me</i>	<i>a</i>	—	—	I
44	(nongerm.)				
891-51	<i>AR ME</i>	—	<i>b</i>	+	III
52	<i>ar me</i>	—	<i>a</i>	+	II
53	<i>AR me</i>	—	<i>b</i>	+	III
54	(nongerm.)				

* Bisexual.

† Not examined.

Because the concealed mating types of strains 714-41 and 753-44 were unisexual, the bisexuality must have arisen from strain S-2141.

In order to test this hypothesis, the concealed mating potency of strain S-2141 was re-examined. This strain was positive in sporulation and reacted with neither *a* or α by the mass mating method, but it mated with both *a* and α by the minimal plate mating method. Moreover, the sister spore cultures which arose from the same ascus were also bisexual. Therefore, the original culture of *S. chevalieri* strain No. 9 is apparently homozygous with regard to bisexuality.

Assuming that bisexuality might be related to the presence of the *D* gene, a new hybrid (H-1162) was produced by the spore-to-spore copulation technique.

	S-2141	<i>D AD AR GA SU ma me</i>							
H-1162:	H-965	<i>HM₁</i>	<i>hm₂</i>	<i>ad</i>	<i>ar</i>	<i>GA</i>	<i>su</i>	<i>MA</i>	<i>ME</i>

Only two ascospores in each ascus of H-1162 germinated normally, and every character except mating type segregated out in general in a 1:1 ratio. Mating types of the single spore segregants were either unisexual by mass mating or bisexual by minimal plate mating as seen in Table 3. None of the segregants

belonged to Type II; this was expected due to the absence of the *HM₂* gene in this material.

Hybrids H-981 and H-986 were produced by crossing cells of S-2141 with cells of 733-51 and 733-53 according to the minimal plate mating method. The cell sizes of the resulting hybrids are larger than any of the parental strains (Table 4 and Figure 1), and they seem to be triploids. Their segregation pattern will be reported elsewhere.

DISCUSSION

As stated before, the starting strain S-2141, a single spore culture of *S. chevalieri*, is homozygous with regard to bisexuality; it mates with cell of both *a* and α strains of heterothallic haploids as well as with spores of concealed *aa* and $\alpha\alpha$ strains of homothallic diploids. Therefore, this strain of *S. chevalieri* is bisexual both in the ascospore state and the vegetative cell state.

All of the diploid segregants obtained from H-1162 were bisexual. Consequently, gene *D*, which was reported by WINGE and ROBERTS (1949) as responsible for the self-diploidization of single ascospore cultures in *S. chevalieri*

TABLE 2
Segregation data of H-899

Segregant	Segregation	Sexual behavior			Type
		Mass mating type	Concealed mating type	Sporulation	
899-11	<i>ad ar me</i>	—	<i>b</i>	+	III
12	<i>AD AR ME</i>	<i>a</i>	—	—	I
13	<i>ad ar me</i>	—	α	+	II
14	<i>AD AR ME</i>	—	<i>b</i>	+	III
899-21	<i>ad ar ME</i>	—	α	+	II
22	<i>ad AR ME</i>	—	<i>b</i>	+	III
23	<i>AD AR me</i>	—	<i>b</i>	+	III
24	<i>AD ar me</i>	—	α	+	II
899-51	<i>AD ar ME</i>	<i>a</i>	—	—	I
52	<i>AD AR ME</i>	—	<i>a</i>	+	II
53	<i>ad AR me</i>	—	<i>b</i>	+	III
54	<i>ad ar me</i>	—	<i>b</i>	+	III
899-61	<i>AD AR me</i>	—	<i>b</i>	+	III
62	<i>ad ar ME</i>	—	<i>b</i>	+	III
63	<i>AD AR me</i>	α	—	—	I
64	(nongerm.)				
899-71	<i>AD AR me</i>	<i>a</i>	—	—	I
72	<i>ad AR ME</i>	—	<i>b</i>	+	III
73	<i>AD ar me</i>	—	α	+	II
74	<i>ad ar ME</i>	—	<i>b</i>	+	III

TABLE 3
Segregation data of *H-1162*

Segregant	Segregation	Sexual behavior			Type
		Mass mating type	Concealed mating type	Sporulation	
1162-31	<i>ad AR su me</i>	—	<i>b</i>	+	III
32	<i>ad AR su ME</i>	α	—	—	I
1162-41	<i>AD AR SU ME</i>	<i>a</i>	—	—	I
42	<i>AD ar su me</i>	—	<i>b</i>	+	III
1162-51	<i>AD AR su ME</i>	<i>a</i>	—	—	I
52	<i>AD ar su me</i>	<i>a</i>	—	—	I
1162-61	<i>AD AR su me</i>	—	<i>b</i>	+	III
62	<i>AD AR SU ME</i>	<i>a</i>	—	—	I
1162-71	<i>AD AR SU me</i>	<i>a</i>	—	—	I
72	<i>AD ar SU ME</i>	α	—	—	I
1162-81	<i>ad ar SU me</i>	—	<i>b</i>	+	III
82	<i>ad ar SU ME</i>	α	—	—	I
1162-101	<i>ad ar SU ME</i>	—	<i>b</i>	+	III
102	<i>ad ar SU me</i>	—	<i>b</i>	+	III

TABLE 4
Check of marker traits as evidence for conjugation between *S-2141* and haploid strains

Strain	Genotype or phenotype								Cell size (volume)
733-51	α	<i>d</i>	<i>ad</i>	<i>ar</i>	<i>GA</i>	<i>su</i>	<i>MA</i>	<i>ME</i>	$4.35\mu \times 5.25\mu$ ($52.2\mu^3$)
733-53	a	<i>d</i>	<i>ad</i>	<i>ar</i>	<i>ga</i>	<i>SU</i>	<i>MA</i>	<i>ME</i>	$4.40\mu \times 5.25\mu$ ($52.8\mu^3$)
S-2141	<i>D</i>	<i>AD</i>	<i>AR</i>	<i>GA</i>	<i>SU</i>	<i>ma</i>	<i>me</i>		$3.88\mu \times 12.60\mu$ ($128.8\mu^3$)
	<i>D</i>	<i>AD</i>	<i>AR</i>	<i>GA</i>	<i>SU</i>	<i>ma</i>	<i>me</i>		
H-981	$\left(\frac{733-51}{S-2141} \right)$		<i>ADE ARG GAL SUC MAL MEL</i>						$5.85\mu \times 10.45\mu$ ($161.4\mu^3$)
H-986	$\left(\frac{733-53}{S-2141} \right)$		<i>ADE ARG GAL SUC MAL MEL</i>						$5.65\mu \times 9.40\mu$ ($156.1\mu^3$)

might have something to do with the mating potency. One possibility is that this gene converts both mating types to a neutral type (*n*), which is able to mate with both *a* and α types. If one assumes that the following cross is made:

$$nD \times ad$$

then the meiotic products of the hybrid could be:

- (1) *aD* $\rightarrow$ *aaDD*
- (2) *ad*
- (3) *nd*
- (4) *nD* $\rightarrow$ *nnDD*.

As matter of fact, the diploids were all bisexual. Therefore if this hypothesis is correct, *aaDD* must have been converted into *nnDD*. To explain the rather frequent appearance of the α mating type in this material, transformation from

FIGURE 1.—Assumed triploids and their parents. (1) haploid: 733-51. (2) haploid: 733-53. (3) diploid: S-2141. (4) triploid: H-981 (733-51 $\times$ S-2141). (5) triploid: H-986 (733-53 $\times$ S-2141).

nd to *ad* must also be taken into account. However, since here the accompanying gene is *d*, rather than *D*, and since gene *n* is considered as neutral, the transformation might possibly take place.

In connection with this hypothesis, the authors wish to refer to WINGE and ROBERTS's statement (1958), according to which the ascospores arising from an *aaDD* culture were able to mate with every ascospore from an *aaDD* culture;

aD thus mated with both aD and αD , and this fact is consistent with the present view. However, the above hypothesis will be studied further.

None of the HM genes appear to be identical with the gene D . Strains with $HM_1 hm_2 hm_3$ and $hm_1 HM_2 hm_3$ are haploids and strains with $HM_1 HM_1$, $HM_2 HM_2 hm_3 hm_3$ and $hm_1 hm_1 HM_2 HM_2 HM_3 HM_3$ are diploids and either a or α . However, as mentioned previously (TAKAHASHI, SAITO, and IKEDA 1958) homothallic strains with HM genes were sometimes asexual by the minimal plate mating method, and, in addition, the sexual reaction of polyploid yeasts becomes weaker with increase in ploidy, so that it might be supposed that the diploidization genes such as D and HM could produce a common effect upon the expression of mating potency.

As seen in Table 1 and 2, a few heterothallic haploid cultures were produced from the hybrids between two homothallic *Saccharomyces*. This may be due to the appearance of segregants carrying either the HM_1 or the HM_2 gene, because the crosses were made between strains with $HM_1 HM_2$ and strains with $hm_1 hm_2$. Thus the production of heterothallic yeasts from the homothallic yeasts is indicated.

SUMMARY

In order to examine whether or not the concealed mating type found in a homothallic strain of *S. cerevisiae* is common among another species of homothallic yeasts, the mating potency of *S. chevalieri* was studied.

It was found that this yeast is bisexual, being able to mate with both a and α strains in the ascospore and the vegetative cell states. Among the segregants from the hybrids between a D gene strain and a Carbondale heterothallic strain, the diploids were invariably bisexual. It was concluded that the D gene was different from any of the HM genes, and its connection with bisexuality was discussed.

Heterothallic haploid cultures were produced from the hybrid between two homothallic *Saccharomyces*. These haploid cultures were presumed to carry either the HM_1 or the HM_2 gene.

ACKNOWLEDGMENT

The authors wish to express their thanks to DR. C. ROBERTS for her valuable comments and criticism of this manuscript.

LITERATURE CITED

- FOWELL, R. R., 1952 Sodium acetate agar as a sporulation medium for yeasts. *Nature* **170**: 578.
HAWTHORNE, D. C., 1955 The use of linear asci for chromosome mapping in *Saccharomyces*. *Genetics* **40**: 511-518.
LINDEGREN, C. C., 1949 *The Yeast Cell, Its Genetics and Cytology*. Educational Publishers, Inc., St. Louis.
LINDEGREN, C. C., and G. LINDEGREN, 1943 A new method for hybridizing yeast. *Proc. Natl. Acad. Sci. U. S.* **29**: 306-308.

- SAITO, H., and T. TAKAHASHI, 1957 Gene controlled adenine dependence epistatic to color production in *Saccharomyces*. *Japan J. Genetics* **32**: 147-152.
- TAKAHASHI, T., 1958 Complementary genes controlling homothallism in *Saccharomyces*. *Genetics* **43**: 705-714.
- TAKAHASHI, T., H. SAITO, and Y. IKEDA, 1958 Heterothallic behavior of a homothallic strain in *Saccharomyces* yeast. *Genetics* **43**: 249-260.
- WINGE, Ø., and O. LAUSTSEN, 1938 Artificial species hybridization in yeast. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol.* **22**: 235-...
- WINGE, Ø., and C. ROBERTS, 1948 Inheritance of enzymatic characters in yeasts, and the phenomenon of long-term adaptation. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol.* **24**: 263-315.
- 1949 A gene for diploidization in yeasts. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol.* **24**: 341-346.
- 1958 Life history and cytology of yeasts. pp. 93-122. *The Chemistry and Biology of Yeasts*. Edited by A. H. COOK. Academic Press, Inc. New York.